TOXIC EFFECTS OF ALUMINUM AND CHROMIUM ON SPRAGUE-DAWLEY ALBINO RATS (Rattus rattus)

A Thesis

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By

Ali Kadhum Naama Al-Mosawy
M.Sc. College of Science
University of Basrah
١٩٨٣

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بسم الله الرحمن الرحيم

قَـاْلوُا
سُبْحَاْنكَ لاَ عِلْمَ لَنَااِلاّ مَا عَلَّمْتَنَا اِنَّكَ
اَنْتَ اْلعَليمُ اْلحَكيمُ

صدق الله العظيم

((البقرة ٣٢))

التأثيرات السمية للألمنيوم والكروم على الجرذ الأبيض (Rattus rattus)

أطروحة مقدمة إلى مجلس كلية العلوم-جامعة بابل
وهي جزء من متطلبات نيل درجة الدكتوراه-فلسفة
في
علوم الحياة/الحيوان

من قبل

علي كاظم نعمه الموسوي

ماجستير علوم حياة
كلية العلوم_جامعة البصرة
١٩٨٣

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SUMMARY

Acute and subchronic toxicity of aluminum as aluminum chloride and hexavalent chromium as potassium dichromate were studied separately in male Spraque-Dawely rats. Somatic, reproductive, histochemical, histopathological and immunological parameters were evaluated to investigate their possible alteration due to aluminum or chromium intoxications.

Calculated oral median lethal dose (LD٥٠) of aluminum chloride was to be ٥٥٠ and ٤٣٣.٤ mg(Al)/kg body weight for male and female respectively. Acute toxic symptoms were characterized by depression and cessation of appetite followed by difficult breathing and muscular convulsion.

Calculated LD٥٠ of potassium dichromate was to be ٦١.٥ and ٥٥.٠ mg (Cr(VI))/kg body weight for male and female respectively. Acute toxic symptoms were characterized by depression and cessation of appetite followed by hypersalivation, lacrimation and diarrhea.

Two separate experiments were carried out with oral administration of a single dose (٧٥٪ of LD٥٠) to induce acute toxicity of Aluminum and hexavalent chromium. Twenty four male rats for each experiment were used. Evaluation of the enzymes histochemical activity and changes in lectin mitogenicity were done within ٢٤ hours of toxicity.

Effects of subchronic exposure to aluminum and hexavalent chromium were investigated using ٤٨ male rats for each exposure experiment. Animals were subdivided into ٤ patches of ١٢ rats, first represented as control group, and other three received in drinking water, ١٢٥, ٢٥٠ and ٥٠٠ppm(Al) respectively in case of aluminum exposure experiment and ٥, ١٠, and ٢٠ppm (Cr(VI)) one. Evaluation of enzyme histochemical activity, organo-somatic index (OSI), some reproductive parameters and histopathological changes were done.

Aluminum and hexavalent chromium caused noticeable changes in the OSI of investigated organs and they induce a significant decrease in the relative weight of liver and testis.

Aluminum and chromium (VI) produced significant effects on male rat reproductive system. Sperm counts from rats exposed to aluminum (٧.٦٤٨x١٠٦/g epididymis) and chromium (VI) ٧.١٩٥x١٠٦/g epididymis) were lower than control groups (٢١.٦٤١x١٠٦/g epididymis). Exposure of rats to aluminum and chromium (VI) also significantly increase the sperm abnormality from ٠.٠٣٦٪ in the control groups to (٠.١٦٣-٠.٣٢٠٪) and (١٨.٣٣-٢٧.٣٣٪) in aluminum and chromium treated rats respectively. There was significant decrease in the diameters of seminiferous tubules in exposed

rats and remarkable alteration in the percentage of spermatogenesis elements were observed.

Both acute and subchronic aluminum and hexavalent chromium exposure produced marked histochemical alterations in the activity of SDH and –GPDH in kidney, liver, spleen, muscle and lung. A marked decrease in the activity of SDH was observed in all investigated organs except lungs, where an increase in the activity of SDH occurred, and that may be due to phagocytic activity of alveolar cells. On the other hand, a notable increase in the activity of GPDH was observed in kidney, liver and spleen.

Apparently the histological analysis of liver, spleen and muscle did not evidence any relevant morphological alteration induced by aluminum or hexavalent chromium during ١٢ weeks of treatment. On the other hand, subchronic exposure to ٥٠٠ppm (Al) induced intracytoplasmic vacuolization, inerstitial edema and degeneration of the tubular epithelial cells of kidney. Subchronic exposure to ١٠ and ٢٠ppm(Cr(VI)) caused a chronic bronchitis and interstitial pneumonitis in the lung of rats. Also, both metals produced some morphological alterations in testis of rats as thickening of seminiferous tubules and evidence of early fibrosis.

Lectins suppress the i*nvivo* T-cells mitogenic activity in animals exposed to hexavalent chromium acute toxicity in comparison to controls, and rice lectin showed marked suppression as compared to other lectins.

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LIST OF ABBREVIATIONS

Al	Aluminum
AS	Ascending limb
B.W	Body weight
Bp	Base of pyramid
CD	Collecting duct
Cr	Chromium
CRD	Complete randomized design
CT	Collecting tubule
Cv	Central vein
DCT	Distal convoulated tubule
DS	Descending limb
FG	Fast glygolytic
FOG	Fast oxidative glycolytic
Gl	Glomerulus
GPDH	Glycerophosphate dehydrogenase
LD٥٠	Median lethal dose
MA	Macula densa
Mr	Medullary rays
NPhT	Naso-pharyngeal tube
OSI	Organo-somatic index
Pa	Portal area
PCT	Proximal convoulated tubule
ppm	Part per million
Rp	Red pulb
S.D	Standard deviation
SDH	Succinate dehydrogenase
SO	Slow oxidative
Tb	Trabecula
TNBT	Tetra nitroblue tetrazolium
Wp	White pulb

CERTIFICATION

I certify that this thesis was prepared under my supervision at the Department of Biology, College of Science, Babylon University, in partial requirements for the Degree of Doctorate of Philosophy of Science in Zoology and this work has never been published anywhere.

Signature:	Signature:
Name: Dr. Ibrahim M.S.Shnawa	Name: Dr.Karim H. Rashid
Title: Professor	Title: Professor
Address: Department of Biology College of Science Babylon University	Address: Department of Biology College of Science Babylon University
Date:	Date:

In the view of the available recommendation, I forward this thesis for debate by the Examination Committee.

Signature:

Name: Dr.Karim H. Rashid

Title: Professor

Address: Head of Department of Biology
College of Science
Babylon University

Date:

Examination Committee

We, the examination committee, after reading this thesis and examining the student Ali Kadhum Naama Al-Mosawy, in its content, have found that it meets the standard and requirements as a thesis in fulfillment for the Degree of Ph.D.in Biology/Zoology.

Signature :

Name: Dr. Ibrahim M.S.Shnawa
(Supervisor)
Title : Professor

Signature:

Dr.Karim H.Rashid
(Supervisor)
Professor

Address: Department of Biology
College of Science University Of Babylon

Signature :
Name:Dr. Alaa J.Hassen
(Member)
Title :Assistant Professor
Address: Department of Biology
College of Scienc , Univ.Of Babylon

Signature:
Dr. Assad A.H. Al-janabi
(Member)
Assistant Professor
Department of Pathology(Head)
College of medicine, University of Kufa

Signatue

Name: Abdul-hady Salall M.Al-Abodi
(Member)
Title :Assistant Professor
Kufa Technical Institute

Signature :

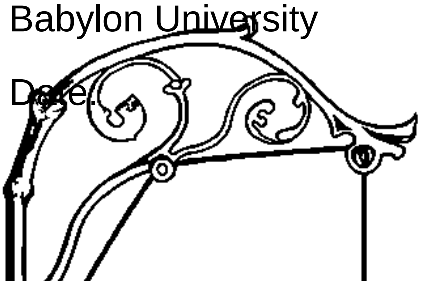
Abdul –Hakim A. Al-Rawi
(Member)
Assistant Professor
College of Science For Girls, Univ. of Baghdad

Signatue
Name: Dr.Ismael K.Ajam
C H A I R M A N
Title : Professor
Address: Department of Biology
College of Scienc , Babylon University

Approved for the College Committee of Graduate Studies.

Signature:
Name: Dr. Oda M.Y. Al-Zamely
Title: Assistant Professor
Address: Dean of College of Science
Babylon University

Date



To all my loved ones,

Who conquer all the hardships that faced me
To them I present the fruit of my effort.

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MOST IMPORTANTLY,I WISH TO THANK GOD FOR GUIDING MY PATH IN LIFE ACCORDING TO HIS WILL

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Ali
٢٠٠٤

١-INTRODUCTION

١-١.GENERAL INTRODUCTION

It is now well realized that environmental problems have increased exponentially in recent decades mainly because of rapid growth in human population and increase demand for several household materials. On one hand technological development has improved the quality of life, on the other hand it has created a number of health hazards. The toxic discharge of the chemicals into air; water and soil reached the food chain in the environment, by entering into the biological system. They disturb the biochemical processes leading to health abnormalities, and in some cases may lead to fatal consequences.

