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Effect of Hydatid Cyst Infection on Oxidative Stress in Producing Mice.

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﴿ بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ﴾

﴿وَأَنزَلَ ٱللَّهُ عَلَيْكَ ٱلْكِتَٰبَ وَٱلْحِكْمَةَ وَعَلَّمَكَ مَا لَمْ تَكُن تَعْلَمُ ۚ وَكَانَ فَضْلُ ٱللَّهِ عَلَيْكَ عَظِيمًا﴾

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Certification

We certify that the thesis entitled (**Effect of Hydatid Cyst Infection on Oxidative Stress in Producing Mice)** was prepared by (**Abbas Nasser Hussein**) under our supervision at the Department of Biology , College of Science , University of Babylon , as a partial fulfilment of the requirement for the degree of Ph.D in Biology.

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Dedication

To …….

Greater ……..…. Allah

My father ……. with respect

The pulsing heart…. My mother

my brother and sisters … thank you for your support

To….My dear wife, I'm so blessed of having you. Thank you for your love, care, support and every little effort you are doing for me is simply amazing.

To…. The givers of science and life growers all over the world.

ABBAS

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Summary

Oxidative stress of life history theory represents as a physiological mechanism drives the tradeoff between reproduction and somatic protection. Cumulative data from wild mammals support this theory. However, findings from laboratory mammals don't support it. One of the possible reasons could be related to lack of increased oxidative stress among lactating lab mammals is an experimental design being used.

The design of previous studies focused on testing oxidative stress between animals with different reproductive status or different reproductive efforts. It has been questioned that the above design may not be enough to explore the oxidative cost of reproduction as the lactating animals may not be limited to an internal trade-off (physiological costs), but may be realized through other exogenous forces such as an increased exposure to predators or parasitic infection (ecological costs).

In this study, we suggested a new design for testing the oxidative stress of life history theory by measuring the levels of different kinds of antioxidants and oxidative damage using varieties of internal tissues in lactating mice that exposed to parasitic infection and comparing to non-reproducing mice. Forty females of BALB/C mice (Mus musculus) were intraperitoneally infected with a parasite (viable Protoscolices 2000 live of *Echinococcus granulosus* /per mouse) and another 19 females were uninfected.

All mice were housed in a cage (one mouse per cage) and food and water were given ad libitum. As a result, the 16 lactating female mice and 16 non-reproducing mice were randomly allocated into four groups (N=8 for lactating infected (LI), N=8 for lactating uninfected (LU), N=8 for non-

reproducing infected parasitized (NRI) and N=8 for non-reproducing uninfected (NRU). Body mass during baseline showed a significant difference between the groups. During pregnancy, the body mass of pregnant mothers increased significantly, and body mass during lactation showed a significant difference between the groups.

The food intake showed a significant difference between the groups during the baseline period. During pregnancy , food intake of pregnant mothers increased significantly, During lactation was a significant difference between the groups.

The litter size (LS) at birth (2 day after birth) was a significant difference between (LI) and (LU), (LS) at 18 day of lactation was a significant difference between two groups.

The litter mass (LM) at birth (2 days after birth) was a significant difference between (LI) and (LU). (LM) at the 18th day of lactation was also a significant difference between the two groups.

Pup mass (PM) at birth (2 days after birth) was showed no significant difference between the (LI) and (LU), (PM) at day 18 of lactation was showed no significant difference between the two groups.

At day of weaning (day 18 of lactation), liver, heart and brain tissues were collected and levels of antioxidants (SOD, CAT, GPx) and markers of oxidative damage (Protein carbonyl for protein damage (PC), Malondialdehyde for lipid damage (MDA), and 8- Hydroxyguanosine for DNA damage (8OHDG) were measured.

Results on the levels of antioxidants measured in the liver and brain tissues indicated that the levels of SOD and CAT were not significantly influenced by reproductive status and parasitic infection, while the levels of

liver GPx in the (NRU) group were significantly reduced compared to the values in the (NRI) group.

Levels of brain GPx were significantly reduced in the (LI) group compared to other groups. Levels of antioxidants in heart tissue indicated that both SOD and GPx were also not significantly influenced by both reproductive status and parasitic infection, while the levels of CAT were significantly greater in the (LI) group compared to the (NRU) group.

Findings on measures of oxidative damage indicated that the levels of both (protein and DNA) damage were not significantly changed by reproduction and parasitic infection measured in the liver and heart, while the levels of liver lipid damage were significantly reduced in the (NRU) group compared to the levels observed in the (LI) group.

Levels of brain (DNA and lipid) damage were not significantly differed among groups, while the levels of protein damage were significantly reduced in the (LI) group compared to the (NRU) group. The levels of heart lipid damage were significantly greater among (LI) group compared to the (NRU) group.

Liver function, GPT levels were not significantly influenced by infections status. However, lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected).

GOT levels were not significantly influenced by reproductive status. However, lactating mice (infected only) had significantly greater (higher compared to the non-reproducing mice (infected and uninfected). ALP levels were not significantly influenced by infections status and reproductive status.

Brain function was measured by measuring the level of BDNF protein, BDNF levels were not significantly influenced by infections status.

However, BDNF levels were significantly influenced by reproductive status. lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected). Heart function measured by Brain Natriuretic Peptide (BNP) , BNP levels were not significantly influenced by infections status. However, BNP levels were significantly influenced by reproductive status. lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected).

CTn levels were not significantly influenced by infections status. However, CTn levels were significantly influenced by reproductive status. lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected).

In conclusion, the parasitic infection in lactating mice could be associated with increase oxidative damage measured in different tissues, indicating that this design could expand our understanding on the role of ecological cost in the context of oxidative stress theory for driving the trade –off between reproduction and somatic protection.

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List of Abbreviations

Abbreviation	Meaning
ALP	Alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BDNF	Brain derived neurotrophic factor
BM	Body mass
BNP	Brain Natriuretic Peptide
CAT	Catalase
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbant assay
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
H2O2	Hydrogen peroxide
K.R.S.	Kreb's - Ringer's Solution
LI	Lactating infected parasitized
LM	Litter mass
LS	Litter size
LU	Lactating uninfected parasitized
MDA	Molandialdehyde
NRI	Non-reproduce infected parasitized
NRU	Non-reproduce uninfected parasitized
PBS	Phosphate Buffer Saline Solution

PC	Protein carbonyls
PM	Pup mass
SOD	Superoxide dismutase
CTn	Troponin
8-OHdG	8-hydroxy-2-deoxyguanosine
TBARS	Thiobarbituric Acid Reactive Substances

CHAPTER ONE
INTRODUCTION

Introductions:

Cystic hydatid disease (CHD) is caused by *Echinococcus granulosus*, A zoonotic disease that has been largely ignored despite its negative impact on human health and cattle ranching across the globe, (Grosso, *et al* ,2012; Mandal, & Mandal,.2012) The larval stage of *Echinococcus granulosus*, known as the metacestode or hydatid cyst, It develops in mammalian intermediate hosts' lungs and livers and causes organ and tissue damage. (McManus., *et al;*2003).

The hydatid cyst is a cavity that is filled with fluid with an inner germinal layer and a laminar layer that is rich in carbohydrates surrounds each cell. (Koziol & Brehm,;2015). Stem cells compose the germinal layer that can develop into protoscoleces (PSCs), which are forms found before an individual becomes an adult. The parasite has a strong hold on its intermediate host and has successfully adapted to its environment., surviving and developing despite adverse host reactions for decades (Frider,1999). To do so, the parasite devised methods to undermine the immune response of the host (Siracusano, *et al*, 2012; Daz, *et al*, 2016).

Parasites live part of their lives on or inside their hosts and obtain their resources from the living bodies of other organisms (Price 1980). As a result, resources from the host that would have been needed for upkeep, survival, and reproduction will be used by the parasites instead. Thus, it is hypothesized that parasites will have a negative impact on the fitness of their hosts (Mller, Allander & Dufva 1990; Lehmann 1993). Due to the high costs associated with both parasite defense and reproduction, an animal must balance how much of its resources are allocated to both (Mller, 1997).

Life-history theory assumes that reproduction is costly and competes for resources with other costly activities performed by individuals. An individual cannot simultaneously maximize all life-history traits since energy is a limiting resource that has to be optimally allocated among different functions (Williams 1966; Stearns 1992).

during reproduction, when both intake and expenditure increase to meet the increased energy demands of growth of the offspring and protection of the somatic cells. according to life history theory, reactive oxygen species(ROS), are responsible for oxidative stress and the damage it causes to macromolecules., were produced in direct proportion to metabolic rate. Due to this, an increase in metabolic rate combined with increased reproduction causes an increase in ROS production.(Selman, Blount *et al*. 2012). If this is the case, energy allocation between the growth of the offspring and physiological antioxidant defense against elevated ROS levels may be compromised. Although inconsistent and contradictory, the results of some recent studies may not be apparent in the oxidative stress life cycle. (Nussey *et al*, 2009; Garratt *et al*, 2012; Fletcherr *et al*, 2013).

antioxidant defenses are absolutely necessary., ROS and RNS are formed during the immunological response of the host, while RNS is formed during the oxidative metabolism of the intracellular organelles. ROS and RNS are both reactive oxygen and reactive nitrogen species, respectively., are harmful to the components of tissue. This is due to the fact that ROS and RNS are both capable of causing damage to DNA, proteins, lipids, and carbohydrates, which in turn causes the functions of these components to change (Pisoschi, & Pop, 2015).As a consequence of this, In order to protect themselves from the damaging effects of oxidative stress, Enzymatic and non-enzymatic defensive mechanisms

have been produced by both unicellular and multicellular species . Glutathione are example of non-enzymatic activities, whereas peroxiredoxins and superoxide dismutases are involved in enzymatic mechanisms. (Mehtta, & Gowder, 2015).

In the case that parasite effects occur, the story may be different depending on the amount of oxidative and antioxidant stress that is created.

Aim of study:

A new test for an oxidative stress of life history theory under parasitic infection.

Objectives

Investigate in a comparative way:-

1. How parasitic infection is related to reproductive performance of mice (mother food intake, mother body mass, fecundity, litter mass, pup mass , and number mortality)
2. How parasitic infection is related to antioxidants status among different tissues of lactating mice.
3. How parasitic infection is related to oxidative damage status among lactating mice.
4. How the interaction between parasitic infection and reproduction is related to organ function among different tissues of lactating mice.

CHAPTER TWO
LITERATURE REVIEW

2.Literature review

2.1.Echinococcus granulosus

The hydatid disease, which is carried on by the larval stage of the *Echinococcus granulosus* tapeworm, can be found in nearly every country on the planet (Morris and Richards.,1992). Hippocrates, who lived from 460 to 377 BC, was the first person to make a detailed description of hydatid cysts (Hosemann *et al.*,1928, Eckert and Thompson,2017).

Hydatid cysts in the liver were compared to fluid balloons by Al-Rhazes, a Persian physician who practiced medicine about the year 900 AD (Kattan,1977),assuming that the cysts were either insect eggs or embryos or cystic tumors at the time. The hydatid cysts were separated into adherent and nonadherent types by Peter Pallas in 1776, and dubbed Taenia hydatigena cysts. The cysts were considered to be caused by lymph gland dysfunction (Grove,1990). Goeze discovered protoscoles in hepatic cysts in 1782, and it was discovered that they appeared in the shape of granules on the inner surface of the cyst, with a similarity to the front end of the tapeworm, leading to the conclusion that the tapeworm is the organism that causes the condition (Elderdiri,2014). The hydatid cysts in sheep were later recognized as Hydatigena granulosa by Batsch (1786). (Grove *et al.*,1990).

The name Echinococcus was suggested by Rudolphi (1801), and it comes from a Greek phrase that means "spine and berry" (Craig *et al.*,2006).

In 1855, Haubner noticed the development of cysts in pigs experimentally infected with parasitic eggs E. granulosus, which was

followed by Krabbe's results, who successfully infected sheep with parasitic eggs experimentally (Tappe *et al*.,2010).

Nannun discovered adult worms in the intestines of dogs after feeding them the contents of a hydatid cyst taken from an infected human in 1863, proving the link between human and animal infestations with hydatid cysts; also, Leuckart and Virchow determined the morphology features of the cysts and their contents, which led to the discovery of hydatid cysts. (Guillebeau,1890, Boemke,1939, Tappe *et al*.,2010).

The risk of the infection lies in the fact that the affected person does not have clinical signs despite the growth and development of cyst within the body tissues until they reach a certain size, causing pressure on the neighboring organs due to the length of time for those cysts (Zhang *et al*., 2003). Classification The parasite was classified into followings by (Lewall and Mccorkell.,1985)

Kingdom Animalia

Subkingdom Eumetazoa

Phylum Platyhelmynthes

Subphylum Cestodes

Class Cestoda

Subclass Eucestoda

Order Cyclophyllidea

Family Taeniidae

Subfamily Echinococcinae

Genus Echinococcus

Species *Echinococcus granulosus*

There are a variety of species that were classified as belonging to the genus Echinococcus in the family Taeniidae according to the following criteria: (Craig *et al*.,2003): Family Taeniidae Ludwig, 1886 *Echinococcus multilocularis* Leuckhart, 1863; produces alveolar echinococcosis, *Echinococcus oligarthrus* Diesing, 1863; causes polycystic echinococcosis, *Echinococcus vogeli* Rausch and Bernstein, 1972; causes polycystic echinococcosis Genus *Echinococcus Rudolphi*, 1801 *Echinococcus granulosus* Batsch, 1786; causes cystic echi, *Echinococcus canadensis*, *Echinococcus equinus*, *Echinococcus ortleppi*, *Echinococcus felidis*, and *Echinococcus shiquicus* are the five additional species that have been reported in recent years (Hüttner etal., 2008; Shi *et al.*, 2019).

Echinococcus has several features that distinguish it from the other genus in the Taenia family; the adult tapeworm is few millimetres in long (usually 2- 7 mm), whereas other Taenia species can grow to a length of several meters and consist of several thousand proglottides (Eckert *et al*.,2001).

The body is consisting of a scolex on the anterior end, followed by a neck and a strobili; the scolex has four muscle suckers that used to attach the body to the host's intestinal wall and a rostellum with two rows of hooks around it (25-50 hooks) (Thompson, 2017), the strobila consists of three to six proglottides; the proglottides slowly develop towards the

back end of the worm and the last is normally a gravity section of uterine eggs (Patkowski *et al*.,2017).

Echinococcus species lack digestive and respiratory systems, so that metabolic activity takes place through the tegument; this structure covers the body and protects the parasite from host enzymes and immune reactions (Sazmand and Joachim.,2017).

The adult has reproductive ducts that open on a commonly lateral genital pore, and it is hermaphrodite.; the genital pore is proximal to the center of the proglottide; the cirrus sac-located horizontally or internally deviated; vitellarium is globular; uterus distended after fertilization that occupies most terminal proglottides when the eggs are fully developed (Eckert *et al*.,2001).

2.1.1.Metacestode (Hydatid cyst) It is composed of the following components:

2.1.1.1. The outer layer (peri cyst):

It is made up of tightly packed host cells, fibroblasts, giant cells, and eosinophils, all of which combine to produce a stiff structure and many millimetres thick protective layer prevents the parasitic secretions from the passage into the immune system (Pakala *et al*.,2016, Budke *et al*.,2017).

2.1.1.2 Laminated Layer:

A cellular, elastic, and stiff material that is two millimeters thick., According to the majority of research, this layer is formed by the parasite and not by the host. (Reinehr *et al*.,2020).

It contains mixture of thick granules of microfibrial unknown chemical structure , this layer protects the parasite against the immune response of the host (Pittini *et al*.,2019).

2.1.1.3 Germinal Layer:

Thin about (20-25 μm in thickness) one translucent layer of cuboid cells Ahmadi and Badi (2011), regulate and monitor the permeability of the wall, allowing other ions to reach the cyst and eventually regulate the osmotic pressure within the cyst, this layer considers the laminated layer source and the creation of protoscoles (Higuita *et al.*,2016).

2.1.1.4 Broad Capsule:

Vesicular structures develop through endogenous budding from the germ layer, its diameter about (250-500) microns with protoscolices, hydatid sand may be detached into cyst fluid (Khalil *et al.*,2015)

2. 1.1.5 Daughter Cysts:

Formed within larger original cysts; however, the cyst growth rates can vary between cysts in the same organs and hosts in different regions, because of mutation, endogenous budding might not produce offspring that are genetically similar to the mother's cyst because to chemical substances, radiation, or immunological differences. Grubor *et al*.,(2017), new cyst may spread into other sites after cyst rupture and the escape of protocols (Malik and Bari.,2019).

2.1.1.6 Protoscolices:

There are seven steps of the formation of protoscolecies, The hydatid cyst germ layer gives rise to the formation of cellular buds, each of which is made up of a cluster of cells., the buds are elongated and seem to diminish in number (Romig,2003).

The anterior (scolex) and caudal (body) parts of elongated buds emerge extremely early on a furrow., hooks are the first structures to be completely distinguished in the apical area of the nascent scolex, the scolex is displaying circular projections and depressions in an advanced stage which grows into suckers. Acone can be seen in the center of the hooks later, A structural neck between the scolex and the body is visible when the body has expanded. (Galindo *et al*.,2002).

During the protoscolecies, remains attached through a stalk to the germinative substrate (Galindo *et al*., 2003), the stalk is cut off when completely differentiated and the infectious protoscolecies are now free in Hydatid fluid (Díaz *et al*.,2011),

2. 1.1.7 Cyst Fluid:

It is characterized by colorless – yellow fluid, pH (6.7 – 7.2) and degree of freezing (-53 °C) (Iqbal *et al*.,2004, Aziz *et al.*,2011). Cyst fluid biochemical research shows that it consists of glucose, protein , fatty acids, uric acid, triglyceride, phospholipids, Ca, Na, K, Mg, , Cyst fluid that passes through membranes contains cholesterol (Vatankhah *et al*.,2003).

According to (Gamboa *et al*.,2019), some enzymes include lipase, protease, amylase, oxidase, and phosphotase. Albumin, NaCl, and NaSo4

are also reported, specially IgG globulins that can be diffused by cyst membranes (Kocherscheidt *et al.*,2008).

The presence of inorganic elements, which are significant nutritional co-factors in metabolic processes and have the potential to obstruct host immune responses, (Ferreira *et al*.,2000), nevertheless, Zn is found in large amounts that play amajor role in immunological processes, metabolism and Co-factors to many enzymes (Kıvanç *et al*.,2018). Fe and Cu have importance in respiratory process, Ni, it can also be contained in cyst fluid and attatched to RNA (Erkan *et al*.,2004, Zhange *et al*.,2008). The biochemical components of hydatid fluid represent variations in strains of various intermediate hosts, glucose, creatinine and Ca is higher in camel cyst fluid (Izadi *et al*., 2006), while uric acid is higher in man cyst fluid (Juyi *et al.*, 2013), the urea in renal cyst fluid is higher while the bile compounds in liver cyst fluid are higher (Barnes *et al.*,2007).

2.2. Reproduction cost:

Physiological costs of reproduction can be divided into two different types:

2.2.1. Direct Costs:

The first is direct costs that stem from satisfying the demands of the reproductive event itself. These demands at their simplest level are the energy and nutrients that the parental animal needs to acquire to successfully reproduce.

2.2.1.1 Increased demand for energy and nutrients:

2.2.1.1 .Energy:

Probably the most detailed data available for the energy demands of reproduction are derived from domesticated rodents. This is primarily because these animals are easily kept, and even quite invasive measurements can be made on them without the risk that they will desert their offspring. (Johnson *et al.* 2001a). Food intake increased during pregnancy to approximately 8 g, compared with 5.5 g in the non-breeding females prior to reproduction. Although the pattern of foetal growth is essentially exponential between conception and birth, the pattern of food intake does not mirror this, but rather rises to a peak approximately 2–3 days earlier and then declines slightly before the day of parturition. The reason for this pattern is uncertain, but one hypothesis is that the expanding foetal mass competes for space with the alimentary tract in the abdomen, limiting the food intake.

This may suggest that resources to support the gestation become limited in late pregnancy. Under such limitation, competing demands may have detrimental effects on the success of the pregnancy. One such competing demand may be the level of basal energy requirements (basal metabolic rate, BMR). that mice with higher BMR have a greater likelihood of mass anomalies occurring during pregnancy. These mass anomalies probably reflect foetal resorption events. Because BMR is correlated with body mass, this relationship might only be an artefact of both resorption rate and BMR being affected by mass, but the effect of BMR is also evident if the effect of mass is removed statistically (Johnston *et al*. 2007).

The most dramatic increase in food intake occurred during lactation. During the initial 10 days, this increase was linear, but then it reached a plateau at approximately 23 g of food per day. Food intake at the plateau (between days 10 and 18) was related to litter size. Small litters reached plateau intakes of less than 23 g , but as the litter size increased food intake also increased to a plateau at approximately 23 g of food per day. The mice seemed to reach a limit in Costs of reproduction in small mammals their food intake at this level. Although litter sizes increased, food intake did not increase in parallel. that this limit in food intake is not mediated via the aspects of the cage the animals live in (Speakman & Krol 2005), and therefore appears to be a physiological feature intrinsic to the animals themselves. This apparent physiological limit in the capacity of the mouse to ingest food at peak lactation may underpin an important life-history trait (maximum litter size) and an important life-history trade-off. Since the maximum asymptotic food intake at peak lactation is fixed, the energy that can be devoted to milk is also fixed. As the litter size increases, this milk must be divided between more and more offspring and, consequently, the pups wean at progressively smaller body masses (Johnson *et al*. 2001a).

Understanding the physiological basis of this maximal intake limit is therefore of critical importance. Two hypotheses concerning the nature of this limit were proposed in the early 1990s (Peterson *et al.* 1990; Weiner 1992; Hammond & Diamond 1997). The first hypothesis was that the limit was imposed by the capacity of the alimentary tract to absorb food (energy) and process it into a form for mobilization. This was called the 'central limit hypothesis'.

The second hypothesis was that the limit is imposed at the peripheral site where the centrally supplied energy is used: the mammary glands.

This was called the peripheral limit hypothesis.(Hammond & Diamond ,1992) manipulated litters of Swiss Webster mice and found that females did not elevate their food intake when given up to 23 pups.

Similarly, mice given up to 19 pups also could not breach the 23 g limit that they reached during unmanipulated lactations with litters of greater than 10 offspring (Johnson *et al*. 2001a). Faced with this problem of a litter size that is 'too large', females will often cull their offspring rather than eat more food (Johnson *et al.* 2001a; Gandelman & Simon 1978).

In a separate experiment, Hammond & Diamond (1994) prevented pups from weaning at their normal weaning age and hence their increasing growth demands needed to be supplied by the mother until the pups were 24 days old. In these conditions, the mothers also could not upregulate their food intake. The absence of an increase in the intake when pup numbers are increased or lactation extended confirms there is a physiologically imposed limit, but does not separate between the peripheral and central limits hypotheses. Perrigo (1987) compared the reproductive strategies of house mice Mus domesticus and deer mice Peromyscus maniculatus by forcing females to run a preset number of revolutions (between 75 and 275) on a wheel to obtain each pellet of food.

Despite the combined demands of lactation and locomotor activity, neither house nor deer mice exceeded the upper limit of food intake compared with unmanipulated mothers, given free access to food.

As a result of the decreased amount of energy available for reproduction, the wheel-running house mice routinely killed some of the offspring throughout the first 12 days of lactation, whereas deer mice

extended lactation well beyond normal weaning age. (Johnson *et al*., 2001b) followed the intakes of mice that had been mated immediately post-partum and found that the mice concurrently lactating and pregnant did not respond to the increased energy burden by elevating their food intake. Instead, they delayed implantation at the start of the second pregnancy and the length of this delay was directly related to the numbers of pups.

The animals therefore 'avoided' overlapping their energy demands, perhaps because they could not elevate their total intake at peak lactation to accommodate both. Similar observations were made in Rockland-Swiss mice (Biggerstaff & Mann 1992), and in rats (Rattus norvegicus: Koiter *et al*. 1999)

When food intake in late lactation was actually reduced in those rats that carried a simultaneous pregnancy, relative to rats just lactating. Together, these activity and pregnancy studies indicate that the limits are centrally, rather than peripherally, mediated; although, in case of concurrent pregnancy, the evidence is less strong as the animals avoided the problem. However, when Hammond et al. (1994) exposed lactating Swiss Webster mice to 8 C (approx. 22C below the lower critical temperature), they found that food intake increased dramatically beyond the supposed centrally imposed limit. Similar observations have since been made in deer mice (Hammond & Kristan 2000), MF1 mice (Johnson & Speakman 2001) and cotton rats (Sigmodon hispidus; Rogowitz 1998). The capacity of the mice to elevate their food intake in the cold was completely at odds with the central limitation hypothesis. To test whether the limit was imposed peripherally, (Hammond *et al*.,1996)

2.2.1.2 Organ remodelling to meet these demands

The alimentary tract has a more limited capacity to process energy than the mouth has capability to supply it. This is why the gut includes storage organs like the crop and stomach at the entry to the tract to take on board the intake at this elevated rate. If an animal requires to increase its food intake from approximately 5 g per day, prior to breeding, to 23 g per day at peak lactation, it could probably achieve this intake immediately, at the level of the mouth.

There are no obvious changes in the morphology of the mouth as the animals progress through reproduction. However, while a nonbreeding mouse might be capable of eating 23 g each day, its alimentary tract would probably be unable to process the intake. Hence, animals need to modify their internal architecture to cope with the altered demands. That lactating animals modify their tracts and associated organs in this manner has been known for at least 45 years. During lactation, there is an increase in the sizes of both the liver (Kennedy et al. 1958) and the pancreas (Jolicoeur et al. 1980).

The most dramatic changes however are in the alimentary tract itself, which involve major morphological increases in the absorptive surface of the intestinal mucosa and also growth in the length of the tract (Cripps & Williams 1975; Burdett & Reek 1979; Prieto et al. 1994; Speakman & McQueenie 1996; Hammond 1997).

These changes are paralleled by alterations in the transport capacity at the cellular level (Larradale et al. 1966; Dugas et al. 1970). The growth of the mucosal layer of the alimentary tract in lactation includes both hypertrophic (cell expansion) and hyperplastic (cell proliferation) responses (Fell et al. 1963; Cairnie & Bentley 1967; Prieto et al. 1994).

These changes effectively allow the lactating female to continue to extract the same amount (%) of energy from the ingested food independent of the rate of intake (e.g. Campbell & Fell 1964; Hammond et al. 1994).