Humans are exposed to an array of chemicals whether as medicines, industrial or environmental chemicals or naturally occurring substances. All substances have potentially harmful effects, which are referred to as toxic or adverse effect. A chemical agent does not produce adverse or toxic effects in a biologic system unless that agent or its biotransformation products reach appropriate sites in the body at a concentration and for a length of time sufficient to produce the toxic manifestation (Klaassen, ١٩٨٦a). Several terms can describe the adverse effects, or toxicity, of chemicals. In general sense, the toxicity of chemical could be defined as the capacity to cause a harmful effect in a living organism .A highly toxic substance will damage an organism if administered in very small amounts; a substance of low toxicity will not produce an adverse effect unless the amount is very large. Thus, toxicity cannot be defined without reference to the quantity (dose) of a chemical to which we are exposed, the way in which this quantity reaches us (inhalation, ingestion, dermal), the duration of exposure (single dose; repeated dose), the type and severity of adverse effects, and the time needed to produce these effects (Loomis, ١٩٧٤). Health effects caused by exposure to toxic substances are usually differentiated based on whether the adverse effect occurs after long-term (chronic exposure) or short-term (acute exposure). Toxicologists usually divide the exposure of animals to chemicals into four categories: acute, sub acute, sub chronic, and chronic (Albert, ١٩٦٥; Hayes, ١٩٧٥; Klaassen ١٩٨٦b). A cute exposure is defined as exposure to a chemical for less than ٢٤ hours, and examples of exposure routes are intraperitoneal, intravenous, and subcutaneous injection, oral intubations, and dermal application. Whereas acute exposure usually refers to a single administration, repeated exposures may be given within a ٢٤hours period for some slightly toxic or practically nontoxic chemicals. An extreme example is acute exposure by inhalation, which refers to continuous exposure for less than ٢٤ hours, most frequently for four hours. Repeated exposure is divided into three categories: Sub acute, sub chronic, and chronic. Sub acute exposure refers to repeated exposure to a chemical for one month or less, sub chronic for one to three months' exposure and chronic for more than three months exposure. These three categories of repeated exposure can be gained, achieved by any route, but most often it is by the oral route, with the chemical added directly to the diet.

١-٢ How Toxins Work (The Receptor Theory)

All living organisms operate through highly integrated sets of biochemical reactions, which are sensitive to conditions including temperature, pH, and the concentration of other chemicals in the system. Some chemicals, such as strong acids and bases, are toxic simply because they denature proteins and dissolve living tissue. Other chemicals, however, exert their toxic effect by binding with specific receptors in cells, thereby disrupting normal biochemical reactions

In multicellular animals, including humans, the body has many finely tuned regulatory systems to ensure things work properly in response to external conditions. This tendency of an organism to maintain normal stability or equilibrium is called homeostasis (Randall *et al*., ١٩٩٧).

Some factors can disrupt homeostasis, such as external physical circumstances (extreme heat or cold) or internal chemical or biological agents. For example, heavy metals, organophosphate compounds, and insecticide affect homeostasis by deactivating an enzyme responsible for the uptake of the key neurotransmitter Acetylcholine. The nervous system becomes over stimulated and induces the activity of sweat glands and the body's muscles to twitch or convulse. It is this loss of homeostasis that produces the symptoms of poisoning or disease. (UEO & EHW, ٢٠٠١ and Zakrzewski, ١٩٩١).

١-٣ The Dose-Response Relationship.

The reaction of an organism to exposure to a toxic substance is called the response. The observed response can be any measurable physiological change such as nausea, blindness, sterility, birth defects, or death or deprived lymph cell activity, that can be evaluated.

In animal studies, the easiest response to measure is the number of deaths in a population of organisms. Because no two individuals of the same species respond to given dosage identically, the dose-response function is expressed in statistical terms. The comparative potency of different substances is often expressed as Lethal Dose Fifty ($LD_{٥٠}$), the dose at which death is observed in ٥٠ percent of the experimental organisms in question (Doull, ١٩٨٠)

١-٤-AIM OF THE STUDY

As far as we know, histochemical evaluation of the dehydrogenase enzymes in rat tissues when exposed to Aluminum and Chromium (VI) has not yet been described.

The aim of the present work is to:

١-Investigate histochemical alterations of Succinate and α-Glyceroldehyde Dehydrogenases activities in kidney, liver, spleen, skeletal muscle and lung.

٢-Evaluate the histopathological changes induced by aluminum and hexavalent chromium in the studied organs.

٣-Determine some parameters of male rat fertility, which included sperm counts, sperm abnormalities and histological observations of testicular tissue

٤-Calculate the Organo-Somatic index of kidney, spleen, liver, lung, testis, epididymes and seminal vesicle.

٥-Evaluation of changes in the lectin mitogenicity in Cr toxicity.

٣-MATERIALS AND METHODS

٣-١ ANIMALS:

Healthy young adult albino Sprague-Dawley rats breed and maintained in animal house of Kufa Medicine College were used in the present experiments. They were fed with standard pellet diet and water ad libidum. A batch of animals was used in each experiment approximately of the same age (١٠-١٢ weeks) and total body weight fall within ٢٠٪ of the mean weight of all animals. The first batch usually served as the control.

٣-٢ CHEMICALS:

ITEM	MANUFACTURE	COUNTRY
Acetone	MERCK	GERMANY
Aluminum chloride	FLUKA	SWITZERLAND
Disodium-α-glycero-٣-phosphate	FLUKA	SWITZERLAND
Disodium hydrogen orthophosphate	KOACH-LIGHT LAB.	GERMANY
Disodium succinate	FLUKA	SWITZERLAND
Eosin stain	REIDLE	GERMANY
Ethanol	BDH	ENGLAND
Ether	BDH	ENGLAND
Formaldehyde	BDH	ENGLAND
Gelatin	DIFCO	U.S.A.
Glycerol	FLUKA	SWITZERLAND
HCL	BDH	ENGLAND
Necrosin stain	BDH	ENGLAND
Phenazine methosulfate	FLUKA	SWITZERLAND
Potassium dichromate	FLUKA	SWITZERLAND
Polyvinyl alcohol (MW ٤٠٠٠٠)	SIGMA	U.S.A.
Sodium azide	BDH	ENGLAND
Sodium dihydgen orthophosphate	FLUKA	SWITZERLAND
Sodium citrate	DIFCO	U.S.A
TNBT	FLUKA	SWITZERLAND

٣-٣ INSTRUMENTS

ITEM	MANUFACTURER	COUNTRY
Analytical balance	SARTORIUS	U.K.
Cryostat, Sakura freezing Micro tom (“Cold tone” model CM-٤١)	SAKURA	JAPAN
Haemocytometer	CHANCE PROPPER LTD	ENGLAND
Incubator	MEMMERT	GERMANY
Liquid nitrogen bottle	BRIGHT INSTRUMENT Co Ltd	U.K
Light microscope with camera	OLYMPUS	JAPAN
Micropipette ٥٠ µ	VOLAC	U.K.
Pediatric nasopharyngeal tubes	BECTON DICKINSON	SPAIN
Oven	MEMMERT	GERMANY
Top balance	SARTORIUS	U.K.
WATER BATH	TAFESA HANNOVER	GERMANY

٣-٤ LD٥٠ DETERMINATIONS:

LD٥٠ experiments were carried out on separated sets of ٣٦ adult males (weighing١٩٦-٢٣٥gm (٢١٣±١٢.٨) and ٣٦ females (weighing approximately ١٧٦-٢٠١ gm (١٨٩±٧.١) rats of ١٠ weeks of age for each aluminum and chromium.

Each set was divided into six groups of ٦ animals each selected for each dose level chosen. ١٢-١٦ hours prior to dosing rats were fasted. After fasting, rats were individually weighed. Whole body weight was taken and individual doses were calculated based on the weight of animal. Aqueous solution of aluminum chloride or potassium dichromate in single dose was administered orally by intragastric intubations (using pediatric nasopharyngeal tubes gage ٨).

The ranges of single oral doses which were used in the determination of LD٥٠ of aluminum chloride are ٣٠٠-٧٠٠ mg Al/kg and ٢٠٠-٦٠٠ mg for Al/kg for male and female respectively and LD٥٠ of potassium dichromate are ٤٠-٩٠ mg Cr./kg and ٣٠-٨٠ mg Cr/kg for male and female respectively. The LD٥٠ of both Al and Cr (VI) were determined according to the formula employed by Behrens and Karber (١٩٥٣):

$$LD_{٥٠} = \text{Biggest dose} — \Sigma (a \times b)/n$$

Where
a= differences in doses between two successive groups.
b= the mean of dead animals in two successive groups.
n= the number of animals in the group.

٣-٥ ACUTE TOXICITY EXPERIMENTS:

Six groups each with ٤ male rats, weighing ٢٠٢-٢٢٩(٢١٦.٧±٨.٠٤) and aged ١١-١٢ weeks, were selected to study the acute toxicity of chromium and aluminum. Five groups received orally a single dose of ٧٥٪ of oral LD٥٠. The sixth group (control animals) received an equal volume of distilled water. Distilled water was used as the vehicle since both potassium dichromate and aluminum chloride are highly soluble in water .The concentration was adjusted to have a maximum ingestion volume of ١-٢ ml. Three rats were sacrificed at ٠, ١, ٣, ٦, ١٢, and ٢٤ hours after oral administration. Two pieces of tissue were cut immediately from kidney, liver, spleen, lung and skeletal muscle; one piece was frozen in liquid nitrogen for later histochemical study and another piece in formalin for histological investigations. This study was conducted on survivors and the animals died earlier to predetermine time intervals were not taken into account.

٣-٦ SUB-CHRONIC TOXICITY EXPERIMENTS.

Two experiments were performed to conduct the sub-chronic toxicity of Al and Cr. Each experiment was performed on ٢٤ males rats divided into ٨ groups of ٣ animals each.

First experiment was carried out in male rats of ١٠-١٢ weeks of age and weighing ١٨٤-٢٢٧gm (٢٠٣.٨±١٩.٢٣). Aluminum chloride was administered for three months in drinking water at doses of:

-- ٠ppm for two groups as control
-- ١٢٥ppm, ٢٥٠, and ٥٠٠ ppm, each for two groups of animals.

Second experiment was carried out in male rats of ١٠-١٢ weeks of age and weighing ١٩٢-٢٢٩gm (٢١١.٢ ±١٧.٦). Potassium dichromate was administered for three months in drinking water at doses of:

-- ٠ppm for two groups as control
-- ٥, ١٠, and ٢٠ppm, each for two groups of animals.

At the end of the ١st, ٢nd and ٣rd month of exposure initiation, ٤ rats from each dose level were weighed and sacrificed under ether anesthesia. Organs in a choice were quickly excised, weighed and prepared for tissue processing.

٣-٧ ORGANO-SOMATIC INDEX (OSI)

The weights of organs of respective groups of animals were recorded at the end of sub chronic exposure period. From these values the organo-somatic index was calculated by the following formula:

$$OSI = \frac{\text{WEIGHT OF THE ORGAN (g)}}{\text{TOTAL BODY WEIGHT (g)}} \times ١٠٠$$

٣-٨ TISSUE PROCESSING

For male fertility parameters study; testes, epididymis and seminal vesicles were excised , removed access fat around them and weighed. Right testes, epididymis and seminal vesicle were fixed in ١٠٪ formaldehyde for later histological preparation, the left epididymis put in normal saline for later preparation. Left epididymis was teasing apart into small pieces and put in to manual homogenizer to release the sperm.

For histochemical analysis, fresh tissue pieces from each kidney, liver, spleen, lung, sartorius muscle and abdomen muscles were cut out rapidly, labeled and immediately frozen and stored in plastic tubes in liquid nitrogen bottle until use. Cryostat sections ١٠µ thick were cut at -٢٠C. Other routine histological examinations based on tissue pieces preserved in ١٠٪ formaldehyde were carried out.

٣-٩ REPRODUCTIVE PARAMETERS DETERMINATIONS

Male rats involved in sub chronic experiments were used to study effects of aluminum and hexavalent chromium on male fertility. At the end of the administration period, the animals were sacrificed and sperm counts, organ weight and histopathological changes in the reproductive organs were recorded. The testis, epididymis and seminal vesicles were dissected out from each male and immediately placed in normal saline. The organs were carefully made free from surrounding fat and connective tissue and weighed up to the nearest ١ mg on an electronic balance. The organs from one side of each animal were fixed in Bouin fluid (replaced after ٢٤ hour by ١٠٪ formalin) for histological study, whereas epididymes from the other side was separated and used for sperm counting.

٣-٩-١ SPERM COUNTS

Sperms were counted according to Seed *et al*., (١٩٩٦). Epididymis was weighed, cut apart into small pieces to release the sperm, homogenized and transferred to a test tube containing ٠.٢m sperm counting solution and ٩.٨ normal saline. After mixing the spermatozoa were counted by using haemocytometer slide under (٤٠X) object lens of light microscope. Sperm number (N) calculated in ٨٠ small squares of haemocytometer, were used to measure sperm counts of rat by using the following equation:

Sperm counts=N/٨٠ X ٤٠٠ X ١٠ X ١٠ X ١٠٠٠.

Where values in equation represents:

٨٠=counted small field of haemocytometer.
٤٠٠=total area of small fields.
١٠=depth of slide.
١٠=dilution rate.
١٠٠٠=to convert sperm number in ١ cc.

٣-٩-٢ SPERM ABNORMALITY

Sperm suspension was first pipetted onto the glass plate and dried. The morphologic characteristics of spermatozoa were evaluated by air dried, stained smears using light microscope (Seed, et al. ١٩٩٦). Minimums of ٥٠٠ spermatozoa were observed per rat and the level of sperm abnormality was calculated.

٣-٩-٣ SEMINIFEROUS & EPIDIDIMALTUBULES DIAMETERS

Randomly measurements of ٥-١٠ tubules from testes and epididymis were taken by using ocular micrometer (calibrated with stage micrometers).

٣-٩-٤ PERCENTAGES OF SPERMATOGENESIS ELEMENTS.

According to (Seed, *et al*. ١٩٩٦), percentages of spermatogenesis cells (spermatogonia, spermatocytes, spermatids, and spermatozoa) were determined for ١٠-١٢ tubules from each sample.

٣-٩-٥ SPERM COUNT SOLUTION:

Sperm count solution was prepared according to (Seed, *et al*. ١٩٩٦) as following:

Add ٠.٢ml eosin stain solution to ٩.٨ml formal saline.

١-Eosin stain solution prepared by dissolved ١gm eosin in ١٠٠ml of sodium citrate solution (٦gm sodium citrate dissolved in ٢٠٠ml D.W.).

٢-Formal saline prepared by adding ١٠ ml of ٤٠٪ formalin to ٩٠ ml normal saline.

٣-١٠.ENZYME HISTOCHEMICAL PROCEDURES

Succinate dehydrogenase (E١.٣.٩٩.١) and α-glycerol-phosphate (E١.١.٩٩.٥) dehydrogenases were histochemicaly demonstrated in the present study by using tetrazolium salt method according to Van Noorden *et.al.* (١٩٨٩), and Stoward & Van Noorden (١٩٩١). Histochemical determinations of SDH and α–GPDH activity were used to estimates oxidative and glycolytic energy supply. To apply a dehydrogenase method it is convenient to make stock solution of the tetrazolium salt and of the substrate solutions.

The incubation procedures for the demonstration of enzyme activities can be performed in different ways. Incubation media containing polyvinyl alcohol was used (Van Noorden and Frederiks, ١٩٩٢). Low concentration of polyvinyl alcohol allow colloidal dispersions of sufficiently high concentrations of compounds which are not very water soluble, such as tetranito blue tetrazolium (TNBT) to be maintained in incubation media (Kulger *et. al*., ١٩٨٨).

٣-١٠-١ Preparation of polyvinyl alcohol-containing medium.

١-Dissolve ١٨ gm polyvinyl alcohol (MW ٤٠٠٠٠), in ١٠٠ ml ١٠٠mM phosphate buffer (pH ٧.٢), stirring and heating in water bath until a clear solution is obtained.

٢- Store the clear solution at ٦٠C^{o} in air tight vials.

٣- Cool a desired volume of the solution to ٣٧C^{o} before incubation.

٣-١٠-٢ Stock substrate (١M succinate) solutions.

١-Dissolve ٦.٧٥ gm of Disodium succinate in ٨ml distilled water

٢-Neutralize to pH ٧.٠ with *N* HCL and made up to ١٠ ml with distilled water.

٣-١٠-٣ Stock substrate (١M α -glycero-phosphate) solution .

١-Dissolve ٣.١٥ gm of Disodium- α -glycero-٣-phosphate in ٨ml distilled water

٢- Neutralize to pH ٧.٠ with *N* HCL and made up to ١٠ ml with distilled water.

٣-١٠-٤ Stock ٥ mM TNBT solution.

Dissolve ٥ mg TNBT in ٤٠ mM ethanol by gentle heating.

_٣-١٠-٥ Stock ٥mM sodium azide solution.

Dissolve ٣٣mg sodium azide in ١m distilled water.

٣-١٠-٦ Stock ٠.٢mM phenazine methosulfate.

Dissolve ٨-ml phenazine methosulfate in ١ ml distilled water.

٣-١٠-٧ Final incubation medium.

The final incubation medium consists of:
١- ١٠m ml polyvinyl alcohol containing buffers.
٢- ١٠m ml of substrate stock solution.
٣- ١٠m ml of ٥mM sodium azide.
٤- ١٠m ml of ٠.٢mM phenazine methosulphate.

٣-١٠-٨ phosphate buffer solution (pH ٧.٢)

Preparation of stock solutions.

Stock A: ٠.٢M Sodium dihydrogen orthophosphate (MW ١٥٦)
Dissolve ٣.١٢gm of Sodium dihydrogen orthophosphate
In ١٠٠ml distilled water.

Stock B: ٠.٢M Disodium hydrogen orthophosphate (MW ١٤٢)
Dissolve ٢.٨٣gm of Disodium hydrogen orthophosphate
In ١٠٠ ml distilled water.

Dissolve ١٤.٠ml of stock A and
٣٢.٠ml of stock B and
made up to ١٠٠ml with distilled water

٣-١٠-٩ Glycerin jelly

١-Slowly dissolve ١٥gm of gelatin in ١٠٠ml ٠.٢ phosphate buffer (pH ٧.٢) with moderate heat.
٢-When gelatin dissolves add ١٠٠ml glycerol and mix well.
٣-Filter through glass wool whilst hot.