Studies of wild rodents reveal similar patterns of change, although generally including more modest increases (e.g. Myrcha 1964; Chlethrionomys glareolus; Myrcha 1965 Apodemus flavicollis; Gebczynska & Gebczynski 1971 Clethrionomys oeconomus) reflective of the lower level of increase in food intake between non-breeding and lactation states This is consistent with the fact that the extent of modification of the intestine is related to inter-individual variations in litter size in mice. Several hypotheses have been advanced about how the growth in the gut is stimulated. The first is that the hormones that underpin the increase in food intake during lactation (Speakman & Krol 2005), including elevated prolactin and reduced leptin, directly stimulate the changes in the alimentary organs. Alternatively, the food intake itself may result in the production of local growth factors stimulating tissue proliferation (Datta *et al.* 1995).

Finally, hormones linked to milk production, e.g. oxytocin, may stimulate the growth. Unfortunately, separating these effects is difficult. Injection of hormones, like prolactin, affect food intake (Noel & Woodside 1993),

So eliminating a secondary local effect stimulated by the elevated food intake would need animals to be pair-fed with non-injected controls, and these critical experiments have not yet been performed. Hammond (1997),

However, noted that hypertrophy of the gut is a generalized response to elevated nutritional demands (such as cold exposure) and the hormonal profile in these circumstances is completely different from that in

lactation, favouring the hypothesis that changes are primarily stimulated by a local response, stimulated directly by the food intake. Many other morphological changes occur during pregnancy and lactation, not least of which is the growth of mammary tissue during late pregnancy to facilitate milk production during the subsequent lactation. As might be expected, considerable attention has been paid to this growth process and its hormonal basis, particularly the roles of sex steroids progesterone and 17b-oestradiol (Lamote *et al*. 2004).

Not all changes in the lactating animal, however, include expansion of tissue sizes. In particular, there is a large reduction in the size of the adipose tissue stores (Speakman & McQueenie 1996; Vernon & Pond 1997).

These reductions in the size of adipose tissue stores have generally been interpreted as withdrawal of stored energy to support energy delivery during the lactation event. Certainly, in some species (like the cotton rat: Randolph *et al*. 1977 and the Siberian hamster: Weiner 1987), there is accumulation of fat during pregnancy that is withdrawn later in lactation and this strategy appears to reduce the peak food intake demands in lactation .

However, in other animals, the contribution of fat to the overall energy budget is relatively trivial. In mice, for example, the fat stores decline by approximately 2 g during lactation (Johnson *et al*. 2001c) equivalent to approximately 80 kJ of energy, compared with a metabolizable energy intake over the last 10 days of lactation of approximately 2600 kJ. The increasing recognition that adipose tissue is not only an energy store but also an endocrine regulator suggests an alternative explanation of the reduction in fat mass may be that this reduces production of leptin (and possibly other adipokines) which then act to stimulate food intake. That low levels of leptin during lactation are

involved in stimulating food intake has been demonstrated by repleting leptin levels using miniosmotic pumps. This provision of exogenous leptin blunts the level of increase in food intake during lactation (Stocker *et al.* 2004).

2.2.3.**Indirect Cost**

2.2.3.1**Optional Compensatory Costs**

2.2.3.1.1**Thermoregulatory Demands**

During lactation, many species of small mammals experience large morphological and biochemical changes in their interscapular brown adipose tissue (BAT). These modifications include reductions in the amount of BAT in mice, rats, ground squirrels and hamsters (Agius & Williamson 1980; Wade et al. 1986 Johnson et al. 2001b).

In addition to reductions in overall tissue mass, there are also reductions in BAT mitochondrial mass (Trayhurn et al. 1982; Trayhurn & Jennings 1987a,b). In late lactation, mitochondria specific content of uncoupling protein 1 (UCP-1) is reduced to only 8% of the level found in non-breeding mice (Trayhurn & Jennings 1987a,b, 1988) and to 26% in ground squirrels (Nizielski et al. 1993).

GDP binding, which is a measure of mitochondrial thermogenic capacity, is also reduced in mice and rats (Trayhurn et al. 1982) and ground squirrels (Nizielski et al. 1993), but not in hamsters (Wade et al. 1986). Brown adipose tissue is the key thermogenic organ in small rodents (Cannon & Nedergaard 2004) and the changes observed in lactating rodents mediate a reduction in the noradrenaline-induced non-shivering thermogenesis (Trayhurn et al. 1982; Trayhurn 1983), which is rapidly reversed upon weaning (Trayhurn & Jennings 1987a,b, 1988).

In rats, the extent of decrease in thermogenic capacity is related to litter size (Isler et al. 1984), but this does not appear to be the case in mice (Trayhurn & Wusterman 1987a,b).

These morphological, physiological and biochemical changes in BATappear to be controlled by reduced sympathetic activity in lactation (Trayhurn & Wusterman 1987a,b), which may be responsive to elevated corticosteroid levels (Vernon & Flint 1983). Treatment of lactating rats with exogenous leptin completely reversed the downregulation of UCP-1 gene expression in BAT (Xiao et al. 2004).

Changes in other aspects of BAT physiology are also apparent during lactation, including reduction in the activity of iodothyronine 5'-deiodinase, which catalyses conversion of thyroxine (T4) to triiodothyronine (Giralt et al. 1986). All these changes are consistent with small lactating animals attempting to reduce obligatory heat production from BAT. Trayhurn (1989) interpreted this reduction as an energy-saving mechanism that increased the efficiency of milk production.

However, in the light of our studies of limits to food intake in lactating mice described above, an alternative interpretation is that this downregulation does not save energy which can be used for milk production, but rather reduces the heat burden on the animal, allowing it to elevate milk production. In recent years, a number of additional uncoupling proteins have been described (UCP-2 to UCP-5).

These uncoupling proteins have different tissue distributions, with UCP-2 being very widespread, UCP-3 being restricted to BAT and skeletal muscle, and both UCP-4 and UCP-5 being found only in the brain. The role of these UCPs in resting and thermogenic heat production has been an issue of debate (e.g. Erlanson-Albertsson 2002, 2003).

Studies of the UCP- 1 knockout mouse indicate that the other UCPs cannot reverse the lack of thermogenic capacity brought about by the absence of UCP-1 (Enerba¨ck *et al*. 1997; Golozoubova *et al.* 2001; Nedergaard *et al*. 2001). Although UCP-3 cannot be facultatively upregulated to replace the function of UCP-1, when it is transgenically

overexpressed, the resultant mice have elevated resting metabolism (Clapham *et al*. 2000).

Surprisingly, given the suggested absence of any role for natural levels of UCP-3 in thermogenesis, it has recently been shown that UCP-3 is also downregulated enormously in BAT during lactation, and this is reflected in reduced protein levels as well (Pedraza *et al*. 2000, 2001; Xiao *et al.* 2004), but UCP-2 is unchanged (Pedraza *et al.* 2001). Levels of UCP-3 gene expression in muscle are also decreased in lactation (Xiao *et al*. 2004). These effects appear to reflect circulating levels of free fatty acids (Pedraza *et al*. 2000).

Possibly, UCP-3 is thermogenically insignificant but it is incidentally downregulated because the mechanisms for regulating UCP-1 and UCP-3 are similar. There may be consequences of this downregulation, which the animal cannot avoid (Cadenas et al. 2002), and will be discussed below under 'consequential costs'. Many small mammals are able to make profound savings of energy by relaxing thermoregulation at normothermic levels and entering periods of torpor, during which body temperature is commonly reduced to levels just above ambient (Heldmaier et al. 2004).

While bringing significant benefits in terms of energy saving, torpor is fundamentally incompatible with some of the processes of reproduction as was first demonstrated in a series of elegant experiments in the early 1970s, where bats were forced into torpor for varying periods with the consequence that gestation period was extended by the exact same period that the mice had been made to spend in torpor (Racey 1973).

Foetal growth therefore halts when bats enter torpor. The significance of this effect was demonstrated in wild populations of bats during the early 1980s, when it was shown that adverse weather conditions during early pregnancy slowed foetal growth in wild bat

populations resulting in significant year-to-year differences in the duration of pregnancy (Racey & Swift 1981).

These findings seem to be at odds with the fact that hibernating bears gestate and lactate. However, studies of body temperature in bears during lactation reveal that while metabolism is downregulated to a similar extent as in small mammals, body temperatures only cool to approximately 30–328C.

If the interruption of foetal growth is temperature mediated, this difference in body temperatures during torpor may explain how bears are able to combine their reproductive functions with hibernation.

Despite these apparently negative impacts of torpor in pregnancy, it is used frequently in some species of marsupial (e.g. dunnarts (Sminthopsis macroura):(Geiser *et al*. 2005) and mulgaras (Dasycercus cristicauda): Geiser & Masters 1994), although in these animals the body temperature is still defended at approximately 148C.

The impact of torpor on lactation is less clear. Many species appear to use periodic entry into torpor as an energy-saving mechanism during lactation (Geiser 1994; Turbill *et al*. 2003) and energy budgeting indicates that this effect may be sufficiently great that the species concerned only need to make minor increases in food intake during lactation to achieve an overall energy balance. For example, the mean dry food consumption of non-reproductive brown long-eared bats averaged 1.8 g/day (48 kJ dK1) while lactating bats ate 2.0 g each day (53 kJ), yet the bats in lactation were able to export 22 kJ dK1 of this intake as milk (Mclean & Speakman 1999)

while deriving only on average 1.2 kJ from stored fat. Physiological compensation of energy budgets in this manner, so that food intake requirements are unaltered, may clearly have profound effects on the ecological costs of reproduction. Brown long-eared bats in the wild do

not increase their flight times between pregnancy and lactation (Entwistle *et al*. 1996), a pattern repeated in other species such as the common pipistrelle (Pipistrellus pipistrellus (Zpygmaeus);(Swift 1980) and long-tailed bats (Chalinolobus tuberculatus; O'Donnell, 2002).

If predation risk during reproduction is a simple function of time spent flying, then for these animals there may be no ecological cost at all. This pattern however may be peculiar to bats under particular energy stress (e.g. Turbill *et al*. 2003) or at the margins of their distributions (Speakman *et al*. 1991), since studies of other bat species more centrally in their distributions yield different patterns. Big brown bats (Eptesicus fuscus) and little brown bats (Myotis lucifugus), for example, have increased food intake during lactation (Kurta *et al*. 1989, 1990) and a decreased tendency to enter torpor during lactation (Audet & Fenton 1988; Hamilton & Barclay 1994; Grinevitch *et al*. 1995; Lausen & Barclay 2003). Flight time in northern bats in Sweden is almost doubled in lactation compared with non-breeding individuals (Rydell 1993).

2.2.3.1.1.2.**Physical activity**:

The changes in physical activity that occurred in female rats during reproduction. rats have similar patterns of food intake to mice during pregnancy and lactation, and that wheel-running activity is dramatically suppressed during lactation to approximately onethird to one-half of that in pregnancy.

The suppression of activity however was not proportional to litter size—since rats with small litters (n 6 offspring) ran about the same amount as those with large litters (n 6 offspring) Slonaker (1924).

Mice (MF1) also show similar reductions in spontaneous physical activity during lactation (Speakman et al. 2001). Rabbits also increase the

time spent resting as lactation progresses (Fernandez-Carmona et al. 2005).

Equivalent data on physical activity for nondomestic animals are scarce. This is probably because most activity in wild animals concerns foraging activity, which is generally increased during lactation owing to the elevated food intake requirements (see above).

In domestic animals, physical activity and food intake are not inescapably linked, so modulations of activity without affecting food intake are feasible. However, animals engage in other activities that are not associated with foraging, e.g. grooming.

previous study monitored the behaviour of bats during lactation and found that brown long-eared bats (Plecotus auritus) significantly reduce the amount of time spent grooming when compared with non-breeding females occupying the same roost.

On average non-lactating bats were observed grooming in 26.9% of the behaviour records, while lactating bats were only observed grooming in 11.1% of records. Similar high levels of time spent grooming in non-lactating bats have been observed in some other species (Shen & Lee 2000; Fleming *et al*. 1998).

However, such high levels are not universal (Winchell & Kunz 1996) and reductions in grooming during lactation are also not observed in these species (Winchell & Kunz 1996).

Female long-eared bats groom their offspring during lactation but this time allocation was relatively small and even when this was taken into account, the lactating females we studied still spent less than half of the time spent grooming by nonbreeding individuals (McLean & Speakman 1997).

Grooming behaviour by bats is energetically expensive (Giorgi *et al*. 2001) and plays a role in the removal of ectoparasites. By reducing the

time spent grooming, lactating bats may release significant amounts of energy for lactation, but may pay a price in terms of elevated ectoparasite burden. In the wild, in the mouse-eared bat (Myotis myotis), ectoparasite burden increases during pregnancy and lactation (Christe *et al*. 2000) when it is also related to body mass—suggesting a role for energy balance in driving ectoparasite burden.

However, potential interactions here are complex. (Neuhaus ,2003) experimentally removed ectoparasites (mainly fleas) from female Columbian ground squirrels using a commercially available insecticide. Removing parasites led to an increase in female body condition during lactation and at weaning and an increase in weaned litter size. Hence, removing ectoparasites may be costly, but not removing them may impose other costs (see also Khokhlova *et al*. 2002).

2.2.3.1.1.3.**Oxidative stress**

Animals during lactation have high rates of metabolism, which may result in elevated generation of free radicals. These free radicals may cause oxidative damage to macromolecules if the mice do not simultaneously upregulate their oxidative defence and repair mechanisms. (Wiersma *et al*.,2004) showed that birds feeding nestlings, which is the most energetically expensive phase in avian reproduction, do not upregulate the levels of superoxide dismutase, catalase and glutathione peroxidase, the main defences against radical oxygen species.

However, that study did not include measures of oxidative damage—so the lack of elevation of defence mechanisms may have been because there was no increase in free radical production to defend against. Indeed, the links between radical oxygen species production and metabolic rate are not straightforward (Speakman *et al*. 2002; Speakman 2003), and in

some cases increased metabolism may actually reduce oxidative stress (Speakman *et al*. 2004).

However, the reduction in levels of UCP-1 and UCP-3 in lactation would suggest that mitochondria in lactating animals are more closely coupled—supporting the suggestion that this may be a time of elevated oxidative stress (Demin *et al*. 1998a,b; Brand 2000; Speakman 2003).

2.4.Oxidative stress cost of reproduction (Life history theory of oxidative stress)

2.4.1.Oxidative stress theory

The theory that the metabolism of molecular oxygen (O2) in cells results in the formation of reactive oxygen species is the cornerstone of the oxidative stress theory of disease. This hypothesis forms the basis of the oxidative stress theory of disease. The oxidative stress theory of disease is built on this concept as its underlying premise (ROS). The hydrogen peroxide radical (H2O2), the hydroxyl radical (OH•), and the superoxide radical (O2•-) are the component portions that can be extracted from these reactive oxygen species.

The fundamental concept underlying the OS hypothesis of disease is that any one of these reactive oxygen species (ROS) could potentially cause harm to biological macromolecules as a result of their interactions with those molecules. In response to this requirement, organisms have created antioxidant defense systems in order to get rid of ROS.

These mechanisms can neutralize ROS. This category encompasses low-molecular-weight antioxidants as well as enzymes with names like "superoxide dismutase" (SOD) and "peroxidases" (vitamin E, vitamin C, and GSH). In spite of the fact that the theory of oxidative stress has advanced over the course of the last few years, the concept of oxidative

stress is still usually characterized in terms of a balance, with reactive oxygen species (ROS) on one side of the scale and antioxidants on the other (Jones and Sies, 2015).

The formation of OS is a sign that the body is manufacturing more reactive oxygen species (ROS) than its antioxidant mechanisms are able to eliminate. This is indicated by the presence of OS (either because of increased ROS generation or a decrease in antioxidants).

In 1956, Harman came up with an idea that would later be known as the "free radical hypothesis of aging." According to this theory, the degenerative process of aging shares a mechanism with cancer and radiation toxicity that involves free radicals. Harman made this hypothesis (Harman, 1956).

An organism's life history is a reflection of how the animal incorporated these elements into its life, and it provides valuable information about the "strategy" the species used throughout the course of its lifetime.

Our understanding and interpretation of how life-history strategies arise is, in large part, based on the assumption that trade-offs must always be made between the many aspects of a life history. Our knowledge of how life-history tactics originate and change over time depends on this concept (Fisher 1930).

If these actions are implemented, increases in current reproductive output, such as those brought about by fecundity enhancements, may be at the expense of future survival or future reproductive production (Williams 1966; Stearns 1992; Charnov 1993; Charlesworth 1994).

They work under the premise that animals can't integrate the various elements of fitness in a way that simultaneously optimizes all of them. They thus think that animals are forced to choose between different aspects of fitness. (Johnston *et al*. 2006).

Because reproductive actions not only result in fitness gains in the form of viable offspring, but also incur costs, the idea that there must be trade-offs in different areas of physical fitness originates from the premise that reproductive activities incur costs. This concept resulted in the realization that there must be compromises to be made in various facets of physical fitness. Because of these costs, the nature of the compromise that needs to be reached between the many facets of physical fitness will be determined by (Reznicks *et al*. 1990; Roffe 1992).

There are a variety of potential choices that can be made between various characteristics of an organism's present reproductive effort, such as size against the number of offspring that are generated. One example of this is the trade-off that can occur (Smith & Fretwell, 1974).

It is possible for there to be trade-offs between an organism's current reproduction and characteristics of its future fitness, such as the possibility of survival or fecundity. These trade-offs can take place at any time. Stearns (1992) developed a matrix that illustrated potential trade-offs between 10 distinct characteristics of physical health or life history "traits. "There needs to be a process that connects the various facets of fitness for each and every compromise. This mechanism serves as a metaphor for the "cost" associated with the reproductive event.

Previous studies have found that these costs can be further divided into two categories: physiological costs and ecologically-mediated costs (Zerae & Harshmans 2001).

When an organism goes out to find food in order to gather the energy and nutrients necessary for a reproductive event, such as protein and calcium, there is a greater possibility that it will be consumed by a predator. This is one example of a cost to the environment. Foraging exposes one to this hazard, which is an inherent part of the activity. There are a lot of more instances of things that have an impact on the environment.

The study of life histories has generally been focussed on the estimate of the relative relevance of various trade-offs; On the other hand, the mechanical foundations of these relationships have received an extremely insufficient amount of attention. However, it is becoming more and more obvious that if we want to understand the evolution of life-history patterns, one of the most important things we need to do is differentiate between the relative importance of ecological and physiological factors.

This is one of the most important things we need to do in order to understand the evolution of life-history patterns. Physiological and ecological factors both play a role in In order for us to have any chance of comprehending the development of life-history patterns, this is one of the most essential things that we need to undertake (Zerae & Harshmans 2001; McNamaras & Houstone 2008).

In spite of this, it is of the utmost importance to keep in mind that ecological and physiological components interact with one another in a significant manner and cannot be completely divorced from one another. This is something that should be kept in mind at all times. In spite of the fact that it is common knowledge that physiological costs are the basis for

all life-history trade-offs (for example, Stearnse 1992; Ricklefse and Wikelskie 2002).

2.5.Reproduction and oxidative stress

2.5.1.Reproductive status and oxidative stress

It is usually considered that the fundamental trade-offs in life history are the outcome of the allocation of limited resources among the many different physiological activities (Stearns 1992; Speakman 2008). The time in a mammal's life when it is reproducing and especially while it is breastfeeding, is the most taxing on its energy reserves (Millars 1977; Loudona and Racey 1987; Piersmae and Van Gilss 2011).

Recent studies have pointed to oxidative stress as a possible physiological cost of reproduction that may limit investment in various other areas of life history (Costantinis 2008; Doweling & Simmonss 2009; Monaghan, Metcalfes & Torress 2009; Selman *et al*. 2012).

According to the findings of studies carried out by Finkel and Holbrook in the year 2000, Monaghan, Metcalfe, and Torres in the year 2009, and Metcalfe and Alonso-lvarez in the year 2010, When the rate of creation of reactive oxygen species (ROS) surpasses the capability of antioxidant defense and repair processes, this can lead to oxidative stress., according to Balaban, Nemoto, and Finkel (2005), have the ability to cause damage to a wide variety of biomolecules, some of which include DNA, proteins, and lipids, if they are not neutralized by antioxidants that are either of an enzymatic or non-enzymatic nature (Balabana, Nemotoa & Finkel 2005; Doweling & Simmonss 2009).

Oxidative stress has been established as a critical element that contributes not only to the natural aging process but also to a wide range

of other illness problems. This is due to the fact that oxidative stress has been shown to contribute to the formation of free radicals. (Finkel & Holbrook 2000; Selman *et al*. 2012).

During the process of reproduction, there is an increase in metabolic rate, which, according to Speakman (2008), may result in increased ROS generation and, as a consequence, oxidative stress (Alonso-lvarez *et al.* 2004; Speakman 2008).

On the other hand, reactive oxygen species are not only produced in proportion to the amount of oxygen that is taken in (Speakman etal. 2004, and furthermore, animals may up-regulate a variety of antioxidant defenses in response to increased ROS formation, thereby repairing oxidative damage and minimizing the subsequent impact that it has. (Speakman *et al*. 2004.,Monaghans, Metcalfes & Torress 2009).

As a direct consequence of this, the connection between reproductive effort and oxidative stress could be cloudy and might not always be as cut and dry as one might expect. In recent years, researchers have attempted to study the connection between reproductive effort and oxidative stress both in the field and in the lab. Despite the fact that oxidative stress and the amount of effort put into reproduction have been linked in a number of studies carried out on wild species, such as mammals and birds (Bergeronn *et al*. 2011; Christee *et al.* 2011; Heisse & Schoeche 2012; Fletchers *et al.* 2013)

Other researchers have not found any connection between the two. Two good instances of this are the studies that were conducted by Nussey and colleagues in 2009 and Markó and colleagues in 2011.

However, in the study that was carried out by (Nussey et al. 2009), it is probable that this is due to the fact that plasma samples were collected after reproduction had been finished. This was done in order to exclude any potential confounding variables. Because the contents of plasma are continually changing, oxidative damage assays in plasma have the ability to capture the oxidative state exactly as it is at this very moment.

In contrast to these observations, there is evidence from research that was carried out on breastfeeding female mammals that were held in captivity that suggests oxidative stress may actually decrease after reproduction. For instance, lactation in confined house mice resulted to a reduction in MDA and protein thiols in the liver. MDA and protein thiols are both markers of oxidative damage (Garratt *et al*. 2011).

When compared to non-breeding female bank voles (Myodes glaeolus), the oxidative damage to the lipids in the kidney and muscle was lower, while the oxidative damage to the protein in the kidney, muscle, and heart remained unaffected. This was the case when looking at the oxidative damage to the lipids in the kidney and muscle (Oldakowski et al. 2012).

Research on lactating mammals has been conducted both in the wild and in the laboratory, and the researchers have come up with two possible explanations for the discrepancies they discovered. To begin, reproducing mammals in the laboratory have convenient access to food and, in general, are not subject to the concomitant pressure of immunological challenges or demands placed on their thermoregulatory systems (though see Hammond & Diamond 1992; Johnson & Speakman 2001). (although, see the work that Kristan and Hammond produced in the year 2000).

It is probable that the difficulties of life in the wild will increase the requirements of reproduction and make oxidative stress more obvious. On the other hand, measurements that have been made during field research mainly used serum as the major sample source for measurement, whereas laboratory investigations have mostly concentrated on assessing the degrees of damage that have occurred in the tissues of the body.

The principal study foci of the vast majority of these earlier investigations on mammalian species were oxidative stress and spontaneous variation in reproductive effort. On the other hand, it is well known that changes in reproductive effort that occur naturally or experimentally are not anticipated to correlate with measures of physical condition in the same way. This is due to the fact that different components are predicted to be responsible for natural and experimental variations in reproductive effort. This is due to the fact that natural fluctuations in reproductive effort and those caused by experiments have different root causes (Remick 1992).

For instance, in order for females to lower the likelihood of oxidative damage occurring to their bodies, they can make a less financial investment in the process of reproduction. For example, in order to determine whether oxidative damage and protection are altered in relation to reproductive effort. it may be required to experimentally influence reproductive investment. This is so that the researchers can see whether or not there is a correlation between the two (for example, by manipulating litter size).

This will make it possible to determine whether or not oxidative damage and protection are altered in response to the amount of effort that is put into reproduction (Metcalfe & Monaghan 2013).

In point of fact, a number of studies on birds have found that altering brood size or increasing reproductive effort in birds in an experimental setting results in a decrease in antioxidant activity or tolerance to oxidative stress in the birds. This was the conclusion reached by the researchers after observing the birds over the course of their experiments (Alonso-Alvarez *et al.* 2004, Alonso-Alvarez *et al*. 2006, Bertrand *et al*. 2006, Christe *et al*. 2011). Alterations in antioxidant status are not always an indicator of oxidative stress. This is not always the case (Wiersmaa *et al*. 2004; Monaghan, Metcalfee & Torress 2009).

It is interesting to note that only one previous study assessed the question of whether or not reproductive attempts of small mammals that had been experimentally altered effect oxidative damage and protection (Metcalfe & Monaghan 2013).

The oxidation of proteins in the heart and gastrocnemius muscle was unaffected by manipulating the number of offspring that were produced by wild-derived house mice.

However, manipulating the number of offspring that were produced by mice that had been subjected to intentionally increased levels of reproductive effort did reduce the amount of damage that was done to the liver of those mice (Garratt *et al*. 2013).

prior investigation has shown that reproduction, and more especially lactation, in the Brandt's vole (Lasiopodomys brandtii), is a physiologically costly activity. Specifically, this has been shown to be the case with the vole's ability to produce milk. These investigations also indicated that nursing voles have a greater food consumption and metabolic rate than non-reproductive voles, particularly in those with

naturally large or increased litter sizes (Zhang, Li & Wang 2008; Wu *et al*. 2009; Xu, Yang & Wang 2012).

A rise in the production rate of reactive oxygen species (also known as ROS) is what causes oxidative stress to occur in a system. This happens when the antioxidant defense and repair systems reach their maximum capacity and are unable to cope with the damage (Finkels & Holbrooke 2000; Monaghann, Metcalfes & Torress 2009; Metcalfes & Alonso-Alvarez 2010).

2.5.2. Cost of Parasite infection during reproduction

Wild animals are frequently infected by one or more parasite species throughout their lives (Behnke *et al*., 2001; Cox, 2001). While some parasites exist in the host for a very short time, others can remain for weeks, months or years (Behnke *et al.*, 1992), producing a chronic infection.

Hosts do not always develop life-time immunity to parasites that produce a chronic sublethal infection and so repeatedly incur the cost of parasitism (e.g. Fuller, 1996).

Therefore, chronic infections may have subtle yet important effects on host ecology and life history. For example, costs associated with host immune response and tissue repair resulting from parasitism can alter energy allocation and, if energy is limited, this will leave less energy available for other uses, such as reproduction (Sheldon and Verhulst, 1996; Demas et al., 1997; Nordling et al., 1998).