٣-١٠-١٠ Tetrazolium salt method for the demonstration of dehydrogenase.

١-Mount cryostat sections on cover slips.
٢-Prior to carrying out the reactions, fresh unfixed cryostat sections were treated with cold acetone (±٠C for ٥ min) in order to remove lipids.
٣-Cover sections with final incubation medium.
٤-Incubate for ٣٠ min at ٣٧C.
٥-Rinse thoroughly with ٠.١ M phosphate buffer (pH ٥.٣) at ٦٠C.
٦-Post treats in ٤٪ formaldehyde for ١٠ min.
٧-mounted in glycerin jelly.
٨-Control incubation was carried out in the absence of substrate.
٩-Examine sections immediately.
١٠. Light micrographs of the section were acquired using Olympus camera attached to an Olympus microscope.

٣-١١.HISTOLOGICAL PREPARATIONS:

A piece of tissues intended for histological examination was fixed in ١٠٪ formalin, embedded in paraffin, sectioned and stained with Mayer's haematoxylin and aqueous eosin, according to Bancroft and Stevens (١٩٨٢).

٣-١٢. CHROMIUM IMMUNOTOXICITY:

Lectin solutions were separated and partially characterized through hemaglutination, biurate reaction and carbohydrate binding. ٠.٠٥ml of lectin solution (Table ١) was injected to chromium treated and control rats (in acute toxicity experiments) via s.c., of footpad. Redness and indurations of pad skin was measured up to ١٨ hr post injection (Christe *et al*. ٢٠٠٠ and Al-Mashta, ٢٠٠٣).

TABLE (١): The origin of plant lectins

SCIENTIFIC NAME	COMMON NAME	DESIGNATION
Cucumis melo	Musk melon	B
Oryza sative	Rice (ambur)	R
Panicium miliaceum	Dukhen	D

٣-١٣ STATISTICAL ANALYSIS:

Statistical significance of differences in weight before and after each treatment was analyzed by student t-test. Male fertility data were analyzed by Complete Randomized Design (CRD) and by using LSD to determine significance of results at P<٠.٠٥.

Study protocol

LD50 DETERMINATION

١-Animals:

Four patches, each of six groups, each of the six was male or female, each group received one level of dose.

٢-Chemical: Aqueous solution of Aluminum chloride (as aluminum mg/kg B.W) and of potassium dichromate (as Chromium mg/Kg B.W).

٦ male	٦ male	٦ male	٦ male	٦ male	٦ male
٣٠٠mg	٤٠٠mg	٥٠٠mg	٦٠٠mg	٧٠٠mg	٨٠٠mg

ALUMINUM

٦ female	٦ female	٦ female	٦ female	٦ female	٦ female
٢٠٠mg	٣٠٠mg	٤٠٠mg	٥٠٠mg	٦٠٠mg	٧٠٠mg

ALUMINUM

٦ male	٦ male	٦ male	٦ male	٦ male	٦ male
٣٠mg	٤٠mg	٥٠mg	٦٠mg	٧٠mg	٨٠mg

CHROMIUM

٦ female	٦ female	٦ female	٦ female	٦ female	٦ female
٢٠mg	٣٠mg	٤٠mg	٥٠mg	٦٠mg	٧٠mg

CHROMIUM

Route of administra: Single oral dose via intragastric Pediatric Naso-Pharyngeal Tube (NPhT).

Observations

١-Clinical sign of toxicity.
٢- No. Of dead animals and time of death.
٣- Time of recovery.

ACUTE TOXICITY EXPERIMENTS

Two separate experiments were carried out for induced acute toxicity of

Aluminum & Chromium.

Animals

٢٤ male for each experiment

Dose

٧٥٪ of LD٥٠,

Route of administration

Oral single dose via NPH tube

Sacrificing time & Collection of tissue

٠,١,٣.٦.١٢,and ٢٤ hours after chemical administration .

Experiment analysis

١-Histochemical evaluation
٢-Evaluation of changes in lectin mitogenicity

SUBCHRONIC TOXICITY EXPERIMENTS

Two separate experiments were carried out for induced Sub-Chronic toxicity of Aluminum & Chromium.

Animals

٣ male	٣ male	٣ male

٣ male	٣ male	٣ male

CONTROL

٣ male	٣ male	٣ male
٣ male	٣ male	٣ male

١٢٥ppm Al

٣ male	٣ male	٣ male
٣ male	٣ male	٣ male

٢٥٠ppm Al

٣ male	٣ male	٣ male
٣ male	٣ male	٣ male

٥٠٠ppm Al

A L U M I N U M

٣ male	٣ male	٣ male

٣ male	٣ male	٣ male

CONTROL

٣ male	٣ male	٣ male
٣ male	٣ male	٣ male

٥ppm Cr

٣ male	٣ male	٣ male
٣ male	٣ male	٣ male

١٠ppm Cr

٣ male	٣ male	٣ male
٣ male	٣ male	٣ male

٢٠ppm Cr

C H R O M I U M

Route of administration

Daily exposure in drinking water for ٣ months

Sacrificing time & Collection of tissue

At the end of ١st, ٢nd and ٣rd month of Administration.

Experiment analysis

١-Histochemical evaluation
٢-Organo-Somatic Index
٣-Male fertility parameters
٤-Histopathological changes

٤-RESULTS

٤-١GROSS SOMATIC METAL TOXICITY:

٤-١-١ ALUMINUM EFFECT:

Mortalities as a result of oral administration of different successive graded doses of Aluminum (as Aluminum chloride) are showed in table (٢) and (٣) for male and female rats respectively.

All males given ٧٠٠-٨٠٠ mg (Al)/kg body weight, and females given ٦٠٠-٧٠٠ mg (Al)/kg body weight showed marked toxic symptoms and died within ٢٤ hours of administration. Males and females given ٥٠٠-٦٠٠ mg (Al)/kg and 400-500mg (Al)/kg body weight respectively showed same toxic symptoms but lower percentage of mortality. Aluminum chloride in doses ٣٠٠-٤٠٠ mg (Al)/kg body weight of males and ٢٠٠-٣٠٠ mg (Al)/kg body weight of females also produced similar toxic signs but of less severity.

Toxic symptoms were characterized initially by depression and cessation of appetite followed by difficult breathing and muscular convulsion during first hour of administration and persisted up to ١٢h.A recovery condition and starting appetite were recorded after ٢٤ hours of chemical administration in all survived rats.

LD٥٠ limits were ٥٥٠ and ٤٣٣.٤ mg (Al)/kg body weight for male and female respectively.

٤-١-٢ CHROMIUM EFFECT:

Mortalities as a result of oral administration of different successive graded doses of Chromium (as Potassium dichromate) were showed in table ٤ and ٥ for male and female rats respectively.

All males given ٨٠-٩٠ mg (Cr)/kg body weight, and females given ٧٠-٨٠ mg (Cr)/kg body weight showed marked toxic symptoms and died within ١٢ hours of administration. Males and females given ٦٠-٧٠mg and ٥٠-٦٠mg (Cr)/kg body weight respectively showed same toxic symptoms but lower percentage of mortality. Potassium dichromate of the dose ٥٠ mg (Cr)/kg body weight of males and ٤٠ mg (Cr)/kg body weight of females also produce similar toxic signs but of less intensity, while no toxic symptoms were observed in males and females rats that received ٤٠ mg (Cr)/kg and ٣٠mg (Cr)/kg body weight respectively.

Toxic symptoms were characterized initially by depression and cessation of appetite followed by hypersalivation, lacrimation and diarrhea during first hour of administration and persisted up to ٦ hours. Thereafter the animals remained exhausted and showed fine muscular fasciculation up to ١٢ hours. Survived animals showed recovery after this crucial period without any delayed toxic effect and were apparently normal by the end of ٢٤ hours.

LD٥٠ limits were 61.5 and 55 mg (Cr)/kg body weight for male and female respectively.

٤-2 ORGANOSOMATIC INDEX AND METAL TOXICITY:

٤-٢-١ ALUMINUM EFFECT:

Table (٦) showed the effects of aluminum chloride on the organo-somatic index of studied organs (kidney, liver, spleen, testes, epididymis and seminal vesicle) of male rats under three months induced sub-chronic exposure period.

The percentage of changes in the weight of kidney was decreased significantly (P< ٠.٠٥) in rats that received ١٢٥ppm Al, while no significant differences were observed in the rats that received ٢٥٠ and ٥٠٠ppm Al. Rats exposed to ١٢٥ppm, ٢٥٠ppm, and ٥٠٠ppm Al showed a statistically significant decrease in the relative weights of liver (p<٠.٠٥). A decrease in the percentage of changes showed a close relation with level of exposure dose (i.e. animal group exposed to lower dose showed a lower percentage of changes). The relative spleen weights were significantly decreased in the groups exposed to ١٢٥ and ٥٠٠ppm Al for ٣ months, while animals exposed to ٢٥٠ ppm showed no significant effects on the relative weight of spleen. The percentage of changes in the weight of testes were decreased significantly (P<٠.٠٥) in rats that received ٢٥٠ and ٥٠٠ppm Al, while no significant differences were recorded in the rats that received ١٢٥ppm Al. On the other hand, animals exposed to all doses showed slightly, but no statistically significant changes in the weights of epididymis. Exposure to Aluminum resulted in significant decrease in the relative weights of the seminal vesicle of rats that received ٥٠٠ppm for ٣ months only.