Alternatively, animals may use resources for reproduction and compromise their ability to respond to parasites (e.g. Allander, 1997;

Deerenberg *et al.*, 1997; Ilmonen *et al.*, 1999; Derting and Compton, 2003).

Lactation is an extremely energy-demanding time for mice (Hammond *et al.*, 1994), with an increase in food intake of more than 100% over that consumed by virgin mice (Hammond et al., 1996).

Because much of this extra energy consumed by lactating mice is converted to milk and exported to pups, it does not represent the actual levels of increased energy expenditure, in terms of metabolism, for lactating females (Speakman, 2000).

However, mice must still ingest and process all the calories needed to synthesize milk and must produce and deliver milk to pups, which may account for part of the true increase in resting metabolism observed in laboratory mice during lactation (Hammond and Diamond, 1992; Hammond *et al*., 1994; Speakman and McQueenie, 1996).

Wild house mice in captivity given a 14·h:10·h L:D light cycle can produce approximately one litter each month (Bronson, 1979; D. Kristan, personal observation) and, in nature, mice are not always limited to a specific reproductive season (Bronson, 1979) as is typical for many other small mammals.

Therefore, female house mice are likely to spend much of their lives either pregnant, lactating or both. Importantly, immune function of female mice during pregnancy and lactation is typically diminished compared with non-reproducing mice (Selby and Wakelin, 1975; Ferguson *et al.*, 1982; Sulila and Mattsson, 1990; Medina *et al*., 1993), causing them to be especially susceptible to a broad range of parasites. Numerous nematode species infect wild Mus (Doran, 1955).

Mice respond to the larval stages of the nematode Heligmosomoides polygyrus with both a cell-mediated and antibody-mediated immune response (Liu, 1965; Panter, 1969; Monroy and Enriquez, 1992), and laboratory mice are more susceptible to infection with H. polygyrus when lactating (Shubber et al., 1981).

After adult worms emerge in the mouse small intestine (approximately 9–11·days after ingesting an infective-stage larva; Monroy and Enriquez, 1992),

The mature parasites produce an immunosuppressive factor to suppress worm expulsion (Dehlawi and Wakelin, 1988; Monroy *et al.*, 1989; Monroy and Enriquez, 1992; Scott and Koski, 2000) and presumably to enhance the ability of newly emerging adults to survive.

At this point, the mouse's initial immune response to the larval stage begins to diminish, and the adult H. polygyrus worms can last up to 8·months in the mouse (Ehrenford, 1954).

For many parasites that produce chronic, sometimes lifelong, infections there is often an initial phase with an active immune response by the host followed by a quiescent phase that can be interrupted by periodic reactivation of the parasite (e.g. as seen with Toxoplasma gondii; Luft *et al.*, 1983; BoschDriessen *et al*., 2002).

Some factors affecting host response to a parasite infection include host age, presence of additional parasites and environmental conditions. For example, responses to infection varied when laboratory mice were subjected to caloric restriction (Carlomagno *et al*., 1987; Effros *et al*., 1991; Otesile *et al*., 1991; Peck *et al.*, 1992; Shi *et al*., 1998; Kristan and Hammond, 2001), protein deficiency (Slater and Keymer, 1986; Tetzlaff

et al., 1988; Peck *et al.*, 1992; Boulay *et al.*, 1998; Cintra *et al.*, 1998; Anstead *et al*., 2001) or cold exposure (Banerjee et al., 1999; Monroy *et al*., 1999; Aviles and Monroy, 2001).

A change in resource allocation with parasitism, as evidenced by a change in body composition, suggests that infection with intestinal nematode *Heligmosomoides polygyrus* affects energy use in the laboratory mouse despite no changes in energy input (food intake did not vary with parasite infection). Part of the change in body composition with parasitism resulted from an increase in mucosal mass of the small intestine, which is important because the mucosal layer is responsible for nutrient digestion and absorption. Unlike previous studies (Kristan and Hammond, 2000, 2001),

Parasitized mice in this study were able to attain similar total glucose-transport capacity, despite a decreased rate of glucose transport per gram of tissue, by increasing the total mass of mucosal tissue. One explanation for these contradictory results may be that mice in this study were parasitized for approximately 26 days longer than in previous studies (Kristan and Hammond, 2000, 2001), and the potential negative effects of larval-stage parasites on mucosal tissue function may have been overcome.

For example, if immune responses to larval parasites affect the function of small intestine tissue [perhaps by the proliferation of cells in the small intestine (Symons, 1965) yielding an accumulation of immature enterocytes rather than functional mucosal tissue] then the more time that has passed since the larval stages, the less H. polygyrus should impact on total glucose acquisition.

As in previous studies (Kristan and Hammond, 2000, 2001), the effects of H. polygyrus on small intestine function reached beyond the site of worm occupation to other regions of the small intestine. This is likely to be an indirect effect of parasitism, possibly resulting from changes in the nutrient density of the ingesta, which results in 'nutrient spilling' into more distal parts of the small intestine that will then change the capacity of these regions for nutrient uptake.

In contrast to previous studies (Kristan and Hammond, 2000, 2001), after accounting for mass, resting metabolism did not change with parasite infection. This difference in response to parasitism again may reflect different experimental protocols. In previous work (Kristan and Hammond, 2000, 2001).

Mice were parasitized for 23 days prior to metabolism measures, whereas in the present study mice were infected on average for 49 days.

It is well documented that H. polygyrus elicits an immune response by the host during larval stages (e.g. Monroy and Enriquez, 1992).

In contrast to unparasitized females, infected females that produced large first litters had relatively smaller second litters, which implies that the cost of producing a large litter may be more for parasitized females than for unparasitized females. Importantly, parasitized females that produced small first litters tended to have larger second litters. A shift in optimal allocation of energy between current and future offspring associated with parasitism can occur, and further exploration of relative changes in reproductive effort over the course of numerous reproductive events will provide valuable insight into how parasites may influence this life-history parameter. (Richner and Tripet, 1999)

After accounting for effects of litter size and parity, parasitized mothers had pups that were 4% smaller at weaning (20 days after birth) compared with pups from unparasitized mothers (Kristan, 2002)

2.6.Parasite infection and oxidative stress

Oxidative stress occurs when the rate of production of reactive oxygen species (ROS) exceeds the capacity of the antioxidant defence and repair mechanisms (Finkel & Holbrook 2000; Monaghan, Metcalfe & Torres 2009; Metcalfe & Alonso-Álvarez 2010).

ROS are physiological by-products of normal metabolic processes; their unstable and very reactive nature can cause damaging effects on many biomolecules (e.g. DNA, proteins and lipids) unless quenched by enzymatic and non-enzymatic antioxidants (Balaban, Nemoto & Finkel 2005; Dowling & Simmons 2009).

Oxidative stress has been implicated as a proximate mechanism responsible for the natural ageing process as well as a variety of disease states (Finkel & Holbrook 2000; Selman *et al*. 2012).

During reproduction, metabolic rate is increased (Speakman 2008), which potentially could cause increased ROS production and result in oxidative stress (Alonso-Álvarez *et al*. 2004; Speakman 2008).

However, ROS are not simply generated in direct proportion to oxygen consumption (Speakman *et al*. 2004), and further, animals can potentially up-regulate a variety of antioxidant defences in response to increased ROS production, repairing oxidative damage and limiting its subsequent impact (Monaghan, Metcalfe & Torres 2009).

Consequently, the association between reproductive effort and oxidative stress is potentially complex and not necessarily straightforward.

Recently, both field and laboratory studies have tried to explore the association between reproductive effort and oxidative stress. Some results in free-ranging animals including mammals and birds have indicated that oxidative stress was positively linked to reproductive effort (Bergeron *et al*. 2011; Christe *et al*. 2011; Heiss & Schoech 2012; Fletcher *et al*. 2013), but others have failed to find any association (Nussey *et al*. 2009; Markó *et al*. 2011).

Although in the case of the study by Nussey *et al*. (Nussey *et al*. 2009), this might be because plasma samples were collected after reproduction was completed. Oxidative damage assays in plasma may reflect the recent oxidative state because turnover of plasma constituents is high. However, in contrast to these studies, some studies of lactating female mammals in captivity have indicated that oxidative stress may actually be decreased during reproduction. For example, two measures of oxidative damage (MDA and protein thiols) in the liver were reduced during lactation in captive house mice (Garratt *et al*. 2011).

Similarly, lipid oxidative damage was lower (kidney and muscle) and protein oxidative damage unaltered (kidney, muscle and heart) in breeding female bank voles (*Myodes glaeolus*) relative to non-breeding females (Oldakowski *et al*. 2012).

The contrast between field and laboratory studies of lactating mammals is striking and may have two contributing causes. First, in the laboratory, reproducing mammals have ready access to food and do not generally have other simultaneous pressures like thermoregulatory

demands (but see (Hammond & Diamond 1992; Johnson & Speakman 2001) or immune challenges to cope with (but see Kristan & Hammond 2000).

Rats infected with *Toxoplasma gondii* showed significant decreased in antioxidant (SOD and CTA) in the serum and testis of infected rats, SOD as an important defense against oxygen radicals involved in dismutation of the superoxide radicals to H2O2 which can cause subsequent oxidative damage to various types of macromolecules including lipids, proteins and DNA,CAT activity adversely affected by oxidative stress caused by various diseases such as toxoplasmosis. The MDA level was considerably increased in serum and testis after infection, respectively. Detection of TAC level in serum and tissue may represent the environmental, physiological factors of the oxidative stress in various tissue (55,59,61,65). There are many studies conducted on different mammals that showed the state of oxidation during parasitic infection with different types of parasites infecting different organs in the body of their host.

in reproductive mice that antioxidants were regulated physiologically in response to ROS, by which oxidant-antioxidant balance occurs during peak lactation. Oxidative stress results from an imbalance between oxidative protection and production of ROS and one may speculate that increased oxidative stress was not observed in reproductive animals, because ROS production was reduced or antioxidant protection was upregulated during reproduction. Previous studies have shown increases in activity of catalase (Brzek ,*et al*,2014), SOD (Garratt M, *et al*,2013), or increased levels of glutathione (Garratt, *et al*,2011) in livers of reproductive mice. We found an increase in SOD activity in the brain in reproductive compared to control mice, but a reduction in GPx activity in

liver. These results once more point out the complexities of the relationship between antioxidant protection and oxidative damage and the variability of these responses between tissues of interest (Yang ,*et al*,2013, Xu, *et al*,2014).

Rats infected with *Toxoplasma gondii* in different tissues (liver, heart and brain) that showed As biochemical markers of oxidative stress, endogenous concentrations of GSH, GPX and SOD activity, MDA level, protein carbonyl content and total antioxidant capacity were determined from the mentioned tissues of control and infected rats. post infection the level of hepatic glutathione were significantly decreased in infected rats when compared to control. There was a significant increase in hepatic (GPx)activity and (MDA) level post infection in comparison to uninfected rats. Significant elevation of (SOD) activity and (MDA) level post infection and (PC) and total antioxidant capacity post infection in infected livers were obtained. Significant changes of glutathione level, total antioxidant capacity and protein carbonyls contents were observed in cardiac homogenate. Measured parameters were constant throughout all stages of experiment in brain of infected rats.

Indeed increased production of reactive oxygen species accompanies Toxoplasma infection in liver and heart tissues of experimentally infected rats. Based on this study, antioxidant defense system can probably play a role in parasitic stage interconversion and shifting the toxoplasmosis into the chronic phase. (Delavari, *et al* ,2017)

Mice infected with *Toxoplasma gondii* in liver showed were significantly increased in T. gondii-infected mice compared with the control group hepatic nitric oxide (NO), lipid peroxidation (LPO) levels and caused significant decrease in superoxide dismutase (SOD), catalase

(CAT) and glutathione activities in the liver of infected mice., The excessive accumulation of radicals was suggested in the tissue as the main cause of decreased activity of enzymes (Alajmi,*et al* ,2019).

The probable reason for this difference might be due to activity of enzyme level d of Toxoplasma infection. In fact, the activation of the host immune system against T. gondii and the respiratory burst caused by macrophages lead to the production of active oxygen species by extending the study ,CAT enzyme by the detoxification of reactive oxygen species produced by immune cells prevents the possible damage (Moreira-Souza,*et al* ,2017).

It seems that T. gondii reduced the ROS encoding proteins activity. Also, with the excessive production of these active oxygen species, important enzymes such as CAT and SOD lose their effective function and became inactive , Glutathione, an important non-protein thiol source, can act as substrate for glutathione peroxidase and served as functional protective molecule for oxidative damage in various tissue (Dincel, & Atmaca,. ,2016).

The decrease might be due to elevation of oxidative stress in infected cells, that is why the significant difference between enzymatic markers occurred. Additionally, decreased serum and testis GSH level in chronic toxoplasmosis could change the detoxified capacity of reproductive tissue and then result in oxidative damage of reproductive organs which can adversely affects the fertility capacity (Aldabagh, M. A, *et al*, 2018).

On the other hand, Toxoplasma infection in pregnant women could induce oxidative stress which resulted in marked increase in the blood level of MDA and decrease in the blood level of GSH (Ali, *et al* ,2006).

mice infected with Angiostrongylus cantonensis was examined for kinetic changes in oxidative stress parameters, including reactive oxygen species (ROS), superoxide dismutase (SOD), catalase, malondialdehyde (MDA), 8-isoprostane, and 8-hydroxy-2′-deoxyguanosine (8-OHdG). The ROS increased gradually in the early stage of infection. During days 12–30 post-infection, the infected mice revealed ROS levels significantly higher than that in uninfected controls .The kinetics of MDA, 8-isoprostane, and 8-OHdG concentration changes observed in the CSF of the infected mice corresponded with kinetic changes in ROS levels. Thus, the excess ROS caused lipid peroxidation and DNA damage to cells in the central nervous system (CNS) of mice infected with A. cantonensis despite the increased antioxidant SOD and catalase enzyme activities during parasitic infection. The oxidative stress in the CNS of C57BL/6 mice was apparently increased by diseases associated with A. cantonensis infection.(Chung, *et al* ,2010**)**

camels (Camelus dromedarius) infected with Trypanosoma evansi finding the activity of antioxidant enzymes (SOD and GSH) significant reduce while were no significant difference in CAT activity.(Saleh, *et al*,2009)

Trypanosoma evansi in dromedary bulls causes severe damage to testicular tissue and reduces reproductive hormone levels, resulting in severe morphological abnormalities in sperm due to oxidative stress. Infected Camels infected with Toxoplasma gondii finding that activity of antioxidant enzymes (SOD and GPX) was significant decreased in the blood samples of infected animals camper to uninfected animals.(Amin, *et al* ,2020)

camel infected with cystic echinococcosis of the liver. The index of serum lipid peroxidation, which was measured by the level of malondialdehyde (MDA), was significantly higher in the parasitized group than in the healthy control group. But the parasitized group had significantly lower levels of serum total antioxidant status (TAS) and erythrocyte glutathione peroxidase (GPx). Serum zinc levels were much lower in camels with liver cystic echinococcosis than in healthy camels. in camels, parasitic infection, it was a strong link between MDA, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), and bilirubin. On the other hand, MDA had a negative relationship with packed cell volume (PCV), serum albumin, and zinc. From this study, it was determined that oxidative stress is linked to cystic echinococcosis in camels. The oxidative stress that happens as a result seems to damage hepatocytes, change trace elements, and destroy erythrocytes.(Heidarpour, *et al* ,2012)

Mongolian gerbils experimentally infected with Babesia divergens showed significant decrease in total antioxidant capacity, as indicated by lowered glutathione and catalase levels, increased production of nitric oxide-derived products (nitrite/nitrate) and malondialdehyde, and increased lactic acid dehydrogenase activity and protein carbonyl content in the liver.(Dkhil, *et al* ,2013)

Rapid release of)ROS and NO(plays a critical role in the fight against parasitic infection, but it also causes oxidative injury, resulting in tissue damage and the pathophysiology of the disease , Damage to the intracellular lysosomal membrane caused by oxidative stress is followed by apoptosis or necrosis (Alajmi,*et al* ,2019). Increased lipid peroxidation is linked to decreased function of the defense system preventing tissue

damage from free radicals in parasitic infection seropositive individuals and animals (Atmaca, *et al* ,2015).

Chapter Three
Materials and method

3.1.Materials and Methods

Solutions and Reagents: Table(3-1) lists all of the solutions and reagents that were utilized.

Table (3-1): Solutions and reagents and their suppliers and sources.

No.	Solutions and Reagents	Sources &suppliers
1	Brain drived neurotrophic facor (BDNF) ELISA Kit	Bioassay Technology Laboratory / China/Jiaxing Shi5 / f 2 bldg, 501 Changsheng
2	Brain Natriuretic Peptide (BNP) ELISA Kit	Bioassay Technology Laboratory / China/Jiaxing Shi5 / f 2 bldg, 501 Changsheng
3	Brain Protein Carbonyl ELISA Kit	Bioassay Technology Laboratory / China/Jiaxing Shi5 / f 2 bldg, 501 Changsheng
4	Troponin I,slow skeletal muscle,TNNI1 ELSA Kit	Bioassay Technology Laboratory / China/Jiaxing Shi5 / f 2 bldg, 501 Changsheng
5	Liver Protein Carbonyl ELISA Kit	Bioassay Technology Laboratory / China/Jiaxing Shi5 / f 2 bldg, 501 Changsheng
6	Heart Protein Carbonyl ELISA Kit	Bioassay Technology Laboratory / China/Jiaxing Shi5 / f 2 bldg, 501 Changsheng
7	8-hydroxy-2-deoxyguanosine(8-HDG)Kit ELISA (Brain)	Bioassay Technology Laboratory / China/Jiaxing Shi5 / f 2 bldg, 501 Changsheng
8	8-hydroxy-2-deoxyguanosine(8-HDG)Kit ELISA (Herat)	Bioassay Technology Laboratory / China/Jiaxing Shi5 / f 2 bldg, 501 Changsheng
9	8-hydroxy-2-deoxyguanosine(8-HDG)Kit ELISA (Liver)	Bioassay Technology Laboratory / China/Jiaxing Shi5 / f 2 bldg, 501 Changsheng

10	8-hydroxy-2-deoxyguanosine(8-HDG)Kit ELISA (Heart)	Bioassay Technology Laboratory / China/Jiaxing Shi5 / f 2 bldg, 501 Changsheng
11	Ethyl-alcoho	Milpharm/London/UK
12	Formalin 40%	TEDIA Company INC, USA
13	Iodine	Sigma Chemical Co. (USA)

Apparatuses and Instruments: Equipment and instruments used in this study are summarized in table (3-2).

Table (3-2): Apparatuses used in the experiment and their suppliers.

No.	Apparatuses	Supplier
1	Elisa	Biokit ELx800/USA
2	Centrifuge-CL008	Cypress Diagnostics/Langdorp-Belgium.
3	Cool Centrifuge	Cecil Instruments/Cambridge- England
4	Refrigerator with freezer-20C	LG/Korea
5	Electrical sensitive Balance	CAMRY/China
6	Liquid Nitrogen Container	Chengdu Rhett - Hereby Technology Co., Ltd Sichuan, China
7	Micropipettesand Tips (small, large)	Gilson/ France
8	plane tube	AFCO-DISPO/ Jordan
9	Gel tube	AFCO-DISPO/ Jordan
10		
11	Disposable gloves.	TG Medical /Malaysia.
12	Disposable syringes 5 cc	Medeco® Inject /Abu Dhabi

		Medical Devices Co.L.L.C.
13	Cotton	NFLB/CHINA
14	Plastic Gage	Local market/Iraq
15	Universal container	Jordan
16	Dissection set	China
17	glass slides	China
18	cover slips	China
19	Insulin syringe	Q-ject China
20	Gavage	German
21	Refrigerator	China
22	plastic containers	China
23	Eppendorf tube	China
24	Glass Homogenizers	UK

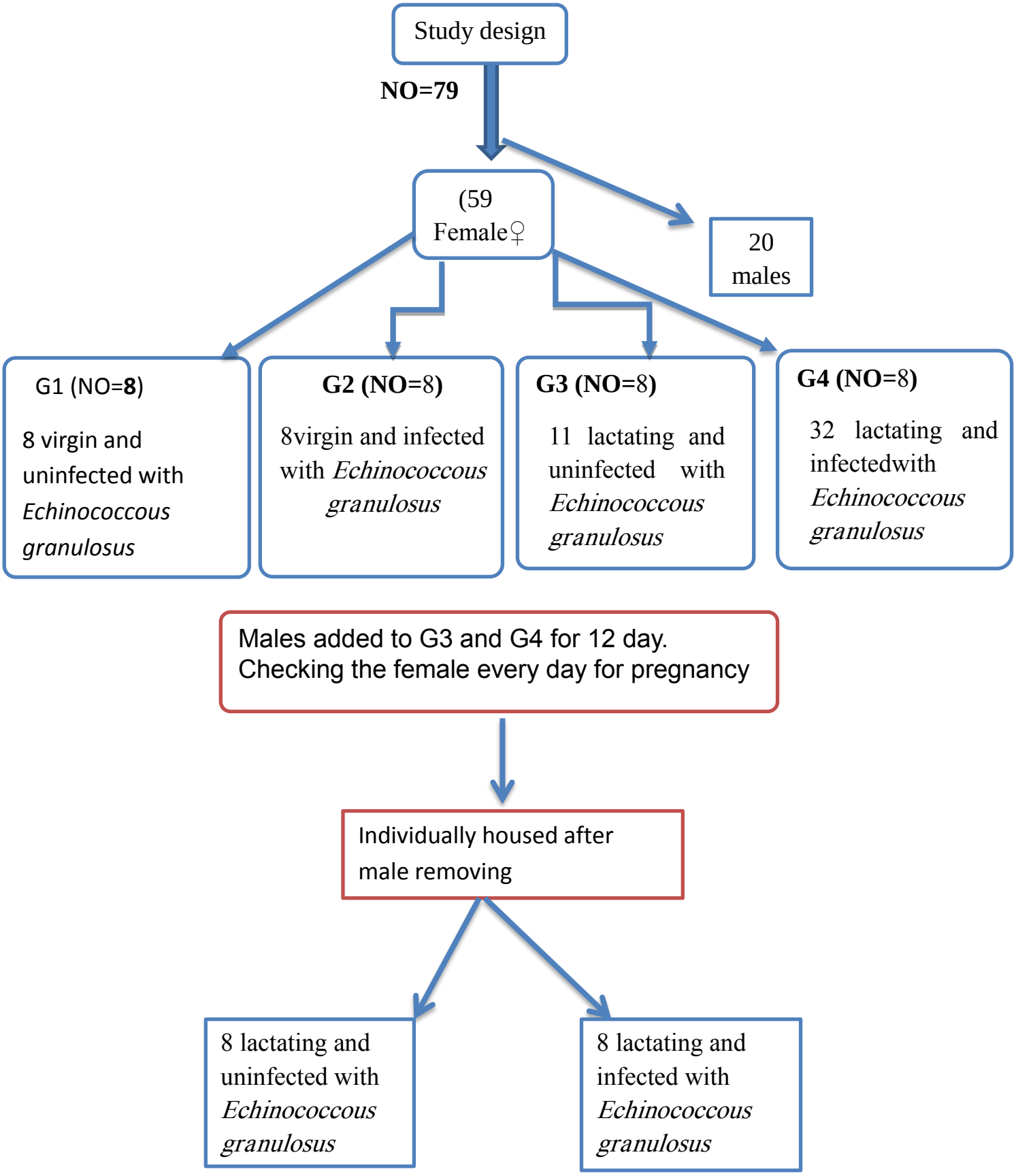

Figure (3-1):study design

3.2Animals Housing:

Fifty nine female mice and twenty males , aged 10-12 weeks, were obtained from animal house at Kufa University and Iraqi central for cancer & Medical Genetics researchers in Al-Mustansiriyah university. The Study Carried out in the Animals House, College of Science, Department of Biology/Babylon University . Mice will be maintained at 12h:12h L:D, 21°C and fed standard rodent diet (LabDiet® 5001, Purina Mills, Inc., St Louis, MO, USA). Housed in 27cm×21cm×14cm polypropylene cages and will be given food and water ad libitum. Forty mice were injected with *Echinococcus granulosus*, The baseline period was about forty days. fifty-nine female mice were assigned into four groups: 8 virgin and uninfected parasitized females, 8 virgin and infected parasitized, 11 lactating and uninfected parasitized and 32 lactating and infected parasitized were mated with males for 12 days, Checking the female every day for pregnancy , then males were moved out. After this period, the total number of lactating females and the parasitic infection decreased. Other deaths occurred during the lactation period, as the total number became 16 females pregnant (lactation, Lac. n=8) (8 lactating –parasitized and 8 lactating –non parasitized) and the rest of females were not paired with males (non-lactation, Non-Lac , n=16)(8 virgin and unparasitized females, 8 virgin and parasitized).

3.3Solutions:-

3.3.1.Normal Saline Solution (N.S):

The germ layer of the hydatid cyst was washed with (N.S) and it were prepared using the following:

1. Sodium Chloride (Na Cl) 9 gm

2. Distilled water1000 ml

Sodium chloride was dissolved in 100 ml of distilled water, then the volume was increased to one liter, bringing the concentration of the solution to 0.009 g/ml. This solution was then sterilized in an autoclave binder USA at 121 ° C and 15 pounds/ang 2 pressure for 15 minutes, and it was then kept in the refrigerator at 4 ° C. (Collee et al., 1996).

3.3.2.Phosphate Buffer Saline Solution (PBS)

This solution was used to wash the germ layer of the hydatid cyst and it was prepared according to the method of Hudson and Hay (1984) using the following materials:

1. aqueous disodium phosphate	$Na_2HPO_4.7H_2O$	2.89 gm
2. potassium dihydrogen phosphate	KH_2PO_4	0.2 gm
3. sodium chloride	NaCl	8 gm
4 .potassium chloride	KCl	0.2 gm
5. distilled water		1000 ml

After gradually dissolving the materials in a certain volume of distilled water, the volume was completed to one liter, then the solution was

sterilized in an autoclave at a temperature of 121°C and a pressure of 15 pounds/ang2 for 15 minutes.

3.3.3Kreb's - Ringer's Solution (K.R.S.):-

Karp Ringer's solution is considered one of the best media for preserving viable Protoscolices outside the vivo after isolating them from hydatid cysts (Al-Rubaie, 1999). This solution was prepared according to the method of Routunno *et al.* (1974) from the following articles:

Sodium chloriide	NACL	0.481 gr
potassium chloriide	KCL	0.157 gr
Calcium chloriide	CaCl	0.0137 gr
Acidic Suodium Carbonate	NaHCo3	0.281 gr
Sodium dihydroggen Phosphate	NaH2Po4	0.097 gr
Sodium monohydroggen Phosphate	Na2Hpo4	0.490 gr
Magnesium sulfate	MgSO4	0.072 gr

All of the above were added to one liter of distilled water and dissolved gradually; no further ingredients were added until the previous ones were completely dissolved. For the next half an hour, the solution was autoclaved at 121 ° C. and 15 pounds per square inch of pressure. Glucose was added to it 0.09 g, streptomycin 200 mg and penicillin. 4000 IU/liter, and these antibiotics were added to prevent contamination by first dissolving the antibiotics, then glucose, and then sterilizing it with Millipore membrane filters with 0.45 micrometer holes. Preservation and measurement of the vitality of the Protoscolices .

3.3.4.Lugol's iodine solution

Dissolve KI in 20 to 30 ml of distilled water. Add iodine and heat while continuously stirring until iodine is dissolved. Dilute to 100 ml with

distilled water. Keep in a dark amber glass container with a cork stopper. (Revision A, 1998).

3.4.1.Collection Hydatid Cyst

Hydatid cysts were obtained from the butchery of Najaf / Najaf governorate, and the bags were isolated from the livers of slaughtered sheep and placed in a container that contained ice to cool it. As soon as these infected livers were delivered to the advanced parasite laboratory at the College of Science / University of Babylon, they were washed with water to remove the blood that was on them, then the bags were opened directly to ensure their fertility through the presence of the protoscolices inside of them, and the vitality of the protoscolices was examined using Lugol's iodine solution dye , and the primary principles whose vitality reached the desired level (Al-Mubarak, (2006).

3.4.2.Collection of Protoscolices

The protoscolies were obtained using the Smyth (1985) method, which involved sterilizing the surface of the hydatid cysts with ethyl alcohol (70%), and then removing the liquid from the bags using a 10 ml medical plastic syringe and a needle that was (21) degrees in angle depending on the size of the cyst. 500 ml in volume. After the cysts were cut open with forceps and scissors, the germ layer was removed and placed in a sterile container with physiological saline solution. The cysts were then drained of hydatid fluid, and the germ layer was repeatedly washed with sterile phosphate saline (PBS) to extract the most protoscolices. protoscolies were collected and discarded using a centrifuge, model 206/1-BL. Antibiotics (Procaine Penicillin at (2000) IU/L and Streptomycin) were applied before beginning the second wash in Sao Paulo, Brazil, three times at (3000) r/min

and for (15) minutes each time. In the second PBS wash, one g/L was added to the washing solution. After centrifugation, the filtrate was emptied, a little amount of sterile phosphate-saline buffer was added to the precipitate, and the Protoscolices was calculated.

3.4.3.Estimation of Protoscolices Viability

The viability of protoscolices was estimated by mixing (10) µl using a micropipette of the suspension with a similar volume of aqueous iodine dye at a concentration (0.1%) on a glass slide and examined directly under microscope a Smitech XSZ -N 107 Malaysia, and the percentage of live protoscolies that appear in bright green color, the dead protoscolices were stained Brown, and three replicates were used for the purpose of calculating the vitality ratio (Smyth & Barrett, 1980).

3.4.4.Viable Protoscolices Count

Protoscolices were calculated using a light microscope under the power of magnification (X40). An average of three readings was taken, and according to the required number, approximately 2000 live Protoscolices were calculated for each dose, as the Protoscolices were calculated in one milliliter as follows: The average number of Protoscolices in the fixed volume used is 10µ = 30.3 heads, so the number of capillaries in one milliliter = 30.3 x 100 = 3030 capillaries, and the required number of 2000 capers in a volume of 0.66 ml was taken by taking the live capillaries and neglecting the dead ones (Smyth, 1985).

3.4.5.Injection of Protoscolices

Live Protoscolices were injected into the intraperitoneal cavity with approximately 2,000 live Protoscolices per mouse, and to all mice, using a sterile (1) mL syringe with a 21-degree needle after sterilization of the injection area with 70% ethanolic alcohol for each mouse (Wangoo et al. ., 1989).

3.4.6. Infection with *Echinococcus granulosus* (hydatid cyst)

Before starting the experiment, we conducted a pilot study. This study involved the use of three doses given to mice subperitoneal to ensure that infection occurred and the animal did not die. The first group was injected with 2000 live protoscolices of hydatid cyst, the second group with 4000 live protoscolices, and the third group with 6000 live protoscolices. As all the mice that were given 4000 and 6000 live protoscolices died a week after giving the two doses, and the first group that was given 2000 live protoscolices survived for three months. Therefore, the dose of 2000 live protoscolices of *Echinococcus granulosus* was adopted, which is the dose used in this study. the infection with *Echinococcus granulosus* **(**hydatid cyst), where we see the cyst prominent in the liver of infected mice.

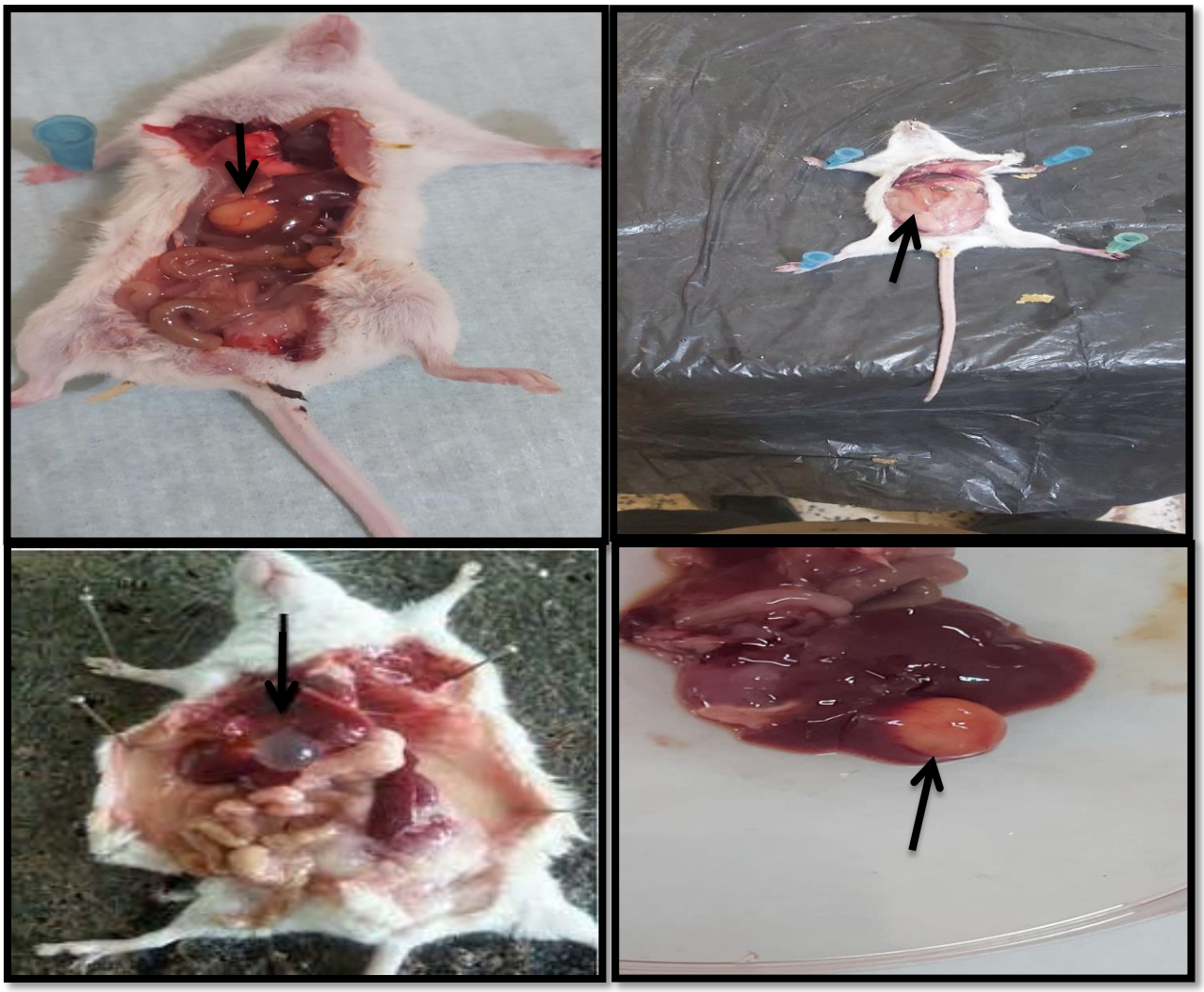

FIG (3-2) : *Echinococcus granulosus* appeared in mice injected with 2000 live protoscolices, where we see the cyst prominent in the liver of infected mice .

3.5.Mating and pregnancy

Forty three female mice in pairs housed (32 female lactating and infected with *Echinococcus granulosus* and 11 female lactating uninfected with *Echinococcus granulosus*) one female and one male in each cage. The mice that were mated were given standard food and water ad libitum. The body mass of female pairs was evaluated every day for 12 days. If the female became pregnant, the male was removed and the pregnant female was placed in a new cage. The pregnant female's daily body mass and food consumption were measured until the day of delivery. Pups were weighed and counted before being returned to their mothers.

3.6.Body mass and food intake

Using a calibrated balance, female body mass and food consumption were monitored daily throughout baseline (forty days), the last five days of pregnancy, and the first 18 days of lactation. When females were confined with males, no assessments of food intake were made. During lactation, litter mass and litter size were recorded daily. The average pup mass was then determined by dividing the litter mass by the litter size.

3.7.1. Tissue collection:

On day of weaning (day 18 of lactation), lactating infected parasitized (LI), lactating uninfected parasitized (LU), non-reproducing parasitized (NRP) and non-reproducing uninfected parasitized (NRUP) mice, After the dissection of dead animal, the internal tissues (brain, liver and heart) were collected in Eppendorf tube and kept in liquid nitrogen until the time of homogenization method which was explained in appendix 1, and analysis of biomarkers (GPx ,SOD,CAT) , protein carbonyl (PC) , lipid damage (MDA), damage DNA (8-OHdG), BDNF, BNP and Troponin I (TNNI1).

3.7.2. Blood collection:-

Blood collection by cardiac puncture Anesthetize the animal thoroughly with the chosen anesthetic agent prior to sample collection. , After the blood is drawn, it is placed in a tube with a yellow cap that contains an anticoagulant. This gel is placed in the yellow tube in a simple, non-concentrated way, in order to prevent blood clotting and also to separate the serum, then , We separate the serum using a centrifuge, taking the clear liquid and discarding the precipitate.

3.8.1Brain derived neurotrophic facor (BDNF)

The level (BDNF) in brain was measured using Mouse Brain derived neurotrophic factor (BDNF) ELISA kit (Manufactured in Bioassay Technology Laboratory / China) steps for the procedure mentioned in kit which were illustrated in appendix II.

3.8.2.Superoxide dismutase (SOD)activity determination:-

The SOD activity in brain ,liver and heart tissues was estimated According to Marklund and Marklund (1974), which was explained in appendix III.

3.8.3.Glutathionne peroxidasse Determination (GPx) activity

The activity of glutathione peroxidase was analyzed using the method proposed by Hafemann et al (1974). It was explained in greater detail in appendix IV.

3.8.4.Malondialdehyde (MDA):

The concentration of malondialdehyde was determined using the thiobarbituric acid (TBA) assay method developed by Buege and Aust in 1978 and measured using a spectrophotometer. It was further upon in the mentioned appendix V.

3.8.5.Protein carbonyls(PC)

The level protein carbonyls (PC) in brain, liver and heart tissues were measured using Mouse protein carbonyl ELISA kit (Manufactured in Bioassay Technology Laboratory / China) steps for the procedure mentioned in kit which were illustrated in appendix VI.

3.8.6. 8-hydroxy-2- deoxyguanosine (8-OHdG)

DNA damage in brain, liver and heart tissues was detected by measuring the levels of 8-hydroxy-2- deoxyguanosine (8-OHdG). 8-OHdG is widely used as a biomarker of DNA oxidative damage because it is recognised to be one of the main oxidative base modifications, in particular, generated by oxidising effects of hydroxyl radical (Cooke et al., 2000; Kasai et al., 2008). were measured using Mouse 8-hydroxy-2-deoxyguanosine (8-OHdG) ELISA kit (Manufactured in Bioassay Technology Laboratory / China) steps for the procedure mentioned in kit which were illustrated in appendixVII .

3.8.7.Mouse Brain Natriuretic Peptide, BNP ELISA Kit:

The level Brain Natriuretic Peptide (BNP) in heart tissue was measured using Mouse Brain Natriuretic Peptide ELISA kit (Manufactured in Bioassay Technology Laboratory / China) steps for the procedure mentioned in kit which were illustrated in appendixVIII .

3.8.8.Troponin I,slow skeletal muscle,TNNI1 ELSA Kit:

The level Troponin I (TNNI1) in heart tissue was measured using Mouse Troponin I,slow skeletal muscle,TNNI1 ELSA Kit (Manufactured in Bioassay Technology Laboratory / China) steps for the procedure mentioned in kit which were illustrated in appendix IX .

3.8.9.Alkaline phosphatase (ALP) :-

Catalyze the hydrolysis of 4-nitrophenylphosphate (4-NPP) by using the alkaline buffer as a phosphate-group acceptor. This will result in the synthesis of 4-nitrophenol and inorganic phosphate. The rate of 4-nitrophenol synthesis is used to kinetically follow the reaction at 405 nm. This rate is proportional to the ALP activity of the material being tested. Combining water and 4-nitrobenzylphosphate 4-Nitrobenzene plus Pi The procedure that is considered to be the standard has been followed in the development of this test by DGKC.

3.8.10.Determination of alanine Aminotransferases (ALT) or (GPT) Activity:

According to the following reactions, GPT activity:

Alanine + α -ketoglutarate → Pyruvate+glutamate

The pyruvate formed is measured in its derivated form, 2,4-dinitrophenylhydrazone at 505nm. number of GPT units/ml in serum were calculated using the standard curve.

3.8.11.Determination of aspartate aminotransferase (AST) or (GOT) Activity: GOT activity according to the following reactions:

GOT activity according to the following reactions:

L–A spartate + α -ketoglutarate → Oxaloacetic + L–glutamate

The oxaloacetate formed is measured in its derivated form, 2,4-dinitrophenylhydrazone at 505nm. number of GOT units/ml in serum were calculated using the standard curve.

3.9. Statistical analysis

Prior to perform the statistical analysis, data were checked for normality using shipro test and the transformation was made for non-normal-distributed data. Statistical changes in daily measured variables such as maternal body mass, food intake, litter mass, pup mass, and liter size throughout the experiment were tested using repeated measures of General Linear Model (GLM) followed by tukey post-hoc test to establish the changes among days. Differences in the maternal body mass, asymptotic food intake, litter mass, pup mass and litter size between groups were assessed using GLM. The changes of antioxidants levels and oxidative damage levels measured in different tissues among all studies groups were statistically tested by using GLM followed by tukey post-hoc test.The effect of both infectious and reproductive status on the levels on organ functions measured in the serum were tested by using GLM in which the reproductive status (RS),infectious status (IS) ,and the interaction between RS and IS were included as fixed factors.If the interaction was insignificant, GLM test was repeated without interaction.. The data were presented as mean ± standard error and the p-value equal or less than 0.05 was considered statistically significant. All analysis was done using Minitab software version 17 (Ryan et al., 1985).

CHAPTER FOUR

RESULTS

4.1: Maternal body mass

At day 1 of baseline, the body mass was not significantly changed among all groups in which the body mass of assigned group for Lactating infected (LI) which mean was 21.558±0.892 (N=8) while average body mass in Non-Reproducing uninfected (NRU) was 18.635±0.700 (N=8), (N=8), (RM GLM groups; $F_{3,31}$=1.91, P=0.151). **(**Table 4-1)

Throughout the baseline ,the body mass was significantly increased by days of baseline and groups assigned of (LI) which mean was 25.918±0.826 (N=8) while average body mass in (NRU) was 23.127±0.700 (N=8), (RM GLM, groups, ; $F_{3,117}$=2.94, P=0.05; days, ; $F_{39,117}$=260.12, P=0.000). **(**Table 4-1) Fig (4-2).

Body mass in 40 day was significantly increased by groups assigned of (LI) which mean was 31.213 ±0.858 (N=8) while average body mass in (NRU) was 27.445±0.739 (N=8), (RM GLM groups; $F_{3,31}$=4.11, P=0.015). **(**Table 4-1)

During pregnancy , on the last 4 days, the body mass of pregnant mothers significantly increased by groups and days assigned of (LI) which mean was 38.144±0.858 (N=8) ,while average body mass in (NRU) was 28.352±0.731(N=8). (RM GLM groups ; $F_{3,9}$=26.04, P=0.000; days ; $F_{3,9}$=7.55, P=0.008;). Fig (4-3) **(**Table 4-1).

At day 1 of lactation, the body mass was significantly increased by groups in which the body mass of assigned group for (LI) which mean was 33.839 ±0.607 (N=8) while average body mass in (NRU) was 28.724 ±0.732 (N=8), (RM GLM groups; $F_{3,31}$=9.38 , P=0.000).

During lactation body mass significantly increased by groups and days assigned of (LI) , which was average body mass of (LI) 32.700±0. 615 (N=8), while average body mass in (NRU) was 29.706±0.737(N=8). (RM GLM groups; $F_{3,48}$=3.42, P=0.030; days; $F_{16,48}$=0.01, P=0.000;) Fig (4-4) **,(**Table 4-1).

Body mass in 18 day of lactation, was not significantly changed among all groups in which the body mass of assigned group for (LI) which mean was 31.485 ±0.646 (N=8) while average body mass in (NRU) was 30.587±0.738 (N=8), (RM GLM groups; $F_{3,31}$=0.63 , P=0.604).

Table 4-1: Body mass of animals in different reproductive stages in the baseline ,pregnancy and lactation of mothers and non- reproducing mice.

Traits	LI	LU	NRI	NRU	F-Value	P-Value
BM baseline	25.918 ± 0.826	25.370 A ± 0.849	23.897 ± 1.129	23.127 ± 0.700	2.94	0.050
BM DAY 1	21.558 ± 0.892	21.063 A ± 0.915	20.488 ± 1.136	18.635 ± 0.700	1.91	0.151
BM DAY 40	31.213 A ± 0.858	30.123 A B ± 0.867	27.588 B ± 1.174	27.445 B ± 0.739	4.11	**0.015**
Bm preg.	38.144 A± 0.901	36.713 b± 1.331	28.640 C± 1.171	28.352 C± 0.731	26.04	**0.000**
Bm lact.	32.700a ± 0.615	32.663a ± 0.925	29.916b ± 1.160	29.706b ± 0.737	3.42	**0.030**

Bm baseline = Body mass baseline , , **Bm preg**.= Body mass pregnant,; **Bm lact**.= Body mass lactation, **LI** =Lactating infected, **LU**= Lactating uninfected, **NRI=**Non-reproduce infected, **NRU=** Non-reproduce uninfected.

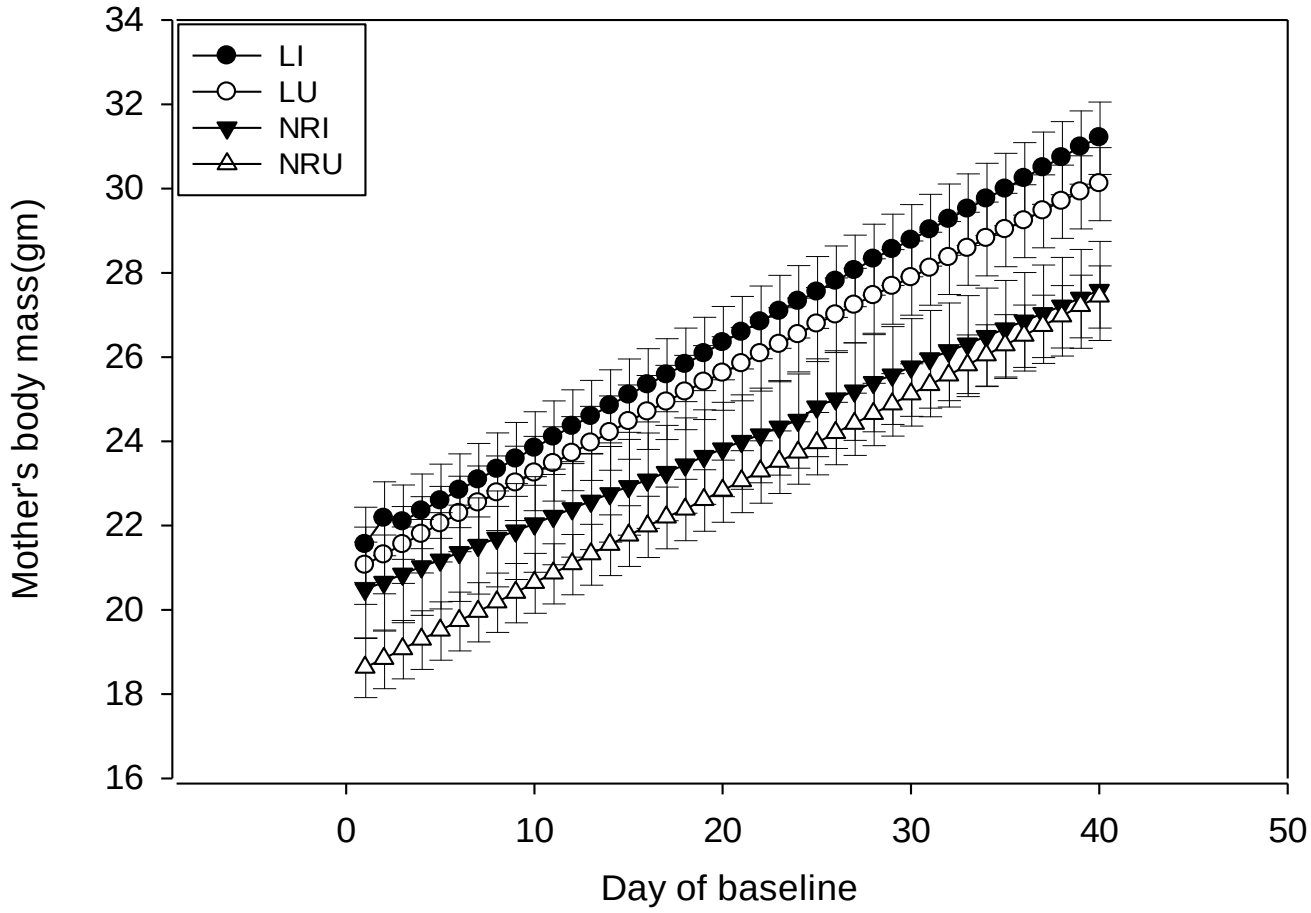

Fig (4-1): Mother's body mass of studied groups of mice throughout the baseline period (LI: lactating infected, LU: lactating uninfected, NRI: non-reproducing infected, NRU: non-reproducing uninfected)

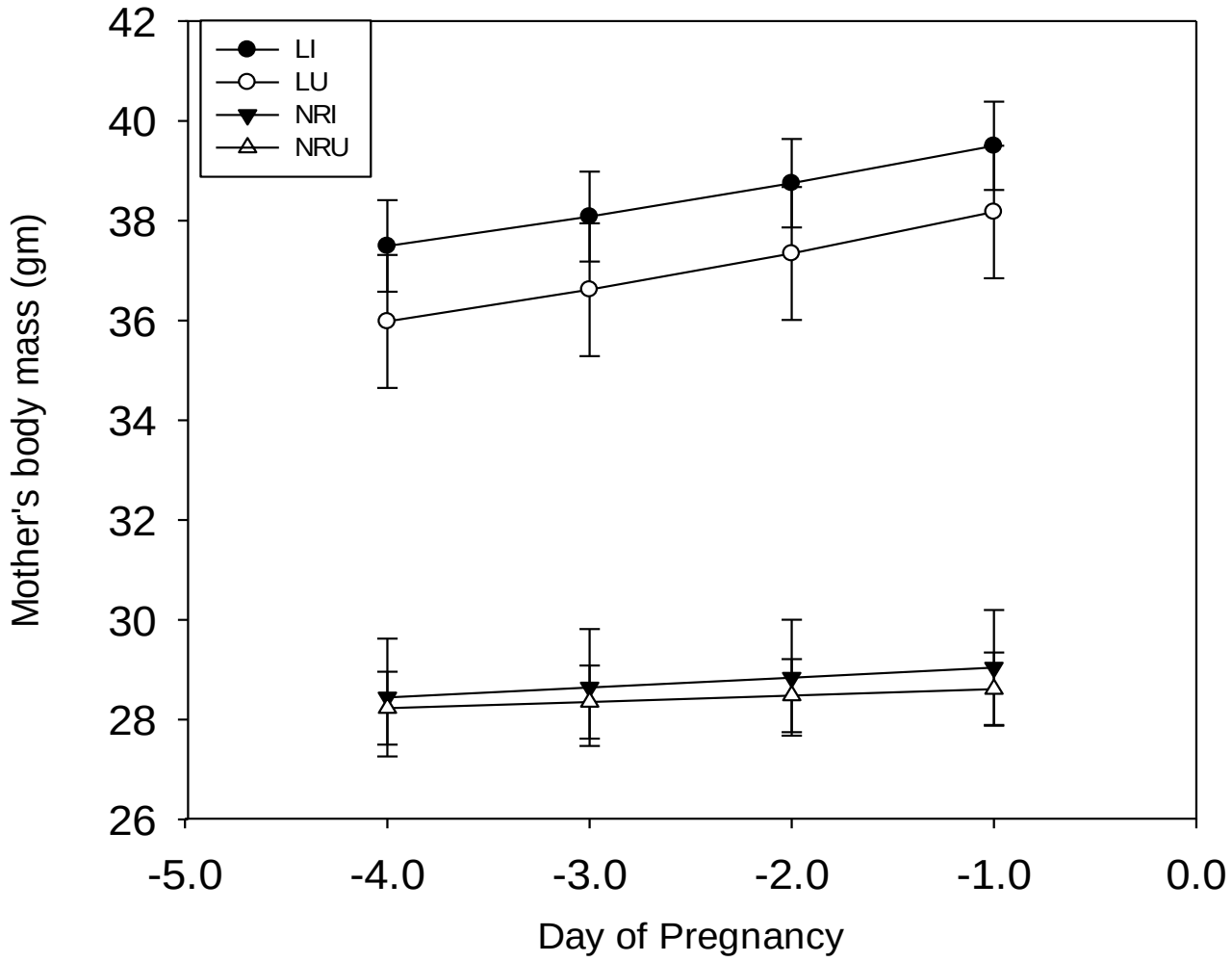

Fig (4-2): Mother's body mass of studied groups of mice throughout the last four days of pregnancy (LI: lactating infected, LU: lactating uninfected, NRI: non-reproducing infected, NRU: non-reproducing