Table (٢): LD٥٠ determination parameters of male Spraque-Dawley rats exposed to Aluminum chloride.

LD٥٠ = ٨٠٠ – ١٥٠٠/٦ =٥٥٠.٠ mg (Al)/Kg body weight.

DOSE mg/kg	NO. ANIMALS	NO. DEAD ANIMALS	a	b	a X b	Σ (a X b)
300	6	0	100	0	0	
400	6	0	100	0	0	
500	6	2	100	1.0	100	
600	6	4	100	3.0	300	
700	6	6	100	5.0	500	
800	6	6	100	6.0	600	1500

Table (٣): LD٥٠ determination parameters of female Spraque-Dawley rats exposed to Aluminum chloride.

LD٥٠ = ٧٠٠ – ١٦٠٠/٦ =٤٣٣.٤ mg (Al)/Kg body weight.

DOSE mg/kg	NO. ANIMALS	NO. DEAD ANIMALS	a	b	a X b	Σ (a X b)
200	6	0	100	0	0	
300	6	0	100	0	0	
400	6	2	100	1.0	100	
500	6	5	100	3.5	350	
600	6	6	100	5.5	550	
700	6	6	100	6.0	600	1600

Table (٤): LD٥٠ determination parameters of male Sprague-Dawley rats exposed to potassium dichromate.
LD٥٠ = ٩٠ – ١٧٠/٦ =٦١.٥ mg Cr/Kg body weight.

DOSE mg/kg	NO. ANIMALS	NO. DEAD ANIMALS	a	b	a X b	Σ (a X b)
40	6	0	10	0	0	
50	6	0	10	0	0	
60	6	3	10	1.5	15	
70	6	5	10	4.0	40	
80	6	6	10	5.5	55	
90	6	6	10	6.0	60	170

Table (٥): LD٥٠ determination parameters of female Spraque-Dawley rats exposed to potassium dichromate.
LD٥٠ = ٨٠ – ١٥٠/٦ =٥٥.٠ mg Cr/Kg body weight.

DOSE mg/kg	NO. ANIMALS	NO. DEAD ANIMALS	a	b	a X b	Σ (a X b)
30	6	0	10	0	0	
40	6	0	10	0	0	
50	6	1	10	0.5	15	
60	6	5	10	3.0	30	
70	6	6	10	5.5	55	
80	6	6	10	6.0	60	150

Table (٦): Effect of Aluminum chloride on Organo-Somatic Index of kidney, liver, spleen, testes, epididymis and seminal vesicle of Sprague-Dawley male rat exposed for three months period.

All values are mean ± S.D of ٤ animals

TISSUE OSI (control)	DOSE (ppm)	ORGANO-SOMATIC INDEX (mean ± S.D)	% CHANGE
KIDNEY ٠.٣٢٤ (±O.٠٢٥)	١٢٥	٠.٢٦١±٠.٠١٥	-١٧.٩٢ *
	٢٥٠	٠.٣١٤±٠.٠٠٣	-٠١.٢٥ N.S
	٥٠٠	٠.٣٢١±٠.٠٠٤	-٠٠.٩٤ N.S
LIVER ٣.٩٧٠ (± ٠.٤٥١)	١٢٥	٣.٣٧١±٠.١٠٦	-١٥.٢١ *
	٢٥٠	٣.٢٠٧±٠.٠٣١	-١٩.٣٤ *
	٥٠٠	٢.٧٩٩±٠.٤٥٣	-٢٩.٦٠ *
SPLEEN ٠.٣٤٠ (±٠.٠٣٨)	١٢٥	٠.٢٨٢±٠.٠١٠	-١٢.١٥ *
	٢٥٠	٠.٣٤٣±٠.٠١٦	+٠٦.٨٥ N.S
	٥٠٠	٠.٢٦٤±٠.٠٢٤	-١٧.٧٥ *
TESTES ٠.٥٣٤ (±٠.٠٤٨)	١٢٥	٠.٤٨١±٠.٠٢٧	-٠٨.٧٣ N.S
	٢٥٠	٠.٤٤٦±٠.٠٢٨	-١٥.٣٧ *
	٥٠٠	٠.٤٤٥±٠.٠١٨	-١٥.٥٩ *
EPIDIDYMIS ٠.٢٢٣ (±٠.٠٣٩)	١٢٥	٠.٢١٨±٠.٠٢٠	-٠٨.٣٦ N.S
	٢٥٠	٠.٢٠٦±٠.٠٠٥	-١٣.٨٠ N.S
	٥٠٠	٠.٢١٤±٠.٠٠٨	-١٠.٤٦ N.S
SEMINAL VESICLE ٠.١٧٣(±٠.٠٣٩)	١٢٥	٠.٢٠٥±٠.٠٣٦	+١٢.٦٣ N.S
	٢٥٠	٠.١٨٣±٠.٠٣٦	+٠٠.٥٥ N.S
	٥٠٠	٠.١١٩±٠.٠١٣	-٣٤.٦١ *

***The significant values as per student's t- test are between control and experimental values at p<o.o٥.**

٤-٢-٢ CHROMIUM EFFECT:

Table (٧) shows the effects of Potassium dichromate on the organo-somatic index of studied organs (kidney, liver, spleen, testes, epididymis and seminal vesicle) of male rats under three months induced sub-chronic exposure period.

Although no significant differences were observed in the percentage of changes in the weight of kidney of all rats exposed to chromium through out the whole period of exposure, a slight increase in the weight of kidney was observed in the group received ٢٠ppm Cr. Rats exposed to ٥ppm, ١٠ppm, and ٢٠ppm Cr showed a statistically significant decrease in the relative weights of liver (p<٠.٠٥) as compared to control rats. The percentages of changes were –٢٠.٤٦, -٢٢.٦٧, and –٢٣.٥٦ at doses of ٥, ١٠ and ٢٠ppm Cr respectively. A decrease in the percentage of changes showed a close relation with level of the exposure dose (i.e.the animal group exposed to a lower dose showed a lower percentage of changes). In all exposed groups of rat, the relative spleen weights were statistically insignificant different in comparison to control. The percentage of changes in the weight of testes were decreased significantly (P<٠.٠٥) in rats that received ١٠ and ٢٠ppm Cr, while no significant differences were recorded in rats that received ٥ppm Cr. The percentage of changes were –٠٧.٧٧, –١٠.١١, and –١٥.٩١ at doses of ٥, ١٠, and ٢٠ ppm Cr. Animals exposed to all doses showed slight, but statistically insignificant changes in the weights of epididymis. Exposure to Chromium resulted in significant decrease in the relative weights of the seminal vesicles of rat that received ٢٠ppm for ٣ months only. The percentages of changes were –٠٥.٢٠, –١٦.٧٦, and –٣٢.٩٤ at doses of ٥, ١٠, and ٢٠ ppm Cr respectively.

Table (٧): Effect of Potassium dichromate on Organo-Somatic Index of kidney, liver, spleen, testes, epididymis and seminal vesicle of Sprague-Dawley male rat exposed for three months period.

TISSUE OSI (control)	DOSE (ppm)	ORGANO-SOMATIC INDEX (Mean ± S.D)	% CHANGE
KIDNEY ٠.٣٢٤ (±O.٠٢٥)	٥	٠.٣٠٦±٠.٠٠٧	-٠١.٨٠ N.S
	١٠	٠.٣٣٠±٠.٠٠٧	-٠١.٨٥ N.S
	٢٠	٠.٣٥٦±٠.٠٧٠	+٠٤.٧٠ N.S
LIVER ٣.٩٧٠ (± ٠.٤٥١)	٥	٣.١٠٢±٠.٠٦٧	-٢٠.٤٦ *
	١٠	٣.٠١٥±٠.٢١٦	-٢٢.٦٧ *
	٢٠	٢.٩٨١±٠.٠٧٢	-٢٣.٥٦ *
SPLEEN ٠.٣٤٠ (±٠.٠٣٨)	٥	٠.٣٠٤±٠.٠١٤	-١٠.٥٨ N.S
	١٠	٠.٣٧٣±٠.٠١٩	+٠٩.٧٠ N.S
	٢٠	٠.٣٦٣±٠.١٣٣	+٠٦.٧٠ N.S
TESTES ٠.٥٣٤ (±٠.٠٤٨)	٥	٠.٤٩٣±٠.٠٠٥	-٠٧.٧٧ N.S
	١٠	٠.٤٨٠±٠.٠١٣	-١٠.١١ *
	٢٠	٠.٤٤٩±٠.٠٣٢	-١٥.٩١*
EPIDIDYMIS ٠.٢٢٣ (±٠.٠٣٩)	٥	٠.٢٢١±٠.٠٠٦	-٠٠.٨٩ N.S
	١٠	٠.٢١٦±٠.٠١٠	-٠٣.١٤ N.S
	٢٠	٠.٢٠٨±٠.٠١١	-٠٦.٧٢ N.S
SEMINAL VESICLE ٠.١٧٣(±٠.٠٣٩)	٥	٠.١٦٤±٠.٠١٦	-٠٥.٢٠ N.S
	١٠	٠.١٤٤±٠.٠٠٩	-١٦.٧٦ N.S
	٢٠	٠.١١٦±٠.٠٠٩	-٣٢.٩٤ *

All values are mean ± S.D of ٤ animals

*The significant values as per student's t- test are between control and experimental values at p<o.o٥.