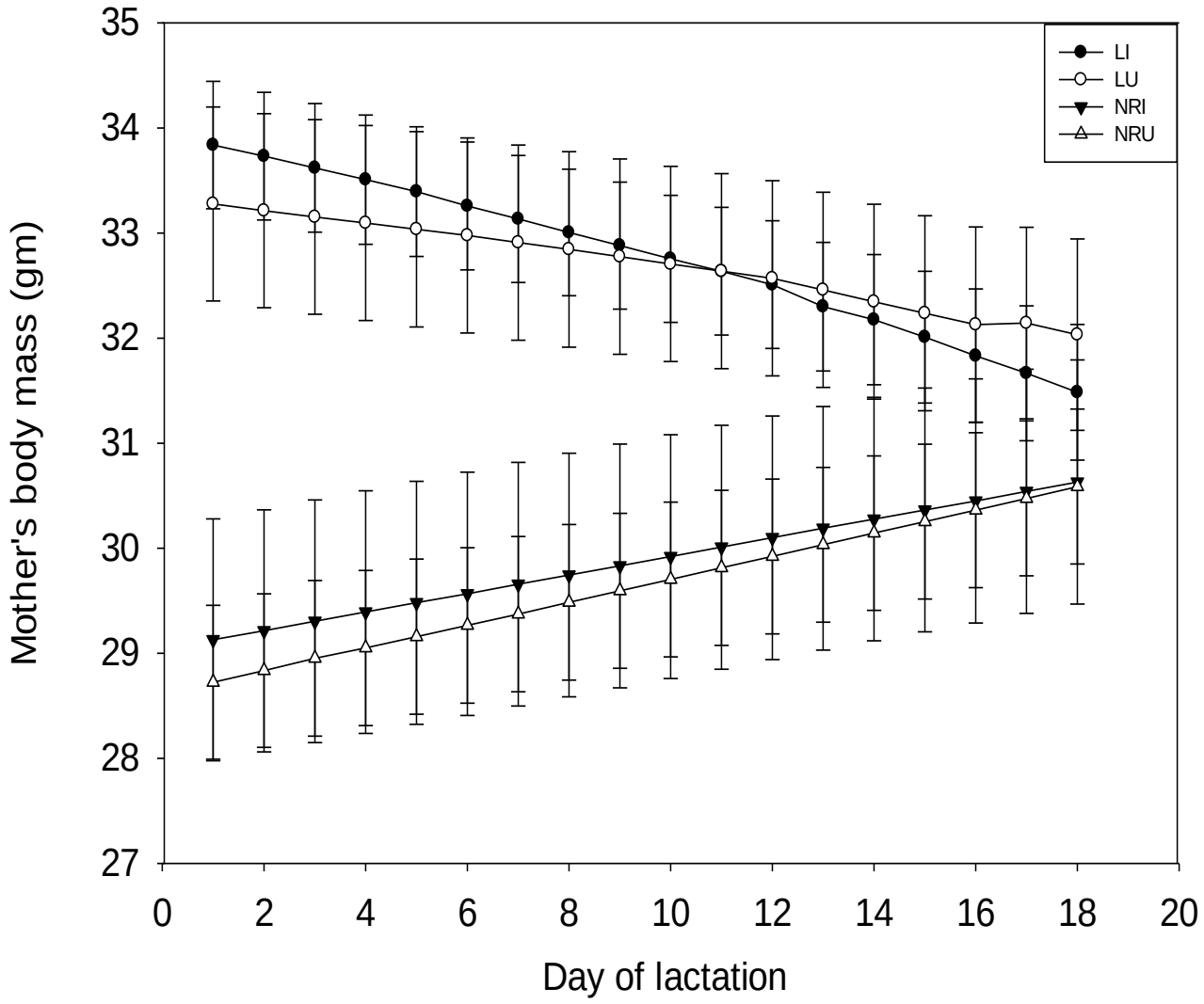

Fig (4-3): Mother's body mass of studied groups of mice throughout the lactation period (LI: lactating infected, LU: lactating uninfected, NRI: non-reproducing infected, NRU: non-reproducing uninfected

4.2.Maternal food intake:

At day 1 of baseline, the food intake was significantly different among all groups in which the food intake of assigned group for (LI) which mean was 4.361±0.204 grams (N=8) while average food intake in (NRU) was 3.988±0.018 grams (N=8), (RM GLM groups $F_{3,31}$=1.91, P=0.151). **(**Table 4-1)

Throughout the baseline, the food intake was significant different by days of baseline and groups assigned of (LI) which mean was 3.741±0.012 grams (N=8) while average food intake in (NRU) was 4.146 ±0.006 grams (N=8)., (RM GLM groups; $F_{3,117}$=150.08 , P=0.000; days; $F_{39,117}$=39. 86 , P=0.000;). **(**Table 4-1) fig (4-5).

Food intake in 40 day was significantly increased by groups assigned of (LI) which mean was 3.506 ±0.013 gram (N=8) while average food intake in (NRU) was 3.970 ±0.005 (N=8), (RM GLM groups; $F_{3,31}$=8.37, P=0.000). **(**Table 4-2)

During pregnancy , food intake of pregnant mothers significantly increased by day-4 Preg. of groups , It was of average food intake assigned of (LI) 6.715±0.310 (N=8) ,while average food intake in (NRU) was 4.512±0.010 (N=8). (RM GLM $F_{3,117}$=79.16 , P=0.000;) fig.5 (Table 4-2) .

During lactation food intake significantly increased by groups and days assigned of (LI) , which was average food intake of (LI) 9.915±0.068 (N=8), while average food intake in (NRU) was 4.930±0.015 (N=8). (RM GLM groups; $F_{3,48}$=1934.82 , P=0.000; days; $F_{16,48}$=1.93, P=0.041;) Fig (4-4) (Table 4-2).

At day 2 of lactation, the food intake was significantly increased by groups in which the food intake of assigned group for (LI) which mean was 10.112 ±0.166 (N=8) while average food intake in (NRU) was 4.9575 ±0.0624 (N=8), (RM GLM groups; $F_{3,48}$=987.80, P=0.000).

During lactation food intake significantly increased by groups and days assigned of (LI) , which was average food intake of (LI) 9.915±0.068 gram (N=8), while average food intake in (NRU) was 4.930±0.015 gram (N=8). (RM GLM groups; $F_{3,48}$=1934.82 , P=0.000; days; $F_{16,48}$=1.93, P=0.041;) Fig (4-4) (Table 4-3).

food intake in 18 day of lactation, was not significantly changed among all groups in which the food intake of assigned group for (LI) which mean was 9.8507±0.0397 (N=8) while average food intake in (NRU) was 4.9278 ±0.0102 (N=8), (RM GLM groups; $F_{3,31}$=1920.54, P=0.000).(Table 4-3**).**

Table 4-2: **food intake of mothers in different reproductive stages in the baseline stage.**

Traits	LI	LU	NRI	NRU	F-Value	P-Value
FI baseline	3.741 A± 0.012	4.103 B ± 0.006	3.708 A ± 0.011	4.146 B ± 0.006	150.08	**0.000**
Fi day 1	4.361 A ± 0.204	3.558 C ± 0.068	3.581 B C ± 0.015	3.988 A B ± 0.018	12.38	**0.000**
Fi day 40	3.506 B C ± 0.013	3.988 A ± 0.012	3.260 C ± 0.247	3.970 A B ± 0.005	8.37	**0.000**

FI baseline =Food intake baseline, **Fi day 1**= Food intake 1 day ,**Fi day 40**= Food intake 40 day.

Table 4-3: **food intake of mothers in different reproductive stages in pregnancy and lactation .**

Traits	LI	LU	NRI	NRU	F-Value	P-Value
Fi preg	6.715a ± 0.310	7.958b ± 0.006	4.617c ± 0.003	4.512c ± 0.010	135.83	**0.000**
FI lact	9.915a ± 0.068	10.845b ± 0.004	4.639c ± 0.148	4.930d ± 0.015	1934.82	**0.000**
asymptotic fi	9.850a ± 0.039	10.073b ± 0.373	4.5217c ± 0.0253	4.927d ± 0.010	1920.54	**0.000**

Fi preg= Food intake pregnant;; **FI lact**= Food intake lactation; **asymptotic fi**= asymptotic food intake.

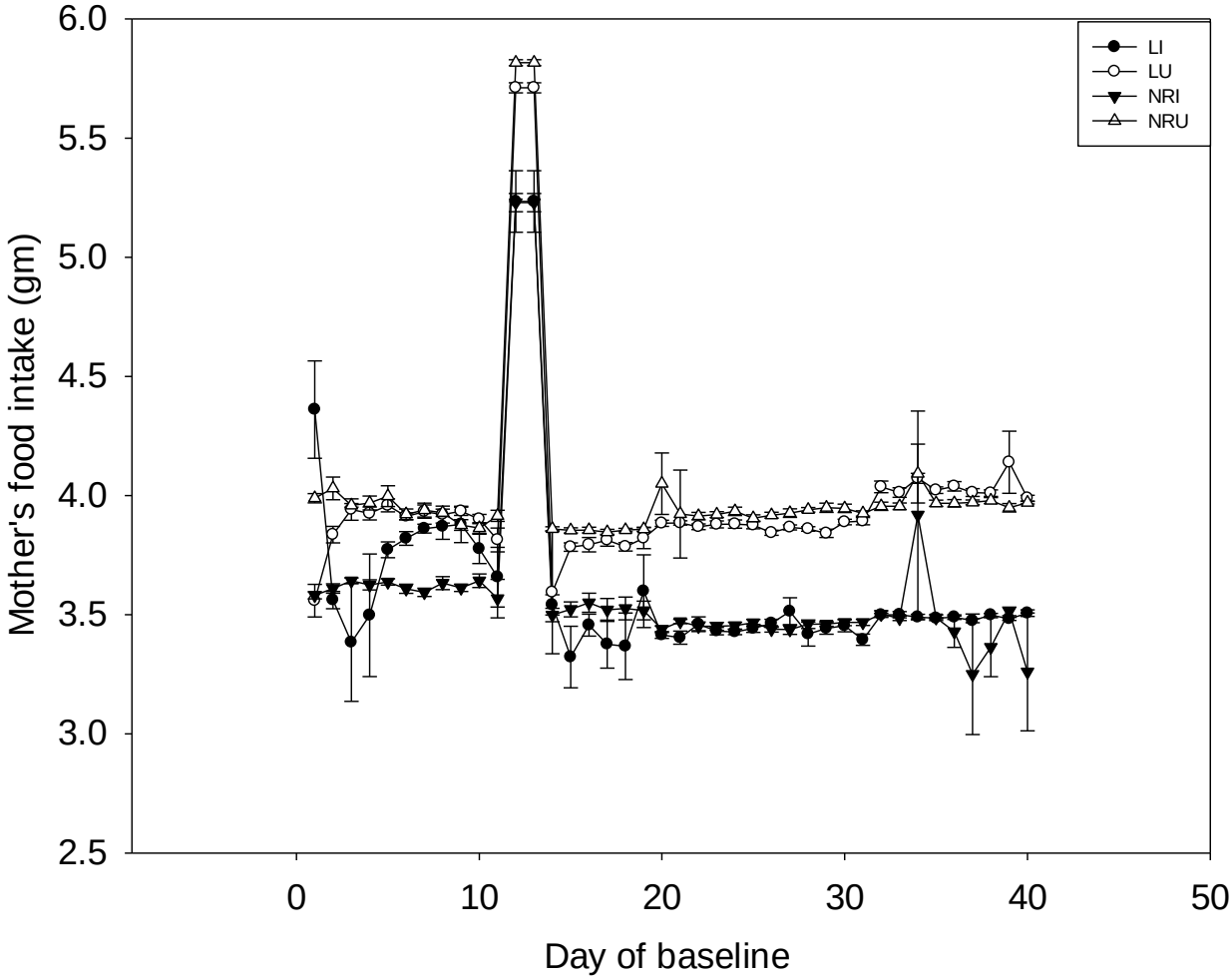

Fig (4-4): Mother's food intake of studied groups of mice throughout the baseline period (LI: lactating infected, LU: lactating uninfected, NRI: non-reproducing infected, NRU: non-reproducing uninfected

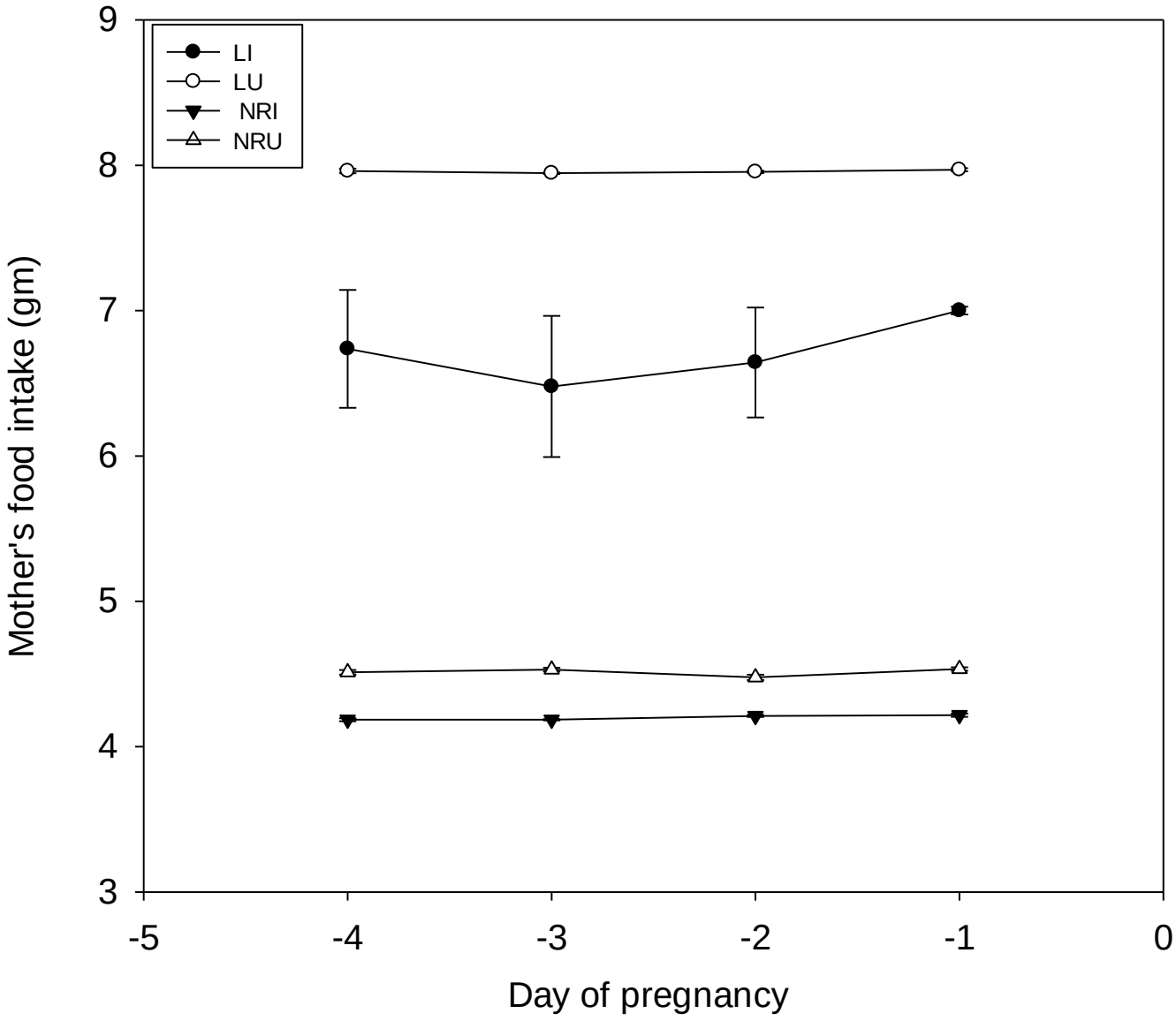

Fig (4-5): Mother's food intake of studied groups of mice throughout the last four days of pregnancy (LI: lactating infected, LU: lactating uninfected, NRI: non-reproducing infected, NRU: non-reproducing uninfected.

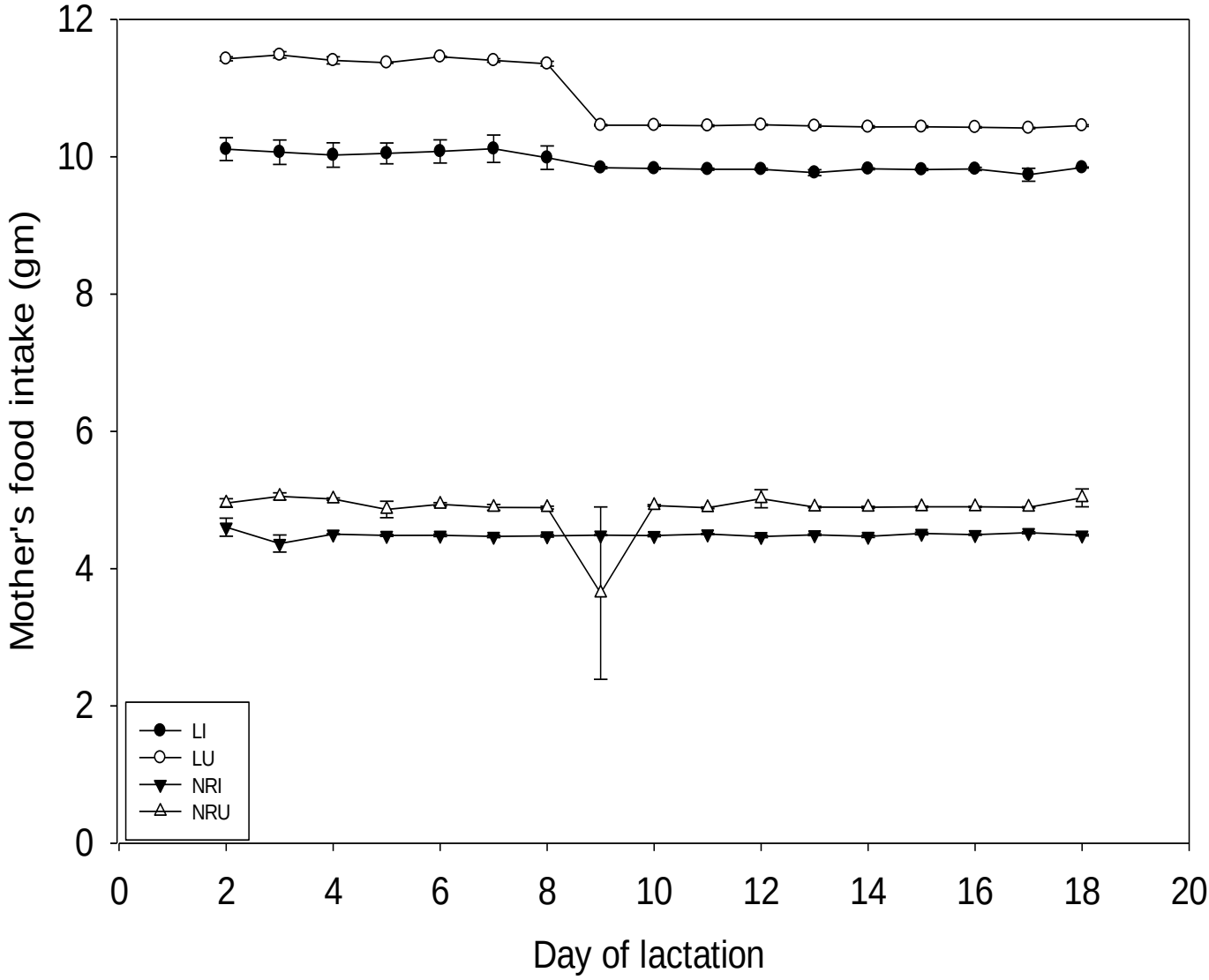

Fig (4-6): Mother's food intake of studied groups of mice throughout the lactation period (LI: lactating infected, LU: lactating uninfected, NRI: non-reproducing infected, NRU: non-reproducing uninfected.

4.3 Reproductive performance:

day 2 after birth, the litter size (LS) was significantly increased by groups in which the litter size of assigned group for (LI) which mean was 5.875±0.915 (N=8) while average litter size (LS) in Lactating Uninfected (LU) was 7.000 ± 0.463 (N=8), (RM GLM groups; F_{10}=22.11, P=0.000).table (4-4).

At day 18 after birth, the litter size (LS) was significantly different by groups in which the litter size of assigned group for (LI) which mean was 4.25±0.56 gram (N=8) while average litter size (LS) in Lactating Uninfected (LU) was 5.88± 0.58. grams (N=8), (RM GLM groups; F_{13}=120.47, P=0.000).table(4-4).

At day 2 after birth, the litter mass (LM) was significantly increased by groups and 2 day of birth in which the litter mass of assigned group for (LI) which mean was 7.16±0.87 (N=8) while average litter mass (LM) in Lactating Uninfected (LU) was 10.10± 0.72 (N=8), (RM GLM groups; $F_{1,15}$=4.02, P=0.066, 2day; $F_{1,15}$=8.26, P=0.013).table (4-4).

During lactation litter mass significantly increased by days and no change in groups assigned of (LI) , which was average litter mass of (LI) 15.780±1.988 gram (N=8), while average litter mass in (NRU) was 20.857±2.170 gram (N=8). (RM GLM groups; $F_{1,15}$=4.01, P=0.064; days; $F_{1,15}$=53.12, P=0.000;) Fig (4-4) (Table 4-4).

At day 18 after birth, the litter mass (LM) was significantly different by 18 day of birth no change in groups in which the litter size of assigned group for (LI) which mean was 24.20±2.98 (N=8) while average litter

mass (LM) in Lactating Uninfected (LU) was 31.72± 4.12 (N=8), (RM GLM groups; $F_{1,15}$=1.39 , P=0.260, 18 day; $F_{1,15}$=120.47, P=0.000).table (4-4).

At day 2 after birth, the Pup mass (PM) was significantly different by 2 day of birth no change in groups in which the Pup mass of assigned group for (LI) which mean was 1.308±0.106 (N=8) while average Pup mass (PM) in Lactating Uninfected (LU) was 1.452± 0.096 (N=8), (RM GLM groups; $F_{1,15}$=8.26 , P=0.066, 2day; $F_{1,15}$=8.26, P=0.013).table (4-4).

During lactation Pup mass significantly different by days and no change in groups assigned of (LI) , which was average Pup mass of (LI) 3.182±0.140 gram (N=8), while average Pup mass in (NRU) was 3.192±0.211 gram (N=8). (RM GLM groups; $F_{1,16}$=0.00, P=0.997; days; $F_{1,16}$=121.87, P=0.000;) Fig (4-4) (Table 4-4).

At day 18 after birth, the Pup mass (PM) was not significantly changed among all groups in which the Pup mass of assigned group for (LI) which mean was 5.695±0.154 (N=8) while average Pup mass (PM) in Lactating Uninfected (LU) was 5.225± 0.322 (N=8), (RM GLM groups; $F_{1,15}$=3.99 , P=0.067, 18 day; $F_{1,15}$=2.74 , P=0.122).table (4-4).

Table 4-4: **Physiological Traits of reproductive performance of mice in different reproductive stages.**

Traits	LI	LU	F-Value	P-Value
LS day 2	5.875a ± 0.915	7.000b ± 0.463	22.11	**0.000**
LS day 18	4.25a ± 0.56	5.88b ± 0.58	120.47	**0.000**
LM Lact.	15.780a ± 1.988	20.857b ± 2.170	4.01	0.064
LM at day 2	7.16a ± 0.87	10.10b ± 0.72	22.11	**0.000**
LM at day 18	24.20a ± 2.98	31.72b ± 4.12	120.47	**0.000**
PM at day 2	1.308 ± 0.106	1.452 ± 0.096	8.26	**0.013**
PM at day 18	5.695 ± 0.154	5.225 ± 0.322	2.74	0.122
PM lact.	3.182 ± 0.140	3.192 ± 0.211	0.00	0.997

LM 2 day = Litter mass 2 day, **LM 18 day=**Litter mass 18 day ;**LM Lact**.=Litter mass lactation .**LS**= Latter size; **LS day 2**= Litter size 2 day; **LS day 18**= Litter size 18 day, **PM lact**.=Pups mass lactation.

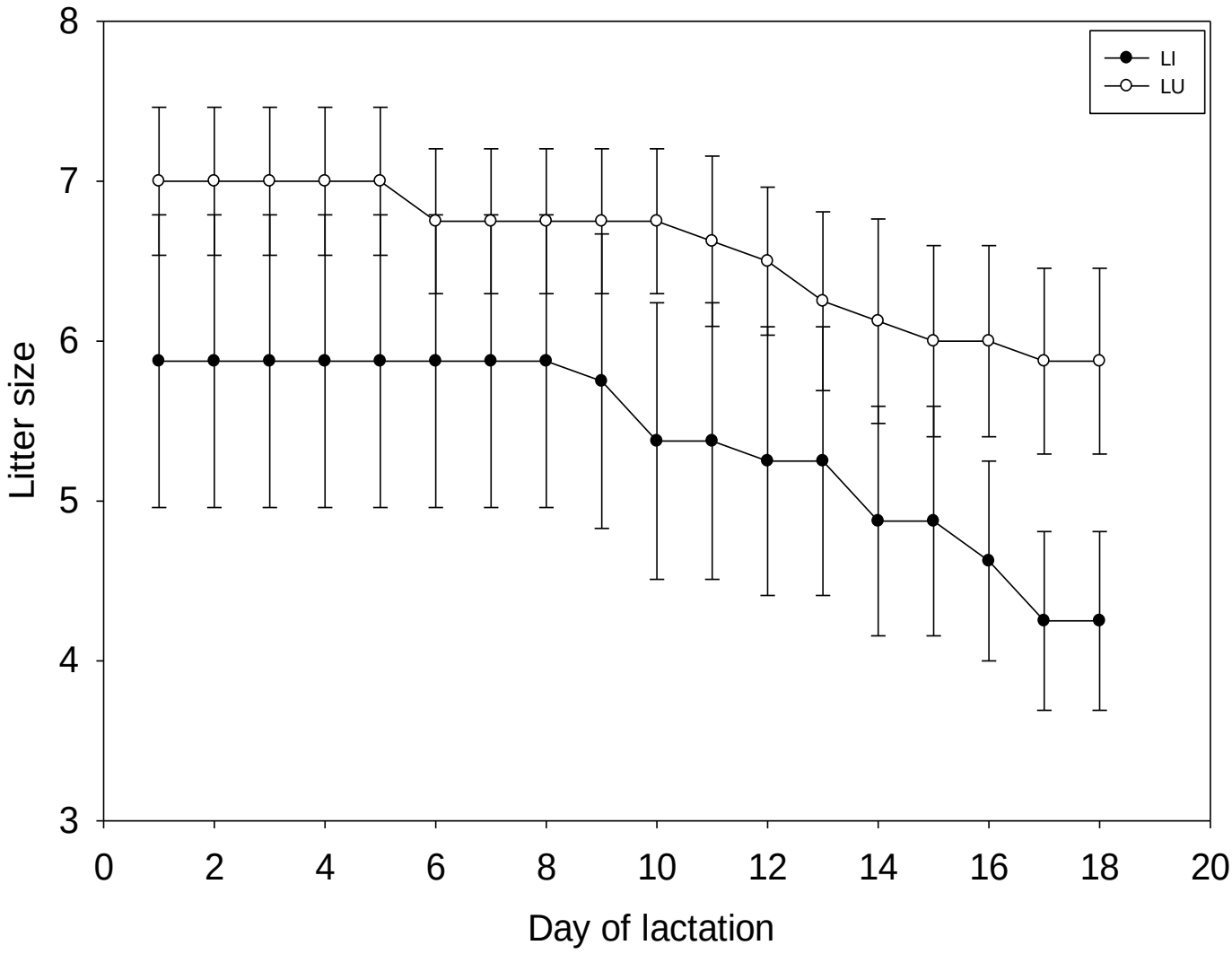

Fig (4-7): The litter sizes of lactating infected (LI) and lactating uninfected (LU) mice throughout the lactation period .

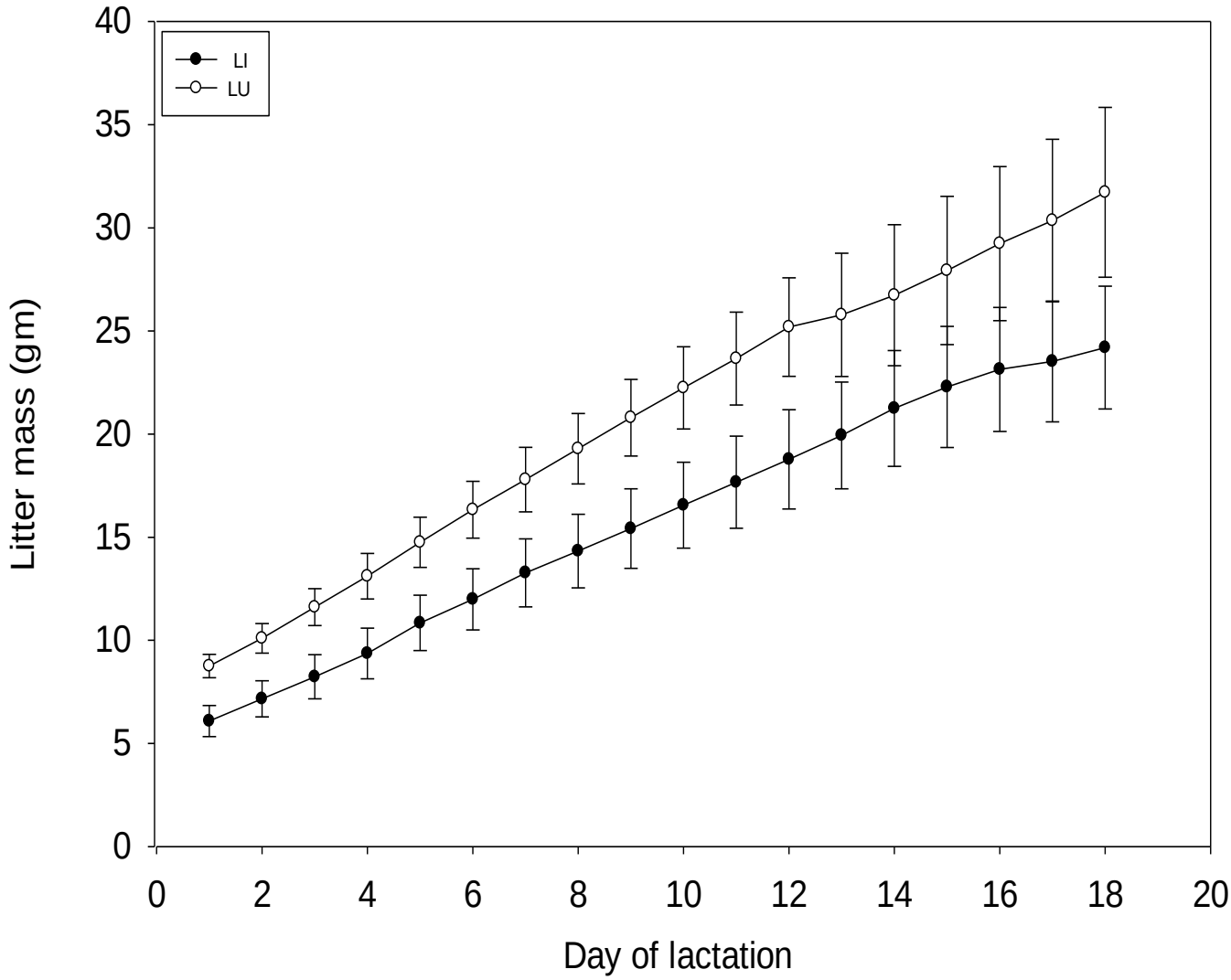

Fig (4-8): The litter masses of lactating infected (LI) and lactating uninfected (LU) mice throughout the lactation period .

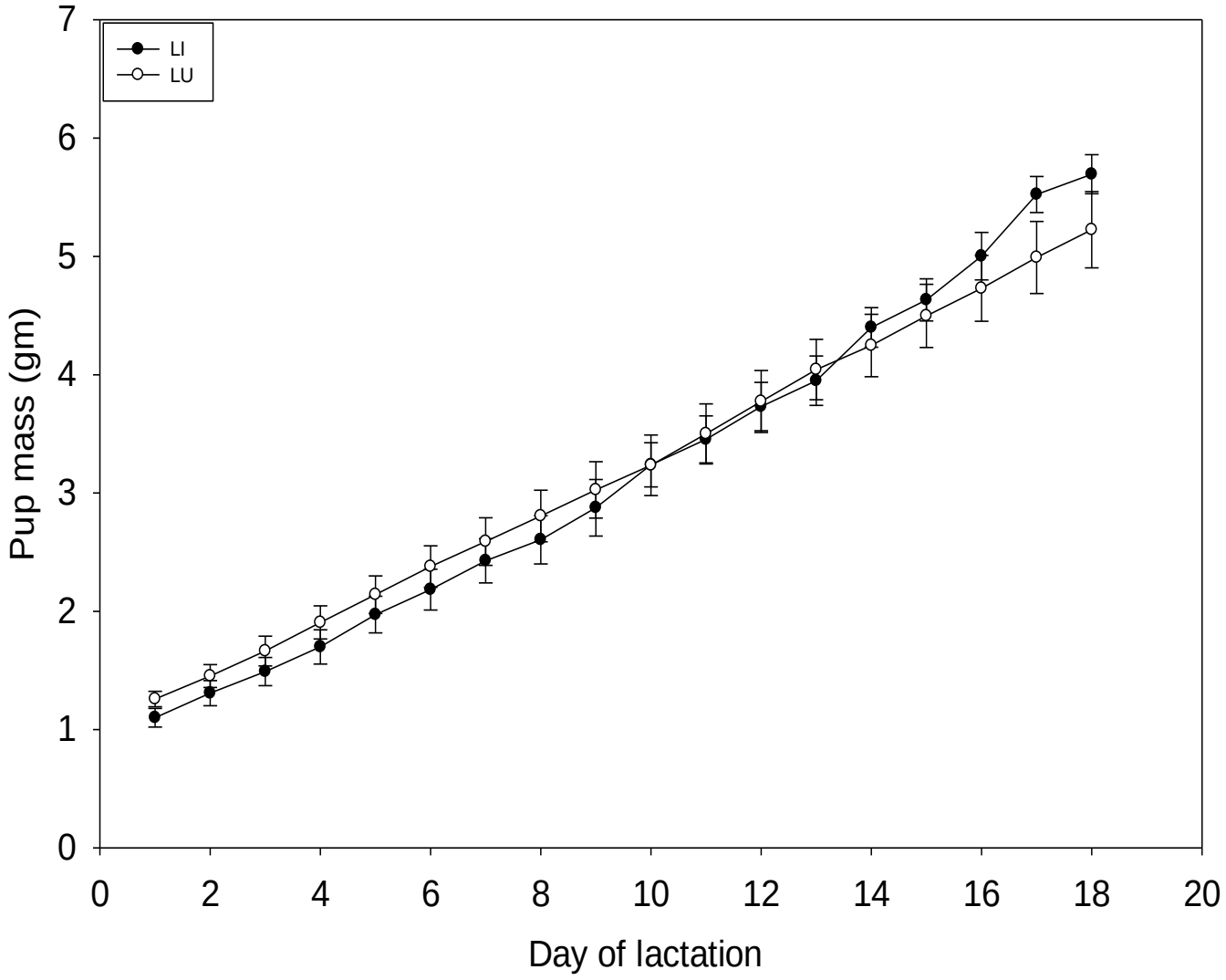

Fig (4-9): The pup masses of lactating infected (LI) and lactating uninfected (LU) mice throughout the lactation period

4.4.Markers of antioxidants

4.4.1.Markers of brain antioxidants:

The results showed that GPx brain was significantly different among groups in which the GPx was significantly decreased in LI mice compared to the levels in other groups. However, the levels of SOD and CAT were not significantly differed among groups (see table 4-5).

Table 4-5: Makers of antioxidants in Brain tissue of all studied groups.

Group	Brain GpX	Brain SOD	Brain CAT
LI	4.19a±1.19	73.58±5.11	72.09± 4.92
LU	10.98b±1.64	64.62± 3.79	88.3±10.5
NRI	12.76b±1.36	74.32±2.79	81.25± 9.80
NRU	11.01b±1.38	71.87± 1.59	68.77±3.90
P value	**0.0001**	0.233	0.298

LI: lactating infected, **LU**: lactating uninfected, **NRI**: non-reproducing infected, **NRU:** non-reproducing , SOD: Superoxide dismutase, CAT: Catalase ,GPx: Glutathione peroxidase.

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4.4.2.Markers of Heart antioxidants:

The results showed that CAT heart was significantly different among groups in which the CAT was significantly increased in (LI) mice compared to the levels in other groups. However, the levels of SOD and GPx were not significantly differed among groups (see table 4-6).

Table 4-6: Makers of antioxidants in heart tissue of all studied groups.

Group	Heart CAT	Heart SOD	Heart GpX
LI	99.9a±12.7	73.13±2.07	8.61±1.82
LU	93.06ab±7.00	70.45±3.67	12.00±1.35
NRI	76.28 ab±5.68	70.48± 3.59	10.32±1.69
NRU	67.32b±5.50	64.59±3.08	12.801±0.826
Pvalue	**0.035**	0.295	0.213

LI: lactating infected, **LU**: lactating uninfected, **NRI**: non-reproducing infected, **NRU:** non-reproducing , SOD: Superoxide dismutase, CAT: Catalase ,GPx: Glutathione peroxidase.

4.4.3.Markers of Liver antioxidants:

The results showed that GPx liver was significantly different among groups in which the GPx was significantly increased in (LI) mice compared to the levels in other groups. However, the levels of SOD and CAT were not significantly differed among groups (see table 4-7).

Table 4-7: Makers of antioxidants in liver the tissue of all studied groups.

Group	Liver GpX	Liver SOD	Liver CAT
LI	12.03ab±1.27	67.69±3.18	55.87±3.30
LU	10.071ab±0.83	66.27± 2.30	51.97± 9.25
NRI	13.556a±0.74	65.51±3.87	55.15± 4.61
NRU	8.746b±0.893	69.95± 2.44	60.5± 10.5
Pvalue	**0.007**	0.741	0.884

LI: lactating infected, **LU**: lactating uninfected, **NRI**: non-reproducing infected, **NRU:** non-reproducing , SOD: Superoxide dismutase, CAT: Catalase ,GPx: Glutathione peroxidase.

4.5.Markers of oxidative damage

4.5.1.Markers oxidative damage in Brain tissue:

The results showed that protein damage measured by Protein carbonyls (PC) in the brain was significantly different among groups in which the PC was significantly decreased in (LI) mice compared to the levels in other groups. However, the levels of DNA damage measured by 8-hydroxy-2-deoxyguanosine (8OHDG) in the brain and Lipid damage measured by Molandialdehyde (MDA) in the brain were not significantly differed among groups (see table 4-8).

Table 4-8: Makers of oxidative damage in brain tissue of all studied groups.

Group	Brain PC	Brain OHdG	Brain MDA
LI	29.57a±1.76	38.88 ±7.80	7.35 ±2.36
LU	43.47ab±4.48	37.06±3.48	7.34±2.10
NRI	47.48b±2.99	41.33±7.32	4.536±0.794
NRU	75.43c±5.86	35.53±7.60	4.320 ±0.449
Pvalue	**≤0.001**	0.938	0.386

LI: lactating infected, **LU**: lactating uninfected, **NRI**: non-reproducing infected, **NRU:** non-reproducing , **PC**: Protein carbonyl , **OHdG**: 8-hydroxy-2-deoxyguanosine , **MDA**: Molandialdehyde.

4.5.1.2.Markers oxidative damage in heart tissue:

The results showed that Lipid damage measured by Molandialdehyde (MDA) in the heart was significantly different among groups in which the MDA was significantly increased in (LI) mice compared to the levels in other groups. However, the levels of DNA damage measured by 8-hydroxy-2-deoxyguanosine (8OHDG) in the heart and protein damage measured by Protein carbonyls (PC) in the heart were not significantly differed among groups (see table 4-9).

Table 4-9: Makers of oxidative damage in heart of all studied groups.

Group	Heart PC	Heart OHdG	Heart MDA
LI	40.29 ±2.84	7.67± 2.53	19.10a±1.47
LU	42.12±2.59	3.72±1.10	12.83ab±2.99
NRI	41.31±3.57	9.60±3.91	9.46b±1.65
NRU	48.50±4.16	5.70±2.23	6.75b±1.18
Pvalue	0.319	0.444	**0.001**

LI: lactating infected, **LU**: lactating uninfected, **NRI**: non-reproducing infected, **NRU:** non-reproducing , **PC**: Protein carbonyl , **OHdG**: 8-hydroxy-2-deoxyguanosine , **MDA**: Molandialdehyde.

4.5.1.3.Markers oxidative damage in liver tissue:

The results showed that Lipid damage measured by Molandialdehyde (MDA) in the liver was significantly different among groups in which the MDA was significantly increased in (LI) mice compared to the levels in other groups. However, the levels of DNA damage measured by 8-hydroxy-2-deoxyguanosine (8OHDG) in the liver and protein damage measured by Protein carbonyls (PC) in the liver were not significantly differed among groups (see table 4-10).

Table 4-10: Makers of oxidative damage in liver tissue of all studied groups.

Group	Liver PC	Liver OHdG	Liver MDA
LI	23.43±3.37	3.448±0.33	8.36 a±1.50
LU	22.92±3.15	3.985±0.63	6.241ab±0.576
NRI	21.21±3.71	3.928±0.84	5.584ab±0.314
NRU	28.04±5.08	2.758±0.18	3.649b±0.483
Pvalue	0.646	0.406	**0.006**

LI: lactating infected, **LU**: lactating uninfected, **NRI**: non-reproducing infected, **NRU:** non-reproducing , **PC**: Protein carbonyl , **OHdG**: 8-hydroxy-2-deoxyguanosine , **MDA**: Molandialdehyde.

4.6.Markers of organ function:

4.6.1Markers of liver function:

GPT levels were not significantly influenced by infections status. However, lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected). GOT levels were not significantly influenced by reproductive status. However, lactating mice (infected only) had significantly greater (higher compared to the non-reproducing mice (infected and uninfected). ALP levels were not significantly influenced by infections status and reproductive status.(see table 4-11).

Table 4-11: markers of organ function in liver tissue of all studied groups.

Group	GOT	GPT	ALP
LI	51.2±109	7.03±3.36	73.8±15.9
LU	23.5± 10.6	18.33± 7.08	100.0±23.5
NRI	30.70±6.47	45.9± 15.9	97.7± 24.2
NRU	18.79± 2.72	21.58± 2.53	54.15±6.27
IS	**P=0.025**	0.493	0.663
RS	0.142	**P=0.032**	0.582

LI: lactating infected, **LU**: lactating uninfected, **NRI**: non-reproducing infected, **NRU:** non-reproducing , **AST/GOT**: aspartate aminotransferase, **ALT/GPT**: alanine aminotransferase, **ALP**: Alkaline Phosphatase, **IS** : Infectious status, **RS :** Reproductive status.

4.6.2.Markers of Brain function:

BDNF levels were not significantly influenced by infections status. However, BDNF levels were significantly influenced by reproductive status. lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected).(see table 4-12).

Table 4-12: marker of organ function in brain tissue of all studied groups.

Group	BDNF
LI	1.929±0.31
LU	1.979±0.97
NRI	4.529±0.88
NRU	4.97± 1.84
IS	0.829
RS	**P=0.019**

LI: lactating infected, **LU**: lactating uninfected, **NRI**: non-reproducing infected, **NRU:** non-reproducing , **BDNF**: Brain-derived neurotrophic factor, **IS** : Infectious status, **RS :** Reproductive status.

4.6.3.Markers of heart function:

heart function measured by Brain Natriuretic Peptide (BNP) , BNP levels were not significantly influenced by infections status. However, BNP levels were significantly influenced by reproductive status. lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected).(see table 4-13)

heart function measured by Troponin (CTn) , CTn levels were not significantly influenced by infections status. However, CTn levels were significantly influenced by reproductive status. lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected).(see table 4-13)

Table 4-13: markers of organ function in heart tissue of all studied groups.

Group	BNP	Troponin
LI	48.4 ± 12.5	1.369±0.18
LU	65.4± 29.5	1.283±0.41
NRI	124.4± 21.9	3.161±0.55
NRU	154.4±66.3	3.09± 1.35
IS	0.538	0.917
RS	**P=0.037**	**P=0.023**

LI: lactating infected, **LU**: lactating uninfected, **NRI**: non-reproducing infected, **NRU:** non-reproducing , **BNP**: Brain Natriuretic Peptide, **IS** : Infectious status, **RS :** Reproductive status.

CHAPTER FIVE
DISCUSSION

5.Discussion

5.1. Maternal body mass

In the current study, the body mass showed a significant difference among the studied groups, and the reason is that some of the groups were infected with parasites, which led to the appearance of this significant difference in body mass among the studied groups during the baseline period. Some groups were infected with the hydatid cyst parasite, and for this reason there appeared A significant difference between the groups, in another study conducted by (Kristan,2002), where it was found that mice infected with parasites were heavier than mice without parasites. Compared to unparasitized mice, , parasitized mice had bigger cecae (22%) and spleens (30%), Mice that have been parasitized had 61% more serosal tissue and 18% more mucosal tissue.

The percentage of mucosa was 6% lower in parasitized mice than in unparasitized mice because serosal tissue increased disproportionally in parasitized animals as compared to mucosal tissue. The size of the small intestine continued to grow during the infection, in contrast to the trend observed with spleen mass. Host morphology can differ between and within organs depending on a number of variables, including the length of the infection. The small intestine's volume did vary, but it did not change consistently along its length or across its layers of tissue. For instance, differences in serosal mass were seen in both H. polygyrus-colonized and non-colonized small intestine areas. Increased serosal mass in infected mice may be caused by enhanced tissue growth in the muscularis at larval-

occupied sites or by any leftover larvae that did not emerge into the lumen before dissection.

The serosal layer variations may instead be the result of changes in the peristalsis patterns brought on by infection with H. polygyrus (depending on the muscularis layers). It is possible that parasitized mice used more frequent peristalsis to move ingesta through the small intestine than non-parasitized mice, which may have accelerated alterations in the majority of the serosal layer throughout the whole small intestine. In fact, H. polygyrus infection can impair food digestion in wild-derived house mice to a small (2%) but considerable extent (although see the most recent studies; Kristan and Hammond, 2003), which is consistent with food passing through the gut more swiftly. It is still unknown how H. polygyrus will affect the host's time for food transit. In terms of their overall body composition and the precise distribution of fat, the wild-derived mice employed in this study may more nearly resemble truly wild mice than domestic mice, where it may be difficult or even impossible to find enough food during periods of high demand like lactation. In addition, (Bronson ,1991).

During pregnancy , The body mass of the pregnant mice increases as a result of the growth and increase in the masses of the pups. During pregnancy, parathyroid hormone-related protein (PTHrP) is one of many growth factors that play important roles to promote fetal growth and development, including stimulation of placental calcium transport. Angiotensin II, acting through the AT1a receptor, is also known to promote placental growth(Wlodek, *et al* ,2005**)**, an increase in the size of the breast, an increase in the size of the uterus, , presence of the amniotic fluid, increase

in the blood volume, increase in the volume of fluids, increase in the fatty mass .(Jungheim,*et al*, 2010**)**

body mass during lactation was a significant difference among the studies groups. The main causes of the increase in body mass during lactation were larger masses of all GI tract components and central processing organs. These have to do with eating more to meet the energy requirements of lactation. Masses of the digestive system will swiftly expand as a result of increased food intake in order to sustain the expanding volume of ingested material (as early as 1-2 days; Toloza *et al*., 1991).

Lactation is associated with increased liver mass and blood volume because the excess food must be processed and transported throughout the body for use in peripheral locations in addition to being eaten and digested (Hammond et al., 1994). (For example, the mammary glands' generation of milk; Hammond *et al*., 1994). (Suzuki *et al.*, 1993; Suzuki *et al*., 2000) .

5.2.Maternal food intake

In our current study, we found that animals that were infected with parasites ate less food than animals that were not infected with parasites during the baseline period. This was confirmed by a previous study(Kristan, & Hammond,2003) During parasitic infection, the ability to intake food decreases, and glucose transport rates decrease,

During pregnancy and lactation , food intake increased significantly, to meet the metabolic demands of the growing fetus and then to produce milk to supply offspring. Moreover, during pregnancy, food intake increases to

allow an increase in fat mass as a proposed potential energy reserve for the extreme metabolic demands of lactation. (Ladyman , *et al* ,2012) .

Another study confirmed that during pregnancy and lactation, the maternal body undergoes many changes in the regulation of appetite, body weight, and glucose homeostasis to deal with the metabolic demands of the growing fetus and subsequent demands of providing milk for offspring.(Ladyman, *et al*, 2018)

5.3.Reproductive performance:

In our current study, litter size (LS) at both tow days (2,18 days after birth) was a significant differ among in (LI) compared to other groups. Litter mass (LM) at both tow days (2,18 days after birth) was a significant differ among in (LI) compared to other groups. Pup mass (PM) at both tow days (2,18 days after birth) was a significant differ among in (LI) compared to other groups.

The immunological response did indeed induce a decrease in the amount of resources allocated to the offspring of lactating mothers. These data imply that the allocation of resources to the immune response throughout pregnancy and during lactation led to a lower allocation to offspring even under conditions when there had ad libitum access to food. In other words, the adaptive immune response causes a trade-off between maintaining maternal condition and offspring development. This is something that has been observed in other animals, such as ageing female wild cavies (Cavia aperea) (Trillmich , *et al*, 2019) and other animals (Bakos ,*et al* 2006, Uller, *et al* ,2006).

The parasitic infection affected on the reproductive performance of the mother, as the offspring of these mothers were less mass than offspring of Lactating Uninfected mothers. The reason may be the fact that the mothers made a trade-off between the growth of the offspring and the development of the immune system, so the offspring of this group mass less than the Lactating Uninfected group. (Lockley, *et al* ,2020)

5.4.markers of oxidative stress and antioxidants activities

5.4.1.Markers of antioxidants:

5.4.1.1.GPx:

The results showed that GPx brain was significantly different among groups in which the GPx was significantly decreased in LI mice compared to the levels in other groups. However, the levels of SOD and CAT were not significantly differed among groups.

The clinical importance of GPx has been underlined by a number of studies. Chabory et al. postulated that individuals with lower GPx activity are predisposed to impaired antioxidant protection, which leads to oxidative damage to membrane fatty acids and functional proteins, and by inference, neurotoxic damage.(Chabory E *etal* 2009) Forgione and colleagues had previously hypothesized that GPX1 deficiency directly induces an increase in vascular oxidative stress, with attendant endothelial dysfunction. (Forgione, *et al* ,2002).

Glutathione peroxidases, particularly GPx1 have also been implicated in the development and prevention of many common and complex diseases, including cancer and cardiovascular disease (Rayman,.2005) .

Actually, the potential associations between the both sides have been observed in the studies previously performed in house mice (Aloise *et al*, 2013; Garratt *et al*, 2011, 2013), Brandt's voles (Xu *et al*, 2014), Mongolian gerbils (Yang et al, 2013), Wistar rats (Rattus norvegicus) (Davidović et al, 1999; Venditti *et al*, 2004) and short-tailed field voles (Microtus agrestis) (Selman *et al*, 2000).

Among the different tissues in the current study, the brain was only one showing a significant increase in oxidative stress, indicated by levels of H2O2 and MDA, which might be partly caused by significant reduction of antioxidant of GSH-PX activity. The decreased GSH-PX activity might impair the oxidant-antioxidant balance. The data from the previous study may also demonstrate in reproductive mice that antioxidants were regulated physiologically in response to ROS, by which oxidant-antioxidant balance occurs during peak lactation. (Li & Wang, 2005; Martin *et al*, 1989; Pedraza *et al*, 2000; Xiao *et al*, 2004).

The results showed that GPx liver was significantly different among groups in which the GPx was significantly increased in (LI) mice compared to the levels in other groups. The enzyme plays a more crucial role of inhibiting lipid peroxidation process, and therefore protects cells from oxidative stress. (Gill &Tuteja , 2010) .

In the current study, mice with infections caused by *Echinococcus granulosis* had higher levels of lipid peroxidation. It is possible to interpret the parasite lactating mice increased lipid peroxidation as an indication of cell damage brought on by *Echinococcus granulosis*. Higher GPx levels in the liver tissue of infected animals can therefore be inferred to be related to

the host's defense against parasite infection.The findings of this study clearly imply that reduced activity of the defense system preventing tissues from free radical damage may be one of the primary causes of higher GPx levels in infected animals.. The elevation of H2O2 in these subjects lead to the speculation that parasitic infection may contribute to a higher level ofOH-induced oxidative stress compared to the controls. Our results can be supported by the findings of previous studies done on other classical parameters of OH-induced oxidative stress such as elevated levels of GPx and SOD in the blood of mice infected with *Trichinella spiralis* (Derda *et al*, 2004).

5.4.1.2.SOD:

The results showed that the levels of SOD in brain ,liver and heart were not significantly differed among groups. It has been suggested that oxidative stress occurs only when ROS production exceeds the capacities of protection, resulting in oxidative damage to macromolecules (Beckman & Ames, 1998; Selman *et al*, 2002, Deponte, 2013).