٤-٣ REPRODUCTIVE AND METAL TOXICITY:

٤-٣-١ ALUMINUM EFFECT:

The effects of aluminum on epididymal sperm count in rats are shown in table (٨). The epididymal sperm number was ٢١.٦٤١X$١٠^{٦}$/g epididymis for control animals. The sperm number decreased significantly (P<٠.٠٥) in all exposed groups. At doses of ١٢٥, ٢٥٠, and ٥٠٠ppm (Al), the sperm counts were ٧.٧٦٧, ٦.٦٤٢, and ٧.٧٧٦x$١٠^{٦}$/g epididymis respectively. However, no variations in sperm counts were observed within the different groups of rats exposed to different doses of Aluminum.

The effects of Aluminum on sperm abnormality in rats are shown in table (٨). The rates of abnormality of 0.163%, 0.285%, 0.32% for rats exposed to 125,250,and ٥٠٠ppm Al respectively, were significantly higher than that of ٠.٠٣٦٪ for the control group (P<٠.٠٥).

The seminiferous tubules and epididymal tubule diameters were changed due to exposure to Aluminum. Figure (1) shows effect of sub chronic exposure of 125,250 and 500ppm Al, tubules diameters decreased significantly (P<0.05) in comparison to control animals (Plate ١,a and b). It may be noted that the diameter of the seminiferous tubules were decreased after Aluminum exposure.

The effect of Aluminum on spermatogenesis elements was shown in figure (٢). Sub chronic Aluminum exposure of male rats caused a significant increase in the percentage of spermatogonia and spermatocytes. On the other hand, a significant decrease in the percentage of spermatids and spermatozoa were recorded in all exposure doses.

Table (٨): Effects of Aluminum on the sperm counts and sperm abnormality of Sprague-Dawley Male rats.

Dose / Elements	Control	١٢٥ppm	٢٥٠ ppm	٥٠٠ppm	LSD
** Sperm Count	٢١.٦٤١	٧.٧٦٧ *	٦.٦٤٢ *	٧.٧٧٦ *	٢.٧٤٨
%Sperm abnormality	٠.٣٦٥ *	٠.١١٣ *	٠.٢٩٤ *	٠.٣٣١ *	٠.٠٣٣٢

**** Sperm counts as number x10^{٦} /g of epididymis.**
***Significant at P<٠.٠٥.**

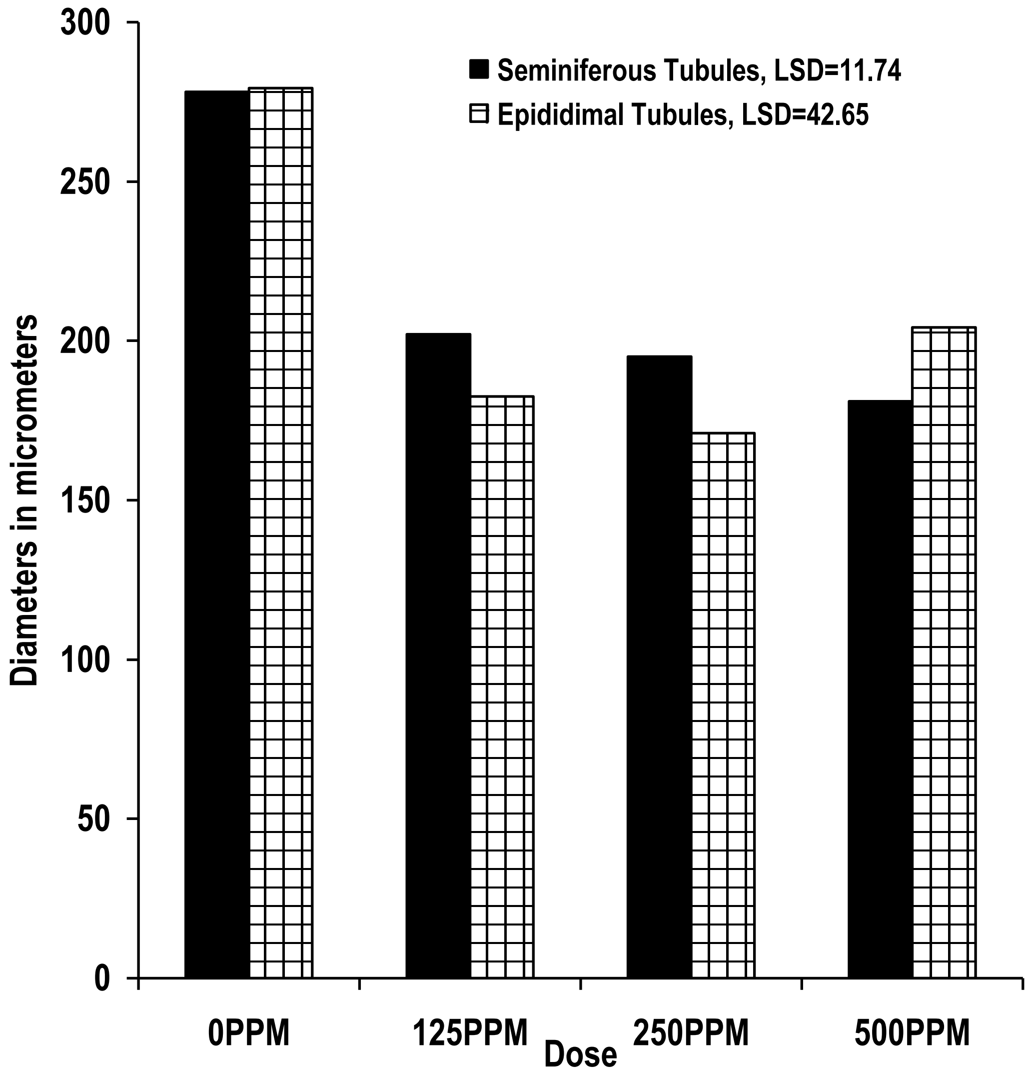
Seminiferous Tubules, LSD=11.74
Epididimal Tubules, LSD=42.65
300
250
200
150
100
50
0
Diameters in micrometers
0PPM
125PPM
250PPM
500PPM
Dose

Figure (١): Effect of Aluminum on Seminiferous and Epididymial tubules diameters of male Sprague-Dawley rats.

PLATE (١): testis sections :(a) from a control rat, (b) from a rat exposed to Al at a dose of ٥٠٠ppm for ٣ months, and (c) from a rat exposed to Cr at a dose of ٢٠ppm for ٣ months.

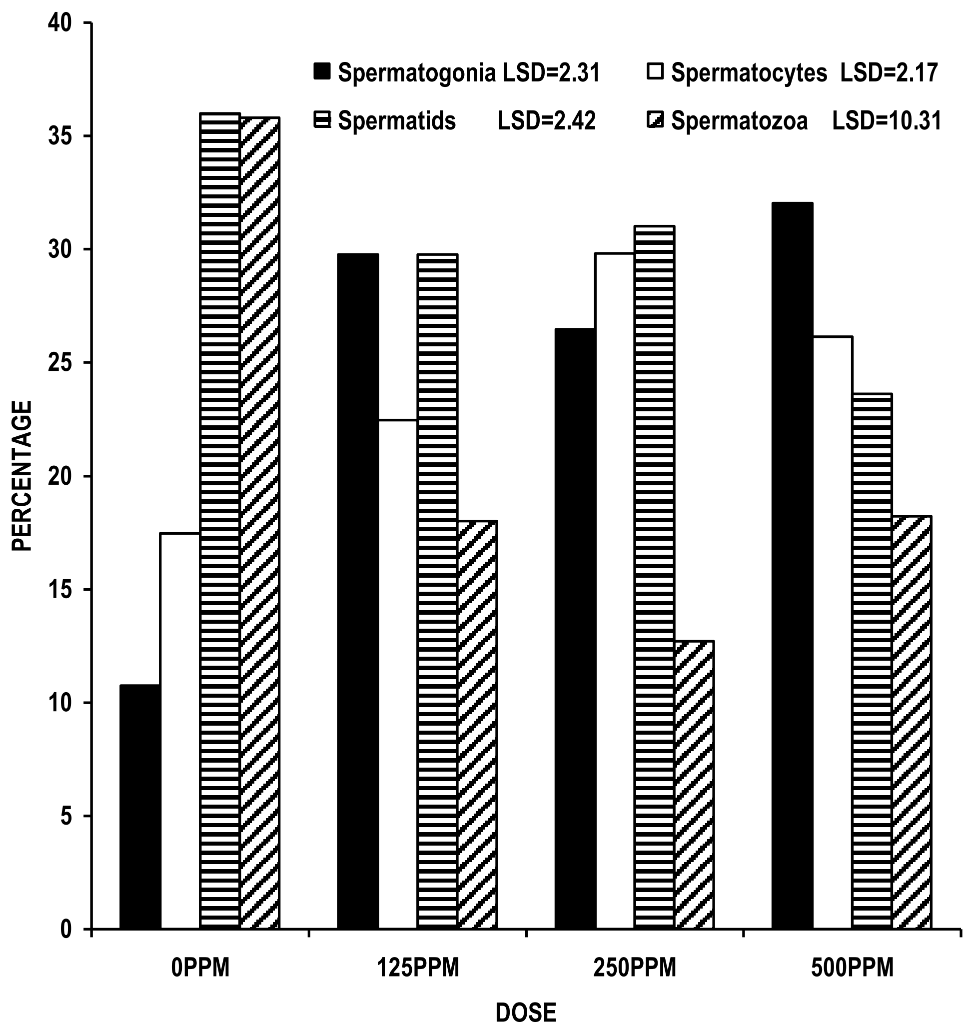

FIGURE (٢): Effect of Aluminum on spermatogenesis elements in male Sprague-Dawley rats.