In another similar study, activities of GSH-PX, SOD and T-AOC, the markers of antioxidants, in lactating mice were almost the same as that observed in non-reproductive mice. Organisms have a variety of defensive mechanisms that can protect against oxidative stress (Garratt ,*et al*, 2011).

Physiological regulation of antioxidant systems are effective mechanisms to maintain the oxidant-antioxidant balance, and consequently may play important roles in preventing oxidative damage to macromolecules (Garratt *et al*, 2011). the main intracellular antioxidant enzyme SOD

specifically transforms superoxide radicals to hydrogen peroxide.(IM Arias *et al*. 1994) .

5.4.1.3. Catalase (CAT):

The results showed that CAT heart was significantly different among groups in which the CAT was significantly increased in (LI) mice compared to the levels in other groups. Increased catalase activity in the heart of infected mice by *Echinococcus granulosus* may be a compensatory reaction to cleanse the heart of the excessive ROS generation. They propose that activated leukocytes in the heart and spontaneous generation of brain cells triggered by ROS are related to higher catalase activities in the hearts of infected mice. Another theory is that in mice infected with this parasite, partial antioxidant enzymes transmigrate into the brain through the damaged BBB. Despite enhanced catalase activity in the heart during lactation, high ROS concentrations were found in the hearts of mice. This is a definite sign of oxidative stress in the infected mouse's heart at this time.(Giorgi, 2018)

The results showed that CAT brain and CAT liver tissue enzymes were not significantly differed among groups. catalase, and other enzymes are typically activated by cells to scavenge free radicals. Catalase detoxifies hydrogen peroxide into water,.(IM Arias *et al*. 1994) .

There are many studies conducted on different mammals that showed the state of oxidation during parasitic infection with different types of parasites infecting different organs in the body of their host. Rats infected with *Toxoplasma gondii* showed significant decreased in antioxidant (SOD and CTA) in the serum and testis of infected rats, SOD as an important defense against oxygen radicals involved in dismutation of the superoxide radicals to

H2O2 which can cause subsequent oxidative damage to various types of macromolecules including lipids, proteins and DNA, CAT activity adversely affected by oxidative stress caused by various diseases such as toxoplasmosis.(Kwok;*et al* ,2004)

5.4.2.Markers of oxidative damage:

Small mammals require the most energy during reproduction, when both intake and output are raised to meet the higher energy needs of offspring growth and somatic defense. Reactive oxygen species (ROS) were said to be formed in direct proportion to metabolic rate, leading to oxidative stress and damage to macromolecules, according to the oxidative stress life history theory, Parasites have different physiological functions for their survival in certain environments of the host. Most of the parasites adapt to the lack of oxygen conditions within the host by utilizing systems other than oxidative phosphorylation for ATP synthesis. Mitochondrial complex II of the parasite acts as a source for anaerobic energy metabolism (Zheng, *et al* 2015;Kita *et al.* 2002).

In general, intestinal parasites (lumendwelling parasites) are anaerobic parasites. They can be divided into 2 major groups namely protozoa and helminths or worms. When a host's immune system is triggered by an infection of parasites, a massive production of ROS or oxidative burst is activated by macrophages that are associated with the inflammatory system (Rosen *et al.* 1995). This provides a first line of defense against these parasites. Macrophage or phagocyte activation causes the release of reactive species that lead to lipid peroxidation, protein damage and DNA strand breaks (Oshima and Bartsch, 1994).

5.4.2.1.MDA :

The results showed that Lipid damage measured by Molandialdehyde (MDA) in the heart and liver were significantly different among groups in which the MDA was significantly increased in (LI) mice compared to the levels in other groups. Level of MDA in the brain was not significantly differed among groups. The rapid increase in MDA suggests that oxidative/antioxidant balance was disrupted, resulting in higher lipid peroxidation. (Sandhir *et al.,* 1994) .

In the current study, mice with infections caused by *Echinococcus granulosis* had higher levels of lipid peroxidation. It is possible to interpret the parasite lactating mice increased lipid peroxidation as an indication of cell damage brought on by *Echinococcus granulosis.* Higher MDA levels in the tissue of infected animals can therefore be inferred to be related to the host's defense against parasite infection.The findings of this study clearly imply that reduced activity of the defense system preventing tissues from free radical damage may be one of the primary causes of higher MDA levels in infected animals.

In a similar study, high levels of urinary MDA in the subjects with parasitic infection compared to the normal individuals may be due to the over production of ROS (during parasitic infection) which causes cellular injuries. Previous studies have reported on increased levels of serum MDA in humans with intestinal parasitic infection such as giardiasis (Dermici *et al*. 2003) and blastocystosis (Kilic *et al*. 2003).

It was also found in a previous study that MDA content was increased and activities of GSH-Px and SOD were decreased significantly in brain,

liver and kidney of lead-poisoned neonatal mice, consistent with previous reports (Shalan *et al*., 2005).

Indeed, in this work, *Angiostrongylus cantonensis*-infected animals showed higher levels of MDA, 8-isoprostane, and 8-OHdG in their CSF. The MDA is a direct indicator of oxidative stress-induced lipid peroxidation in cells.(Gawet *et al* 2004, Murphy, *et al* 2022) Additionally, in a previous study, MDA levels in the CSF were noticeably higher in individuals with bacterial meningitis and cerebral malaria than they were in healthy controls.(Das *et al*., 1991,Menezes *et al.,* 2009).

Oxidative stress raises MDA levels in particular brain areas, as demonstrated by a rat under prolonged stress. (ManoliLP *et al.* 2007).Both living and dead Angiostrongylus cantonensis and worm tracks have been discovered in various areas of the brain during autopsies and experimental research. According to(Lindo.2004 , Wang. *et al* 2005)

As a result, lipid peroxidation took place in the brain of mice infected with Angiostrongylus cantonensis, as demonstrated in this study by the rise in MDA levels in the CSF. Unsaturated fatty acids are abundant in brain tissue and are highly sensitive to ROS damage in membrane phospholipids. The investigation of oxidative stress in a few neurological illnesses using the drug 8-isoprostane has recently been successful. (Greco .,*et al* ,2000 , Minghetti, 2004).

MDA is a decomposition product of lipid peroxidation, and its levels reflect lipid peroxidation in body tissues (Cormard *et al.,* 2005). lipid peroxidation (damage to lipid), is used as an indicator for oxidative stress in the diseased state (Saygili *et al*., 2003). Although much controversy has

appeared in studies concerning the specificity of TBARS toward composites other than MDA, the rapidity of the assay qualifies it as one of the broadly-used assays to determine lipid peroxidation (Siciarz *et al*. 2001).

AOPP in plasma and serum have been extensively used as a marker of free radical-induced protein damage. The low molecular weight AOPP (carbonyl) compounds are possibly excreted under stress conditions (Kalousova *et al*. 2002).

Studies have reported that the level of advanced glycation end-product (AGE) which is known to be analogous with AOPP (Witko-Sarsat *et al.*1998) was elevated in the spleen and liver tissues of mice infected with cysts from tapeworms (Kamalvand and Ali-Khan, 2004).

Thus, in this study, the increase of urinary AOPP levels in parasite-infected subjects indicates that they have higher degree of oxidant-mediated protein damage.

5.4.2.2(8OHDG) :

the levels of DNA damage measured by 8-hydroxy-2-deoxyguanosine (8OHDG) in the heart ,liver and brain were not significantly differed among groups. Oxidatively produced DNA damage can be caused by an excess of ROS. (Marnett ,1999) 8-OHdG is a significant oxidatively generated lesion in the DNA that can lead to primarily G to T transversions. (Nakae, *et al.*, 1997; Shibutani, *et al.*, 1991) .

A previous study is not consistent with the current study, the infected mice displayed significant increases in CSF 8-OHdG along with increased ROS in their CSF, the CNS of mice infected with *Angiostrongylus*

cantonensis may experience excessive 8-OHdG synthesis as a result of oxidative DNA damage. Both direct and indirect methods are used in this study to show that mice infected with *Angiostrongylus cantonensis* are under oxidative stress. Chung and others In mice infected with *Angiostrongylus cantonensis*, there was a kinetic change in the level of oxidative stress. the kinetic change in the quantity of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the cerebral fluid of C57BL/6 mice after infection with 30 third-stage *Angiostrongylus cantonensis* larvae. Compared to indirect evidence from before infection, The excessive ROS production in the CSF of infected mice, despite increased antioxidant enzyme activity, is an illustration of direct evidence. Significantly elevated levels of indicators for DNA damage and lipid peroxidation in the CSF serve as indirect evidence., the oxidative stress time course in *Angiostrongylus cantonensis*-infected mice was further investigated. Infected mice's CSF displayed a comparable kinetic change in ROS level as well as biomarker values of lipid peroxidation and DNA damage. After *Angiostrongylus cantonensis* infection, severe oxidative stress in mice's CNS is highly likely to occur between days 12 and 30. Severe oxidative stress and the length of blood-brain barrier disruption were both present in infected mice. Thus, this parasite illness may be linked to oxidative stress in the CNS of infected mice. The CSF of mice infected with *Angiostrongylus cantonensis* has been reported to drastically rise in several mitogen-activated protein kinases and transcription factors. (Lee *et al*, 2000, Lan ,*et al*, 2004)**.**

5.4.2.3. Protein carbonyls (PC)

(PC) in the brain There was decrease in (LI) compared to the other groups, this decrease give a significant difference between the four groups.

While in the heart muscle, did not give a significant difference between the four groups. In the liver tissue which was PC level did not give a significant difference between the four groups. The brain tissue utilizes about one-fifth of the total oxygen the body consumes (Ames B.N., *etal* 1981) . The brain is especially rich in iron which plays an important role in the generation of reactive oxygen species in the membrane lipids consisting mainly of polyunsaturated fatty acids HallIwell (1989) .

Neurons are particularly vulnerable to free radical attack since impaired defenses or exposure to excess free radicals may lead to neuronal death (Akitane., *et al* ,1994).

Pregnancy is accompanied by an overall increase in the energy demand in different physiological functioning of the body organs. Since brain constitutes the Centre of the nervous system and one of the most metabolically active organs, the metabolism of this tissue is expected to be further enhanced during pregnancy. The tissue oxygen utilization and requirement increases the rate of production of reactive oxygen species (ROS) (Reiter ., *et al* ,1993).

Unlike many other cells, neurons are generally considered to be incapable of mitosis and the damage to these cells by any means, including ROS, would be devastating and may incur permanent lesions. There are a few limited studies reporting high ROS activity in brain during pregnancy (Sainz ., *et al* 2000) .

In a study conducted by a group of researchers(Li & Wang, 2005; Martin *et al*, 1989; Pedraza *et al*, 2000; Xiao *et al*, 2004).

There are many studies conducted on different mammals that showed the state of oxidation during parasitic infection with different types of parasites infecting different organs in the body of their host. in reproductive mice that antioxidants were regulated physiologically in response to ROS, by which oxidant-antioxidant balance occurs during peak lactation. Oxidative stress results from an imbalance between oxidative protection and production of ROS and one may speculate that increased oxidative stress was not observed in reproductive animals, because ROS production was reduced or antioxidant protection was upregulated during reproduction. Previous studies have shown increases in activity of catalase (Brzek ,*et al*,2014), SOD (Garratt, *et al*,2013), or increased levels of glutathione (Garratt,*et al*,2011)

in livers of reproductive mice. We found an increase in SOD activity in the brain in reproductive compared to control mice, but a reduction in GPx activity in liver. These results once more point out the complexities of the relationship between antioxidant protection and oxidative damage and the variability of these responses between tissues of interest (Yang ,*et al*,2013, Xu,*et al*,2014].

Rats infected with *Toxoplasma gondii* in different tissues (liver, heart and brain) that showed As biochemical markers of oxidative stress, endogenous concentrations of GSH, GPX and SOD activity, MDA level, protein carbonyl content and total antioxidant capacity were determined from the mentioned tissues of control and infected rats. post infection the level of hepatic glutathione were significantly decreased in infected rats when compared to control. There was a significant increase in hepatic (GPx)activity and (MDA) level post infection in comparison to uninfected

rats. Significant elevation of (SOD) activity and (MDA) level post infection and (PC) and total antioxidant capacity post infection in infected livers were obtained. Significant changes of glutathione level, total antioxidant capacity and protein carbonyls contents were observed in cardiac homogenate. Measured parameters were constant throughout all stages of experiment in brain of infected rats. Indeed increased production of reactive oxygen species accompanies Toxoplasma infection in liver and heart tissues of experimentally infected rats. Based on this study, antioxidant defense system can probably play a role in parasitic stage interconversion and shifting the toxoplasmosis into the chronic phase. (Delavari, *et al* ,2017)

Mice infected with *Toxoplasma gondii* in liver showed were significantly increased in T. gondii-infected mice compared with the control group hepatic nitric oxide (NO), lipid peroxidation (LPO) levels and caused significant decrease in superoxide dismutase (SOD), catalase (CAT) and glutathione activities in the liver of infected mice., The excessive accumulation of radicals was suggested in the tissue as the main cause of decreased activity of enzymes (Alajmi,*et al* ,2019).

The probable reason for this difference might be due to activity of enzyme level d of Toxoplasma infection. In fact, the activation of the host immune system against T. gondii and the respiratory burst caused by macrophages lead to the production of active oxygen species by extending the study ,CAT enzyme by the detoxification of reactive oxygen species produced by immune cells prevents the possible damage (Moreira-Souza,*et al* ,2017).

It seems that T. gondii reduced the ROS encoding proteins activity. Also, with the excessive production of these active oxygen species, important enzymes such as CAT and SOD lose their effective function and became inactive , Glutathione, an important non-protein thiol source, can act as substrate for glutathione peroxidase and served as functional protective molecule for oxidative damage in various tissue (Dincel, & Atmaca,. ,2016).

The decrease might be due to elevation of oxidative stress in infected cells, that is why the significant difference between enzymatic markers occurred. Additionally, decreased serum and testis GSH level in chronic toxoplasmosis could change the detoxified capacity of reproductive tissue and then result in oxidative damage of reproductive organs which can adversely affects the fertility capacity (Aldabagh, *et al*, 2018).

On the other hand, Toxoplasma infection in pregnant women could induce oxidative stress which resulted in marked increase in the blood level of MDA and decrease in the blood level of GSH (Ali, *et al* ,2006).

mice infected with Angiostrongylus cantonensis was examined for kinetic changes in oxidative stress parameters, including reactive oxygen species (ROS), superoxide dismutase (SOD), catalase, malondialdehyde (MDA), 8-isoprostane, and 8-hydroxy-2′-deoxyguanosine (8-OHdG). The ROS increased gradually in the early stage of infection. During days 12–30 post-infection, the infected mice revealed ROS levels significantly higher than that in uninfected controls .The kinetics of MDA, 8-isoprostane, and 8-OHdG concentration changes observed in the CSF of the infected mice corresponded with kinetic changes in ROS levels. Thus, the excess ROS caused lipid peroxidation and DNA damage to cells in the central nervous

system (CNS) of mice infected with A. cantonensis despite the increased antioxidant SOD and catalase enzyme activities during parasitic infection. The oxidative stress in the CNS of C57BL/6 mice was apparently increased by diseases associated with A. cantonensis infection.(Chung, *et al* ,2010**)**

camels (Camelus dromedarius) infected with Trypanosoma evansi finding the activity of antioxidant enzymes (SOD and GSH) significant reduce while were no significant difference in CAT activity.(Saleh, *et al*,2009)

Trypanosoma evansi in dromedary bulls causes severe damage to testicular tissue and reduces reproductive hormone levels, resulting in severe morphological abnormalities in sperm due to oxidative stress. Infected Camels infected with Toxoplasma gondii finding that activity of antioxidant enzymes (SOD and GPX) was significant decreased in the blood samples of infected animals camper to uninfected animals.(Amin, *et al* ,2020)

camel infected with cystic echinococcosis of the liver. The index of serum lipid peroxidation, which was measured by the level of malondialdehyde (MDA), was significantly higher in the parasitized group than in the healthy control group. But the parasitized group had significantly lower levels of serum total antioxidant status (TAS) and erythrocyte glutathione peroxidase (GPx). Serum zinc levels were much lower in camels with liver cystic echinococcosis than in healthy camels. in camels, parasitic infection, it was a strong link between MDA, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), and bilirubin. On the other hand, MDA had a negative relationship with packed cell volume (PCV), serum albumin, and zinc. From this study, it was determined that oxidative stress is

linked to cystic echinococcosis in camels. The oxidative stress that happens as a result seems to damage hepatocytes, change trace elements, and destroy erythrocytes.(Heidarpour, *et al* ,2012)

Mongolian gerbils experimentally infected with Babesia divergens showed significant decrease in total antioxidant capacity, as indicated by lowered glutathione and catalase levels, increased production of nitric oxide-derived products (nitrite/nitrate) and malondialdehyde, and increased lactic acid dehydrogenase activity and protein carbonyl content in the liver.(Dkhil, *et al* ,2013)

Rapid release of)ROS and NO(plays a critical role in the fight against parasitic infection, but it also causes oxidative injury, resulting in tissue damage and the pathophysiology of the disease , Damage to the intracellular lysosomal membrane caused by oxidative stress is followed by apoptosis or necrosis (Alajmi,*et al* ,2019).

Increased lipid peroxidation is linked to decreased function of the defense system preventing tissue damage from free radicals in parasitic infection seropositive individuals and animals (Atmaca, *et al* ,2015)

5.5.Markers of organ function

5.5.1.Markers of liver function:

GPT levels were not significantly influenced by infections status. However, lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected). GOT levels were not significantly influenced by reproductive status. However, lactating mice (infected only) had significantly greater (higher

compared to the non-reproducing mice (infected and uninfected). ALP levels were not significantly influenced by infections status and reproductive status. The activities of both AST and ALT are wonderful indicators of hepatocellular damage. Normally, these enzymes can be found in the liver as well as in other tissues. There, they play a role in the metabolic process of energy that involves the transamination of amino acids. In the event of cellular injury, however, AST and ALT may release into the general circulation, resulting to increased activity (Adeyemi &Akanji,2011).

Toxoplasmosis is known to carry the risk of developing a number of complications, one of which is hepatic damage (Viranuvatti,1987). These complications include round cell infiltration in the portal sections of the liver, cholestasis, enlarged endothelial cells, and localized necrosis of liver cells. Moreover, the protein fractions of AST and ALT differed according to the degree of inflammation that was brought on by the toxoplasma infection. In keeping with the findings of (Limdi., Hyde,2003 ,Mahmood ,Dawood ,2012) is the observation that there was a substantial elevation in the level of liver enzymes in Toxoplasma positive patients compared to Toxoplasma negative individuals in this investigation. Moreover, the serum ALP activity of patients who tested positive for Toxoplasma was found to be considerably higher than that of individuals who tested negative for Toxoplasma. As hepatic ALP is known to be present on the canalicular and luminal domain of the bile duct epithelium (Limdi &Hyde,2003),

this finding could be explained by the presence of T. gondii in the cells of the bile duct. An damage to the hepatocytes that is caused by malaria may emerge as a considerable elevation in the serum levels of the enzymes

alkaline phosphatase, aspartate transaminase, and alanine transaminase (AST, ALT and ALP) (Al-Salahy, *et al* ,2016) .

It is not entirely clear what causes hepatic dysfunction; however, a reduction in portal venous flow as a result of micro occlusion of portal venous branches by parasitized erythrocytes, intrahepatic cholestasis as a result of reticuloendothelial blockage and hepatic microvilli dysfunction, suppression of bilirubin excretion as a result of parasitaemia or endotoxemia or metabolic.(Fabbri,2013)

Mice infected with *Toxoplasma gondii* in liver showed finding Liver functions estimated by and AST and ALT were significantly increased in T. gondii-infected mice compared with the control group. (Alajmi,*et al* ,2019)

camels with trypanosomiasis had greater mean serum concentrations of GPT, GGT, and GOT than healthy camels. All these findings indicate that T. evansi can cause reproductive failure and fertility.(Amin, ,*et al* ,2020)

5.5.2.Markers of Brain function:

In our current study, BDNF levels were not significantly influenced by infections status. However, BDNF levels were significantly influenced by reproductive status. lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected), (BDNF), a member of the neurotrophin protein family, is an important modulator of neurotransmitter release and synaptic plasticity (Luhder ,*et al* ,2013, Zuccato& Cattaneo , 2009) and has been hypothesized to play a role in the neuroprotective mechanisms of some MS therapies (Azoulay , *et al* ,2005, Jones ,*et al* ,2010, Thöne , *et al*, 2012).

Correale and Farez could demonstrate beneficial immunomodulation by helminths in humans in an observational study of relapsing-remitting multiple sclerosis (RRMS) patients with community-acquired gastrointestinal infections (Correale & Farez, 2007).

They demonstrated that B cells isolated from these helminth-infected MS patients produced greater amounts of BDNF *in vitro* compared to those of normal subjects (Correale , *et al* ,2008)

Especially the BDNF production of immune cells in inflammatory brain lesions is discussed to be a result of body's compensatory neuroprotective mechanism. In line with this understanding is the increase of BDNF levels during relapses in MS (Fleming ,*et al* ,2011) and in stimulated immune cells of patients with higher inflammatory activity in the white matter (Weinstock-Guttman, *et al*,2007).

Neurotrophins, such as BDNF, are involved in adaptive brain plasticity and their expression is modified as a result of stressful challenges (Cirulli and Alleva, 2009). Recent data suggest that, in addition to acting as possible repair mechanism, they might directly participate in the physiological response to a stressor. In particular, it has been hypothesized that BDNF might participate in the hippocampus-hypothalamus-pituitary-adrenal axis response to stress since this neurotrophin increases rapidly following stressful challenges in all these regions (Smith *et al*., 1995; Tapia-Arancibia *et al*., 2004; Cirulli and Alleva, 2009).

Together with the hippocampus, the hypothalamus is also the brain structure that contains the highest BDNF mRNA and protein levels (for a review see Tapia-Arancibia *et al*., 2004).

BDNF mRNA is expressed in the paraventricular nucleus of the hypothalamus (PVN) and anterior and neurointermediate lobes of the pituitary gland, areas important in mediating the endocrine response to stress (Smith *et al*., 1995; Tapia-Arancibia *et al*., 2004).

In this study we found that the effects of a repeated swimming stress procedure on BDNF protein levels were region-dependent and were modulated by early manipulations, such as CR. The most striking effect was the high levels of this neurotrophin found in the hypothalamus, a brain region which represents the final common pathway integrating a number of stress inputs. It has been suggested that BDNF could participate in the stress response. Indeed, there are data to show that in the parvocellular portion of the PVN, neurons coexpress BDNF with CRH while in the magnocellular portion BDNF is coexpressed with vasopressin (Tapia-Arancibia *et al*., 2004).

BDNF is a potent stimulator of neuropeptide synthesis in hypothalamic neurons in vitro. In particular, BDNF might play an important role as co-secretagogue of CRH and vasopressin, themain regulators of ACTH secretion (Tapia-Arancibia *et al*., 2004).

This might explain the meaningful correlation we found between overall CORT output on the last day of the stressing procedure and BDNF protein levels measured in the whole hypothalamic region. It has also been suggested that, in the hypothalamus, BDNF could help to re-establish the hormonal pool (Givalois *et al*., 2004; TapiaArancibia *et al*., 2004).

Indeed, increased levels of BDNF were found in the NH and H groups following the repeated stress procedure. Thus, this neurotrophin could

contribute – likely through increased hypothalamic neurohormones synthesis – to replenish the cellular compartments, once they have been exhausted by a strong demand. This process might help neurons to sustain the demand underlying the stress response. Thus, compared to NH and H groups, CR subjects, which show increased basal BDNF levels, might be endowed by a greater capacity to react to stress and maintain neuroendocrine activity in the face of repeated stimulation. Apart from very traumatic situations, the brain shows some resilience to stress due to its ability to adapt through plastic changes. BDNF might act as a plasticity mediator enhancing this trait which seems to be crucial in adaptive processes. Changes in hypothalamic BDNF levels might thus represent another mechanisms through which early experiences could exert long-term effects on the stress axis, in addition to well described changes in GR (Meaney *et al*., 1989; Plotsky and Meaney, 1993).

A greater reactivity of the HPA axis under conditions of stress might indeed be adaptive for individuals living in crowded conditions, as it would be expected in the case of subjects raised in a communal nest due to increased competition for space and resources. Compared to previous studies here we found no change in hippocampal BDNF levels, which might depend upon the peculiar CR protocol used. By contrast, lower BDNF levels characterized the striatum of the CR group. It has been previously shown that selective, viral knockdown of BDNF in the mesolimbic dopamine pathway can obliterate most of the effects of repeated stress on gene expression within this circuit, with similar effects being produced by chronic treatment with antidepressants. These results have established an essential

role for mesolimbic BDNF in the complex pathophysiology of depression, in addition to the hippocampus and hypothalamus (Berton *et al*., 2006).

In neuronal tissues, BDNF participates in neurogenesis, promoting neurite branching, stabilizing nerve endings, affecting glutamatergic and GABAergic neurotransmission, and has neuroprotective effects associated with its high-affinity binding to tropomyosin-receptor kinase B (TrkB) BDNF ensures the proper functioning of the hippocampus and neocortex. Some authors suggest that abnormal BDNF levels might be due to the chronic inflammatory state of the brain in certain disorders, as neuroinflammation is known to affect several BDNF-related signaling pathways , Brain pathologies are usually associated with a downregulation of BDNF, resulting in reduced levels in the brain and blood , BDNF is critical for neuron survival after injury. It is a neurotrophin that may be produced following inflammatory stimuli as a compensatory mechanism to minimize neuronal damage. (Łanocha-Arendarczyk, *et al* ,2022).

Higher concentrations of NGF have been seen in the hippocampus as a result of brain injury, in part mediated by the effects on astrocytes of pro-inflammatory mediators and cytokines produced by immune cells (Machaliński B., *et al*,2012). Following a brain injury, NGF levels significantly increase IL-8, which stimulates the secretion of this protein in astrocytic tissue cultures (de Sousa Aarão,*et al* ,2018).

There is much less research on the role of NT-3 and NT-4 in the processes occurring in the brain. Neurotrophin-3 promotes the survival of neurons and the repair of nerves (Cong,*et al*,2020, Yan, *et al* ,2021) have shown that NT-3 probably binds to BDNF to regulate neurogenesis and

nerve survival. Neurotrophin-3 also potentiates the neurogenerative effects of NGF and BDNF following a CNS injury (Lin *et al* ,2021).

Neurotrophin-4 plays an important role in the development of the nervous system. NT-4 has a similar role to that of BDNF—it controls the survival and differentiation of vertebrate neurons (Machaliński ., *et al*,2012, Lin.,*et al* ,2021).