٤-٣-٢ CHROMIUM EFFECT:

Rats exposed to Chromium showed a significant decrease (P<٠.٠٥) in epididymal sperm counts in comparison with ٢١.٦٤١x١٠^{٦}/g epididymis of sperm counts for control animals .At doses of ٥, ١٠, and ٢٠ppm (Cr)), the sperm counts were ٦.٥٥١, ٨.٦٩٣, and ٦.٣٤٢x١٠^{٦}/g epididymis respectively (Table, ٩).

The effect of chromium on sperm abnormality in rats is shown in table (٩). The rates of abnormality of ١٨.٣٣٪, ٢١.٣٣٪, and ٢٧.٣٣٪ for rats exposed to ٥,١٠,and ٢٠ppm Cr respectively, were significantly higher than that of ٠.٠٣٦٪ for the control group (P<٠.٠٥). Increasing level of abnormality in exposed groups depended on the dose of Cr (VI) exposure.

Figure (٣) shows effects of sub chronic exposure of ٥,١٠ and ٢٠ppm Cr (VI) on seminiferous tubules and epididymal tubule diameters. Seminiferous tubules diameters decreased significantly (P<٠.٠٥) in comparison to control animals (Plate ١,c). No significant changes were noted in epididymal tubule diameters of rats exposed to different doses of chromium in comparison to control, but a slight decrease in the epididymal tubule diameter of rats exposed to ٢٠ppm Cr for ٣ months was observed.

The effect of hexavalent chromium on spermatogenesis elements was shown in figure (٤). Sub chronic chromium exposure of male rats caused a significant increase in the percentage of spermatogonia and spermatocytes. On the other hand, a significant decrease in the percentage of spermatids and spermatozoa were recorded in all exposure doses.

Table (٩): Effects of Chromium on the sperm counts and sperm abnormality of Sprague-Dawley Male rats.

Dose / Elements	Control	٥ppm	١٠ ppm	٢٠ppm	LSD
** Sperm Count	٢١.٦٤	٦.٥٥١ *	٨.٦٩٣ *	٦.٣٤٢ *	٢.٨٨٩٧
% Sperm abnormality	٠.٠٣٦	١٨.٣٣ *	٢١.٣٣ *	٢٧.٣٣ *	٠.٩٤١

**** Sperm counts as number x10٦ /g of epididymis.**
***Significant at P<٠.٠٥.**

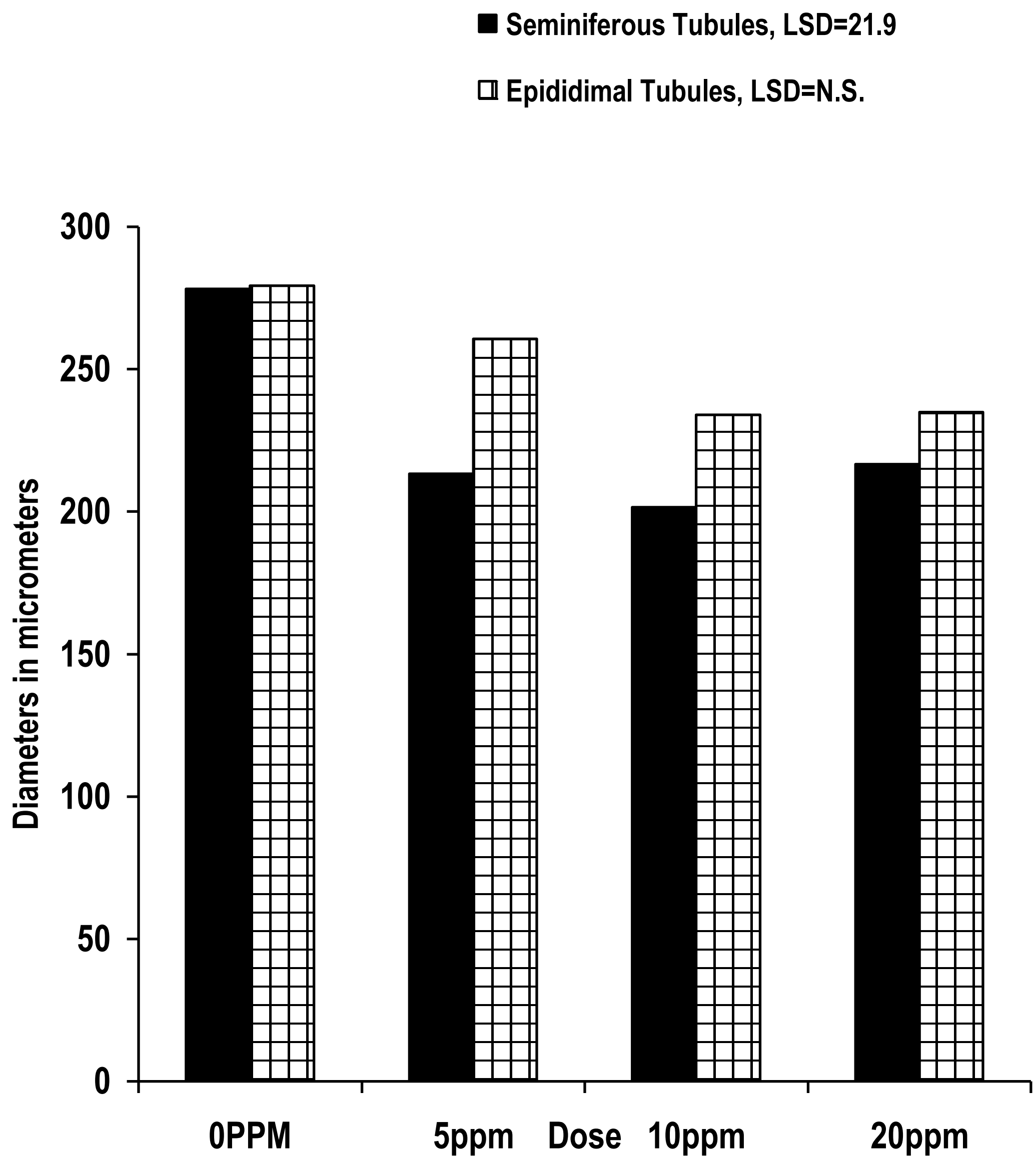

Figure (٣): Effect of Chromium on Seminiferous and Epididymal Tubules diameters of male Sprague-Dawley rats

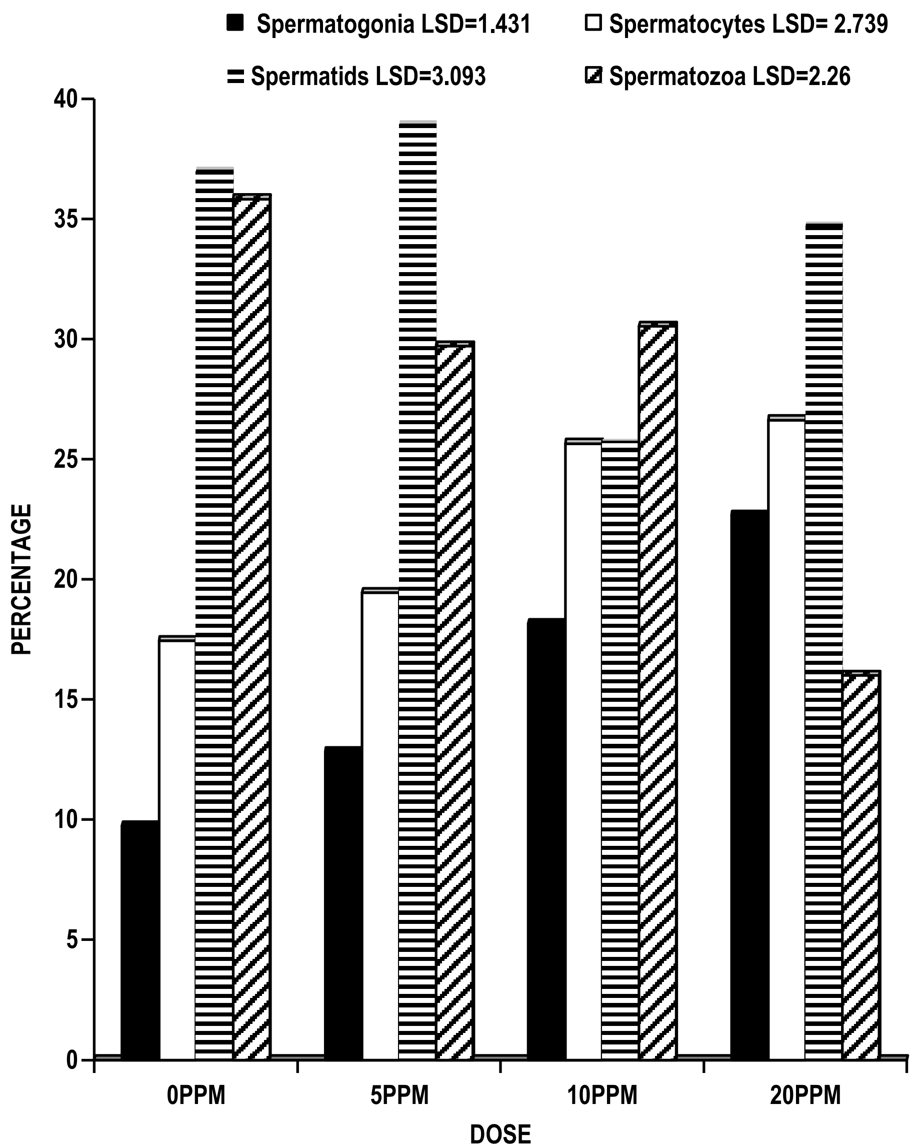

FIGURE (٤): Effects of Chromium+٦ on spermatogenesis elements in male Sprague-Dawley rats.