The activity of neurotrophins has been observed in some protozoan infections. To date, neurotrophin concentrations have been examined in toxoplasmosis (Cordeiro.,*et al* ,2017), cerebral malaria (Linares , *et al* ,2012, McDonald ,*et al* 2017), leishmaniasis (Portes., *et al* ,2016) and American trypanosomiasis (Martinelli ,*et al*,2006).

5.5.3.Markers of heart function:

heart function measured by Brain Natriuretic Peptide (BNP) , BNP levels were not significantly influenced by infections status. However, BNP levels were significantly influenced by reproductive status. lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected).,the main reason was due to (BNP) is a cardiac neurohormone biomarker that is secreted from the ventricles when they are under increased pressure and stress. (Maisel AS, *et al* 2002).

Natriuretic peptides are peptide hormones mostly released by the heart muscles in response to increased volume status and wall stress, which are key elements in cardiovascular physiology (Okamoto , *et al*,2019).

BNP is a commonly measured natriuretic peptide, which is released in conditions that leads to volume expansion and increased myocardial wall pressure. The physiological functions of BNP include relaxation of vasomotor tone, inhibition of sympathetic activity, reduction in cardiac preload, increase in renal blood flow (LR., *et al*,2009)

heart function measured by Troponin (TN) , CTn levels were not significantly influenced by infections status. However, CTn levels were significantly influenced by reproductive status. lactating mice (infected and uninfected) had significantly greater (lower compared to the non-reproducing mice (infected and uninfected). troponin (cTn) is released and is transiently increased in the blood following an acute myocardial infarction (AMI) and other types of acute myocardial injury. The cTns are the preferred biomarkers for the laboratory diagnosis of AMI (Roffi 2016), due to their excellent cardiac specificities, the fact that healthy individuals have only low levels (Cardinaels ,*et al*. 2012),

The (CTn) binds with finite affinity to thin filaments of the sarcomere (Shiraishi *et al*. 1992) . Although release is immediate after membrane damage, the washout of (CTn) is slowed when compared to other cardiac injury biomarkers due to what has been called ‘the trapping effect’ (Starnberg et al. 2014).

This is especially true when there are large volumes of the myocardium that become necrotic, such as after a transmural AMI. These types of insults result in sustained elevations of cTn for days and sometimes weeks (Katus et al. 1991, Laugaudin et al. 2016).

The delayed washout of (CTn) is in contrast to that of cytoplasmic cardiac injury biomarkers , such as lactate dehydrogenase (LDH), creatine kinase (CK) (Katus *et al*. 1989), CKMB (Katus *et al*. 1987) and myoglobin (Sylven and Bendz 1978), which have no affinity for cardiac tissue. Thus, if cardiac tissue is crushed in warm plasma, myoglobin and Tn are released immediately but the washout of Tn takes more time, due to its binding to the insoluble sarcomere. Starting within a few hours, the necrotic cardiac tissue induces local inflammation and accumulation of neutrophils and macrophages that digest damaged tissue, forming granulation tissue and then a stable scar (Pfeffer and Braunwald 1990).

During this later process, CTn release is likely, largely due to degradation of myofibrils. Many of these other proteins, such as CK, are locally degraded and not detected by conventional clinical assays. Consequently, even once blood flow is re-established, they no longer can be appreciated (Clark *et al*. 1978).

early timely limited CTn increases may be the result of scattered cardiomyocyte necrosis and/or apoptosis, or theoretically even reversible cardiomyocyte injury. Prolonged CTn elevations (>24 h), however, may be due to slow washout from local myocardial injury or the delayed necroptosis following an ischaemic event in both granulation tissue and in the remote myocardium undergoing remodelling.(Hammarsten, *et al*,2018).

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1.Parasitic infection in lactating mice had no significant differences on Physiology traits of reproductive performance (litter size, litter mass, and pup mass) compared to the uninfected lactating mice.

2.Parasitic infection in lactating mice could be associated with increase oxidative damage measured in different tissues, indicating that this design could expand our understanding on the role of ecological cost in the context of oxidative stress theory for driving the trade –off between reproduction and somatic protection.

3. Most of the antioxidants markers across different tissues were not influenced by lactating and patristic infection and also the direction of significant changes was tissue –dependent.

4-Parasitic infection had significant effects on the markers of organ functions measured in different tissues and the direction of its effects was tissue and marker-dependent

5- Some of organ function markers measured in different tissues was significantly influenced by reproductive status.

Recommendations

1. Testing the oxidative cost of parasitic infection in lactating animals using other captive species such as rats and rabbits using Echinococcus granulosus (hydatid cyst) as a model.

2. Expand our understanding on the role of ecological cost (parasitic infection) on the oxidative stress during lactation using wild animals.

3- Investigate the potential effects of litter manipulations on the relationship between parasitic infection and oxidative stress using multi-markers across different tissues.

4. Investigate the effects of parasitic infection on the reproductive capacity of pups of mothers that were previously infected.

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APPENDIX

Appendix I

Brain derived neurotrophic facor (BDNF)

Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Mouse BDNF antibody. BDNF present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Mouse BDNF Antibody is added and binds to ACH BDNF in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated BDNF antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Mouse BDNF . The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

1- **Standard** Reconstitute the 120µl of the standard (12.8ng/ml) with 120µl of standard diluent to generate a 6.4ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (6.4ng/ml) 1:2 with standard diluent to produce 3.2ng/ml, 1.6ng/ml, 0.8ng/ml and 0.4ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month .

2- **Wash Buffer** Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved

3.4.2.1.3Procedure

1. All reagents, standard solutions and samples prepared in room temperature before use..

2. Standard solution 50µl was added to standard well.

3. The sample 40µl was added to sample wells and then added 10µl anti-BDNF antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer followed by Incubation at 60 minutes at 37°C.

4. The sealer was removed and washed the plate 5 times with wash buffer by soaking wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

5. Substrate solution A 50µl was added to each well and then added 50µl substrate solution B to each well then plate covered with a new sealer and Incubated for 10 minutes at 37°C in the dark.

6. Stop Solution 50µl was addedto each well, the blue color will change into yellow immediately.

7. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

3.4.2.1.4Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Appendix II

SOD activity determination

1. **Tris buffer (pH 8.0):** was prepared by dissolving 0.258 gm of tris and 0.111 gm of Ethylenediaminetetraacetic acid (EDTA) in dH2O and completing the volume to 100 ml.

2. **Pyragallol solution (0.2 mM):** was prepared by dissolving 0.0252 gm of pyragallol with 10 ml of HCl and completing the volume to 100 ml with dH2O.

Procedure

According to Marklund (1974), reaction mix is consisting of 50 µl crude enzyme extract with 2 ml of tris buffer and 0.5 ml of pyragallol (0.2 mM) which absorbs light at 420 nm. Control solution contains the same materials except for the enzyme extract that was replaced by dH2O. As a blank, dH2O was used. Single unit of enzyme is defined as the amount of enzyme that is capable of inhibiting 50% of pyragallol oxidation. SOD activity was calculated using the following equation (Ma *et al.*, 2009) :

SOD activity (u/ml)= (Vp-Vs)/(Vp*0.5)*(Vt/Vs)*n

Vp=Auto oxidation rate of pyrogallol rate of pyrogallol (control)

Vs= Auto oxidation rate of sample (with enzyme)

Vt=Total reaction volume (ml)

Vs= volume of enzyme used for the assay (ml)

n= dilution fold of the SOD sample

0.5= factor for 50% inhibition

Appendix III

Catalase activity

Catalase activity assay

0.2 ml serum was incubated in 1 ml of substrate (65 μmoL per ml H_2O_2 in 60 mmol/L sodium-potassium phosphate buffer, pH 7.4) at 37 °C for 1 min. The enzyme activity was suspended by adding 1 ml of 32.4 mM ammonium molybdate. The yellow absorption value of the molybdate complex and hydrogen peroxide was measured at 405 nm using a spectrophotometer.

Catalase enzyme activity was calculated according to the following equation:

C.A (KU/l) =(S-B1/B2-B3) * 271

C.A: Catalase activity (KU/l)

S: Sample reading.

B1: Blank 1 reading contained 1.0 ml substrate, 1.0 mL molybdate and 0.2 mL sample.

B2: Blank 2 reading contained 1.0 ml substrate, 1.0 ml molybdate, and 0.2 ml of 60 mmol/L sodium-potassium phosphate buffer, pH 7.4.

B3: Blank 3 reading contained1.0 ml of 60 mmol/L sodium-potassium phosphate buffer pH 7.4, 1.0 mL molybdate and 0.2 ml of 60 mmol/L sodium-potassium phosphate buffer pH 7.4.

Appendix iV

Determination glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was determined according to the method of Hafemann *et al.* (1974).

Principle

The activity of GPx was determined by measuring the decrease in GSH content after incubating the sample in the presence of H2O2 and NaN3.

$H_2O_2 + 2\ GSH \rightarrow \quad 2H_2O + 2\ GSSG$

Procedure

0.1 ml of serum was incubated with 0.1 ml of 5mM GSH, 0.1 ml of 1. 25 mM H2O2, 0.1ml of 25 mM NaN3 and phosphate buffer (0.05 mM, pH 7) in a total volume of 2.5 ml at 37oC for 10 min. The reaction was stopped by adding 2 ml of 1.65 % HPO32- and the reaction mixture was centrifuged at 1500 rpm for 10 min. 2 ml of the supernatant was mixed with 2 ml 0.4 M Na2HPO4 and 1ml of 1mM DTNB. The absorbance of the yellow colored complex was measured at 412 nm after incubation for 10 min at 37oC against distilled water. A sample without the tissue homogenate processed in the same way was kept as nonenzymatic reaction.

Calculation:

$$\text{The residue reduced GSH in test} \quad \text{tube} = \frac{\text{A.test}}{\text{A.STD}} * Conc.of \quad STD$$

Se-dependent glutathione peroxidase activity (µmol of glutathione utilized/min) = Conc. of GSH in STD - Conc. of GSH in test * D.F.

$$\text{Se - GPX activity (µmol of GSH utilized/min)} = \frac{\text{Conc. of GSH in STD - Conc. of GSH in test}}{\text{time(3min)}} * D.F.$$

Appendix V

Estimation of Serum Malondialdehyde (MDA):

Malondialdehyhe was estimated by Thiobarbituric acid (TBA) assay method of Buege & Aust, 1978 on spectrophotometer .

Principle:

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm.

Oxidative damage to protein (Protein carbonyls)

Protein oxidative damage in different tissues of a subsample of lactating mice (N=10 for both lines for each liver and brain tissues) and non-reproductive mice (N=10 for both lines), and mammary gland of lactating MH (N=22) and ML (N=15) was determined by measuring protein carbonyls using the 2, 4-dinitrophenylhydrazine (DNPH) method (nmol protein carbonyls/mg protein; BIOCELL Corporation Ltd., New Zealand). This marker reflects the oxidation of protein by ROS (Stadtman, 2006; Berlett and Stadtman, 1997) and it has been used as a marker of protein damage in a diversity of species (e.g., Selman et al., 2008; Ołdakowski et al.,

2012; Heiss and Schoech, 2012; Archer et al., 2012; Fletcher et al., 2012). Protein quantifications in different samples of tissues were measured using the Bradford assay prior to the oxidative damage assay to determine the amount of sample required.

Appendix VI

Oxidative damage to DNA 8-hydroxy-2- deoxyguanosine (8-OHdG)

DNA damage in liver tissue was detected by measuring the levels of 8-hydroxy-2- deoxyguanosine (8-OHdG). 8-OHdG is widely used as a biomarker of DNA oxidative damage because it is recognised to be one of the main oxidative base modifications, in particular, generated by oxidising effects of hydroxyl radical (Cooke et al., 2000; Kasai et al., 2008).

Reagent Preparation Note:

Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit. Bring all reagents to room temperature (18-25°C) b before use for 30min. Prepare fresh standard for each assay. Use within 4 hours and discard after use. Making serial dilution in the wells directly is not permitted. Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µl for once pipetting. Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

1. Biotin-antibody (1x) - Centrifuge the vial before opening. Biotin-antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of Biotin-antibody + 990 µl of Biotin-antibody Diluent.

2. HRP-avidin (1x) - Centrifuge the vial before opening. HRP-avidin requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-avidin + 990 µl of HRP-avidin Diluent.

3. Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

4. Standard Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1.0 ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 100 pg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µl of Sample Diluent into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (100 pg/ml). Sample Diluent serves as the zero standard (0 pg/ml). Tube S7 S6 S5 S4 S3 S2 S1 S0 pg/ml 100 50 25 12.5 6.25 3.12 1.56 0Centrifuge the sample again after thawing before the assay.

It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.

3. Add 100µl of standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. A plate lay out is provided to record standards and samples assayed.

4. Remove the liquid of each well, don't wash.

5. Add 100µl of Biotin-antibody (1x) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C. (Biotin-antibody (1x) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)

6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash Buffer by aspirating ordecanting. Invert the plate and blot it against clean paper towels.

7. Add 100µl of HRP-avidin (1x) to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.

8. Repeat the aspiration/wash process for five times as in step 6.

9. Add 90µl of TMB Substrate to each well. Incubate for 15-30 minutes at 37°C. Protect from light.

10. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

Note: 1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.

2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added

to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.

4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light. 7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

Appendix VII

Principle Alkaline phosphatase (ALP) :-

catalyze the hydrolysis of 4- nitrophenylphosphate (4-NPP) with the formation of free 4- nitrophenol and inorganic phosphate, acting the alkaline buffer as a phosphate-group acceptor. The reaction is monitored kinetically at 405 nm by the rate of formation of 4-nitrophenol, proportional to the activity of ALP present in the sample.

4-Nitrophenylphosphate + H2O 4-Nitrophenol + Pi This test has been formulated according the standarized method described by DGKC.

Reagent Composition

R1 ALP buffer. DEA buffer 1.25 mol/L pH 10.2, magnesium chloride 0.6 mmol/L. Biocides.

R2 ALP substrate. 4-NPP 50 mmol/L. Biocides.

Reagent Preparation working reagent

Mix 4 mL of R1 + 1 mL of R2. Stable for 5 days at 20-25ºC or 15-30 days at 2-8ºC, depending on the remaining caducity of both reagents. Protect from light.

procedure

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Working reagent	1.0 mL
Sample or control	20 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Incubate for 1 minute and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

Appendix VIII

Mouse Brain derived neurotrophic facor (BDNF)

Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Mouse BDNF antibody. BDNF present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Mouse BDNF Antibody is added and binds to ACH BDNF in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated BDNF antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Rat BDNF . The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

1- **Standard** Reconstitute the 120µl of the standard (12.8ng/ml) with 120µl of standard diluent to generate a 6.4ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (6.4ng/ml) 1:2 with standard diluent to produce 3.2ng/ml, 1.6ng/ml, 0.8ng/ml and 0.4ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month .

2- **Wash Buffer** Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved

Procedure

1. All reagents, standard solutions and samples prepared in room temperature before use..

2. Standard solution 50µl was added to standard well.

3. The sample 40µl was added to sample wells and then added 10µl anti-BDNF antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer followed by Incubation at 60 minutes at 37°C.

4. The sealer was removed and washed the plate 5 times with wash buffer by soaking wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and

wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

5. Substrate solution A 50µl was added to each well and then added 50µl substrate solution B to each well then plate covered with a new sealer and Incubated for 10 minutes at 37°C in the dark.

6. Stop Solution 50µl was addedto each well, the blue color will change into yellow immediately.

7. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Appendix IX

Mouse Brain derived Natriuretic Peptide, BNP ELISA Kit:
Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Mouse BNP antibody. BNP present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Mouse BNP Antibody is added and binds to ACH BNP in the sample. Then

Streptavidin-HRP is added and binds to the Biotinylated BDNF antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Mouse BNP . The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

3- **Standard** Reconstitute the 120µl of the standard (12.8ng/ml) with 120µl of standard diluent to generate a 6.4ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (6.4ng/ml) 1:2 with standard diluent to produce 3.2ng/ml, 1.6ng/ml, 0.8ng/ml and 0.4ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month .

4- **Wash Buffer** Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved

Procedure

1. All reagents, standard solutions and samples prepared in room temperature before use..

2. Standard solution 50µl was added to standard well.

3. The sample 40µl was added to sample wells and then added 10µl anti-BDNF antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer followed by Incubation at 60 minutes at 37°C.

4. The sealer was removed and washed the plate 5 times with wash buffer by soaking wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

5. Substrate solution A 50µl was added to each well and then added 50µl substrate solution B to each well then plate covered with a new sealer and Incubated for 10 minutes at 37°C in the dark.

6. Stop Solution 50µl was addedto each well, the blue color will change into yellow immediately.

7. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Appendix X

Determination of Aminotransferases (ALT) or (GPT) Activity:

GPT activity according to the following reactions:

Alanine + α -ketoglutarate $\rightarrow$ Pyruvate+glutamate

The pyruvate formed is measured in its derivated form, 2,4-dinitrophenylhydrazone at 505nm.

Calculation

number of GPT units/ml in serum were calculated using the standard curve.

Appendix XI

Determination of Aminotransferases (AST) or (GOT) Activity: GOT activity according to the following reactions:

GOT activity according to the following reactions:

L–A spartate + α -ketoglutarate → Oxaloacetic + L–glutamate

The oxaloacetate formed is measured in its derivated form, 2,4-dinitrophenylhydrazone at 505nm.

Calculation

number of GOT units/ml in serum were calculated using the standard curve.

الخلاصة

تمثل نظرية الإجهاد التأكسدي لتاريخ الحياة آلية فسيولوجية تقود المفاضلة بين التكاثر والحماية الجسدية. تدعم البيانات التراكمية من الثدييات البرية هذه النظرية. ومع ذلك ، فإن النتائج من الثدييات المختبرية لا تدعم ذلك. أحد الأسباب المحتملة يمكن أن يكون مرتبطًا بنقص الإجهاد التأكسدي المتزايد بين ثدييات المختبر المرضعة هو تصميم تجريبي يتم استخدامه. ركز تصميم الدراسات السابقة على اختبار الإجهاد التأكسدي بين الحيوانات ذات الحالة الإنجابية المختلفة أو الجهود الإنجابية المختلفة. لقد تم التشكيك في أن التصميم أعلاه قد لا يكون كافيًا لاستكشاف التكلفة المؤكسدة للتكاثر لأن الحيوانات المرضعة قد لا تقتصر على المقايضة الداخلية (التكاليف الفسيولوجية) ، ولكن يمكن تحقيقها من خلال قوى خارجية أخرى مثل زيادة التعرض للحيوانات المفترسة أو العدوى الطفيلية (التكاليف البيئية).

في هذه الدراسة ، اقترحنا تصميمًا جديدًا لاختبار الإجهاد التأكسدي لنظرية تاريخ الحياة عن طريق قياس مستويات أنواع مختلفة من مضادات الأكسدة والأضرار التأكسدية باستخدام أنواع مختلفة من الأنسجة الداخلية في الفئران المرضعة التي تعرضت لعدوى طفيلية ومقارنة بالفئران غير التكاثرية. أصيبت أربعون أنثى من فئران (BALB/C mice Mus musculus) بطفيلي الاكياس المائية (***Echinococcus granulosus***)حيث تم حقن 2000 رؤيس حي (protoscoleces) لكل فار بمنطقة تحت المخلب، ولم تكن 19 أنثى أخرى مصابة. تم إيواء جميع الفئران في قفص (فأر واحد لكل قفص) وتم إعطاء الطعام والماء بكميات كبيرة. نتيجة لذلك ، تم تخصيص 16 فأرة مرضعة و 16 فأرًا غير متكاثرة بشكل عشوائي إلى أربع مجموعات حيث كانت المجموعة الأولى تتكون من 8فئران مرضعات مصابة (LI) ،والمجموعة الثانية من 8 فئران مرضعات غير المصابة (LU) ، والمجموعة الثالثة من 8 فئران غير متكاثرة مصابة بالطفيلي (NRI) و المجموعة الرابعة من 8 فئران غير متكاثرة وغير مصابة بالطفيلي (NRU).

أظهرت كتلة الجسم خلال فترة الاربعين يوما بعد الاصابة فرقا معنويا بين مجموعات الدراسة. في فترة الحمل ، زادت كتلة جسم الأمهات الحوامل بشكل ملحوظ ، وأظهرت كتلة الجسم أثناء الرضاعة فرقا معنويا بين المجموعة المصابة المرضعة والاخرى غير المصابة المرضعة.

أظهر تناول الطعام خلال فترة الاربعين يوما بعد الاصابة فرقا معنويا بين مجموعات الدراسة. خلال فترة الحمل ، ازداد تناول الطعام للأمهات الحوامل بشكل ملحوظ ، وأثناء الرضاعة كان هناك فرق كبير بين المجموعات. اظهر حجم الجراء (LS) عند الولادة (2 يوم بعد الولادة) فرقًا معنويا بين الفئران المصابة بالطفيلي المرضعة (LI) والفئران المرضعة غير المصابة بالطفلي (LU) ، واظهر حجم الجراء (LS) في اليوم الثامن عشر من الرضاعة فرقا معنويا بين الفئران المصابة بالطفيلي المرضعة (LI) والفئران المرضعة غير المصابة بالطفلي (LU).

اظهرت كتلة الجراء (LM) عند الولادة (بعد يومين من الولادة) فرقًا معنويا بين الفئران المصابة بالطفيلي (LI) والفئران المرضعة غير المصابة بالطفلي (LU). اظهرت كتلة الجراء (LM) في

اليوم الثامن عشر من الرضاعة فرقًا كبيرًا أيضًا بين المجموعتين. لم يظهر وزن الجرو (صغير الفار) (PM) عند الولادة (بعد يومين من الولادة) فرقًا معنويا بين المرضعات المصابة بالطفيلي (LI) والفئران المرضعة غير المصابة (LU) ، ولم تظهر كتلة الجرو (PM) في اليوم الثامن عشر من الرضاعة أي فرق معنوي بين الفئران المصابة بالطفيلي المرضعة (LI) والفئران المرضعة غير المصابة بالطفلي (LU).

في يوم الفطام (اليوم الثامن عشر من الرضاعة) تم جمع أنسجة الكبد والقلب والدماغ وتم قياس مضادات الاكسدة (SOD, CAT, GPx) وعلامات الضرر التأكسدي (PC) protein damage و(MDA(lipid damage و (8OHDG) DNA damage .

أظهرت النتائج الخاصة بمستويات مضادات الأكسدة التي تم قياسها في أنسجة الكبد والدماغ إلى أن مستويات SOD و CAT لم تتأثر بشكل كبير بالحالة الإنجابية والعدوى الطفيلية ، بينما انخفضت مستويات GPx في الكبد في المجموعة (NRU) بشكل كبير مقارنةً بـ القيم في المجموعة (NRI). بينما انخفضت مستويات GPx في الدماغ بشكل ملحوظ في المجموعة (LI) مقارنة بالمجموعات الأخرى.

أظهرت مستويات مضادات الأكسدة في أنسجة القلب إلى أن كلا من SOD و GPx لم يتأثروا أيضًا بشكل كبير بالحالة الإنجابية والعدوى الطفيلية ، بينما كانت مستويات CAT أعلى بشكل ملحوظ في المجموعة (LI) مقارنة بـ (NRU).

أظهرت النتائج الخاصة بمقاييس الضرر التأكسدي إلى أن مستويات الضرر (protein and DNA) لم تتغير بشكل كبير عن طريق التكاثر والعدوى الطفيلية المقاسة في الكبد والقلب ، بينما انخفضت مستويات تلف دهون الكبد بشكل كبير في المجموعة (NRU) مقارنة بالمستويات الملاحظة في المجموعة (LI). لم تختلف مستويات تلف الدماغ (الحمض النووي والدهون) بشكل كبير بين المجموعات ، بينما انخفضت مستويات تلف البروتين بشكل كبير في مجموعة (LI) مقارنة بمجموعة (NRU). كانت مستويات تلف دهون القلب أعلى بشكل ملحوظ بين مجموعة (LI) مقارنة بمجموعة (NRU).

إنزيمات وظائف الكبد ، لم تتأثر مستويات GPT بشكل كبير بحالة العدوى. ومع ذلك ، كانت نسبته في الفئران المرضعة (المصابة وغير المصابة) أكبر بشكل ملحوظ (أقل مقارنة بالفئران غير المتكاثرة (المصابة وغير المصابة).

لم تتأثر مستويات GOT بشكل كبير بالحالة الإنجابية. ومع ذلك ، كانت نسبته لدى الفئران المرضعة (المصابة فقط) أعلى بشكل ملحوظ (أعلى مقارنة بالفئران غير المتكاثرة (المصابة وغير المصابة) ، ولم تتأثر مستويات ALP بشكل كبير بحالة العدوى والحالة الإنجابية.

تم قياس وظيفة الدماغ عن طريق قياس مستوى بروتين BDNF ، ولم تتأثر مستويات BDNF بشكل كبير بحالة العدوى. ومع ذلك ، تأثرت مستويات BDNF بشكل كبير بالحالة الإنجابية. كانت

الفئران المرضعة (المصابة وغير المصابة) أعلى بشكل ملحوظ (أقل مقارنة بالفئران غير المتكاثرة (المصابة وغير المصابة).

تم قياس وظائف القلب بواسطة ببتيد الدماغ الطبيعي (BNP) ، ولم تتأثر مستويات BNP بشكل كبير بحالة العدوى. ومع ذلك ، فإن مستويات BNP تأثرت بشكل معنوي بالحالة الإنجابية ، فالفئران المرضعة (المصابة وغير المصابة) كانت أعلى معنويا (أقل مقارنة بالفئران غير المتكاثرة (المصابة وغير المصابة).

لم تتأثر مستويات التروبونين Troponin (TN) بشكل كبير بحالة العدوى. ومع ذلك ، تأثرت مستويات TN بشكل كبير بالحالة الإنجابية. كانت الفئران المرضعة (المصابة وغير المصابة) أكبر بشكل ملحوظ (أقل مقارنة بالفئران غير المتكاثرة (المصابة وغير المصابة).

في الختام ، يمكن أن ترتبط العدوى الطفيلية في الفئران المرضعة بزيادة الضرر التأكسدي المقاس في الأنسجة المختلفة ، مما يشير إلى أن هذا التصميم يمكن أن يوسع فهمنا لدور التكلفة البيئية في سياق نظرية الإجهاد التأكسدي لدفع التبادل بين التكاثر والحماية الجسدية.

وزارة التعليم العالي والبحث العلمي
جـــامـــعـــة بـــــابـــــــــل
كـــــــلـــــيـــة العـــــلـــوم
قـســـم عـــلـــوم الحـــيـــاة

تأثير الاصابة بالأكياس المائية على الجهد التأكسدي في الفئران التكاثرية

رسالة مقدمة الى مجلس كلية العلوم- جامعة بابل كجزء من متطلبات نيل درجة الدكتوراه
فلسفة في العلوم/ علوم الحياة

من قبل

عباس ناصر حسين ادريس الموسوي

باشراف:

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