٤-٤ ENZYME HISTOCHEMISTRY AND METAL TOXICITY:

The intensity of reactions, distribution pattern and site of activities of the investigated Succinate Dehydrogenase (SDH) and **α–** Glycerophosphate Dehydrogenase (α-GPDH) enzymes in various studied organs were mentioned in detail as following:

KIDNEY

Different parts of kidney showed a marked fluctuation in the intensity of reaction of SDH and α-GPDH in the treated rats during induced acute and sub chronic toxicity by Aluminum chloride and potassium dichromate.

٤-٤-١ SDH ENZYME ACTIVITY IN KIDNEY:

In the cortex region of control kidney, a strong reaction of SDH was observed in the proximal convoluted tubules (PCT), distal convoluted tubules (DCT) and macula densa (MD), on the other hand, no reaction was observed in the glomeruli (plate ٢a). In the medulla of control kidney, both thick limbs and thin limbs of henel's loop showed the strong reaction of this enzyme (plate ٢b).

٤-٤-١-١ ALUMINUM EFFECT

Rats exposed to single acute dose of Al showed a marked decrease in the activity of SDH in large numbers of distal and proximal convoluted tubules in the cortex region of kidney after ١ hour of treatment (plate ٣a). A further lowering in the activity of SDH was observed in the cortex region of rat kidney after ٣ hour of treatment (plate٣b). A strong reaction of this enzyme occurred in the medulla during the first and third hours of treatment (plate ٣c). A weak reaction of SDH enzyme was noted in the cortex (plate ٣d) and in the medulla (plate ٣e) of treated kidney after ٦ hour of treatment. A recovery condition was recorded after ١٢ hour of treatment

Sub chronic exposure to Al showed the following effects. A slight decrease in the SDH activity was noted in large numbers of PCT and DCT tubules in the cortex region (plate ٤a), whereas no alteration in its activity was observed in the straight portion of the renal tubules and in the collecting ducts within the medulla (plate ٤b) of treated kidney of rats which received ١٢٥, ٢٥٠, and ٥٠٠ ppm Al in drinking water for ٢ months. Same effects were noticed for groups receiving ١٢٥ ppm Al for ٣ months dosage period. A marked decrease in SDH activity was observed in the cortex region (plate ٤c) and outer edge of medulla (plate ٤d) of rats receiving ٢٥٠ and ٥٠٠ ppm Al for ٣ months.

PLATE ٢
Light micrographs of the succinate dehydrogenase activity in (a) cortex, and (b) medulla, of normal Sprague-Dawely rat kidney.
dct= distal convoluted tubule
pct= proximal convoluted tubule
md= macula densa
gi = glomeruli
bp = base of pyramid
mr = medullary rays

PLATE (٣): Light micrographs of the succinate dehydrogenase activity in (a) Cortex region after ١ hr, (b) cortex region after ٣ hrs, (c) outer medulla region after ٣ hrs, (d) cortex region after ٦ hrs, and (e) outer medulla region after ٦ hrs of Sprague-Dawely rat kidney treated with single acute dose of Aluminum chloride. (Abbreviations as in plate ٢)

PLATE (٤) Light micrographs of the succinate dehydrogenase activity in the kidney of Aluminum chloride treated rats received (a) ٥٠٠ppm AL for ٢ months, (b) ٢٥٠ppm AL for ٢ months, (c) ٢٥٠ppm AL for ٣ months, (d) ٥٠٠ppm AL for ٣ months.

as = ascending limb

cd =collecting duct

٤-٤-١-٢ CHROMIUM EFFECT

Acute exposure to Chromium caused a distinct reduction of SDH activity in the cortical region (plate ٥a) and medulla region (plate ٥b) of kidney after ١ hour of treatment. All elements of cortex and medulla showed this reduction. A gradual increase in the activity of SDH enzyme was noted after ٣ hour of treatment (plate ٥c) and after ٦ hour of treatment (plate ٥d) in the all parts of cortex. After ٢٤ hour of treatment, a recovery of normal enzyme activity was observed in most elements of cortical (plate ٥e) and medullar (plate ٥f) regions of kidney, except collecting tubules which showed a weak enzyme reaction.

A slight decrease in the activity of succinate dehydrogenase was noted in the distal convoluted tubules (DCT) of ١ month treated kidney of rats receiving ١٠ and ٢٠ ppm Cr, while the activity of this enzyme was not affected in the proximal convoluted tubules and macula densa (plate ٦a) and in the thick and thin limbs of Henle loop (plate ٦b). A marked decrease in the activity of SDH enzyme was observed in the whole cortex (plate ٦c) and medulla (plate ٦d) of rat receiving ١٠ and ٢٠ ppm Cr for ٣ months. However, the enzyme activity in kidney of rats receiving ٥ ppm Cr showed the same pattern of SDH activity in the control kidney through the period of exposure.

٤-٤-٢ α -GPDH ENZYME ACTIVITY IN KIDNEYS:

The kidney of the control rat showed a strong reaction of α -GPDH in the outer zone of medulla, thick and thin limbs of Henle's loop (plate ٧a), collecting tubules and collecting ducts (plate ٧b). The reaction was weak in the glomeruli and moderate in the tubules of the cortex (plate ٧c).

٤-٤-٢-١ ALUMINUM EFFECT

A distinct increase of α -GPDH activity was observed in the proximal convoluted tubules (plate ٨a) after ١ hour of treatment with a single oral dose, and in the distal convoluted tubules after ٣ hours (plate ٨b) and collecting tubules in inner medulla after ٦-١٢ hour of treatment (plate ٨c). A strong reaction in the collecting ducts was observed in the kidney after ١٢ hrs persisted until ٢٤ hrs (plate ٨d).

Rats receiving ٥٠٠ppm Al for ٣ months, showed a strong reaction in the whole inner strips of medulla (plate ٨e), and in the collecting ducts in the outer region of medulla (plate ٨f) of kidney. However, no remarkable changes were observed in other groups of rats, which received ١٢٥ and ٢٥٠ppm through out the period of exposure.

PLATE (٥): Light micrographs of the succinate dehydrogenase activity in (a) cortex region after ١ hr, (b) medulla region after ١ hr, (c) cortex region after ٣ hrs, (d) cortex region after ٦ hrs, (e) cortex region after ٢٤ hrs, (f) medulla region after ٢٤ hrs, of Sprague-Dawely rat kidney treated with single acute dose of Potassium dichromate.

PLATE (٦): Light micrographs of the succinate dehydrogenase activity in the kidney of Potassium dichromate treated rats received: (a) ١٠ppm Cr for ١ month, (b) ٢٠ppm Cr for ١ month, (c) ١٠ppm Cr for ٣ months, (d) ٢٠ppm Cr for ٣ months.

PLATE (٧): Light micrographs of the α -Glycerophosphate dehydrogenase activity in the (a, b) medulla (c) cortex of normal Sprague-Dawely rat kidney.

as, ascending limb
ds, descending limb

PLATE (٨): Light micrographs of the α -Glycerophosphate dehydrogenase activity in (a) cortex region after ١-hr (b) cortex region after ٣-hrs, (c) medulla region after ٦-hrs, (d) cortex region after١٢ hrs of rat kidney treated with single acute dose of aluminum chloride, (e) and (f) kidney of rat received ٥٠٠ppm Al for ٣ months.

٤-٤-٢-٢ CHROMIUM EFFECT

A strong reaction was observed in the collecting tubules and macula densa (plate ٩a) in the kidney of rat after ١ hour of treatment with chromium, and a very strong reaction of enzyme was seen also in the collecting tubules after ٣ hour of treatment as well as a slight reaction of enzyme was recorded in comparison to control (plate ٩b). After ٦ hour of treatment an increase in the activity of enzyme was observed in the proximal convoluted tubules while a weak reaction was reported in the other components of the cortex region of kidney (plate ٩c). On the other hand, increased reaction was noted in the collecting tubules of medulla (plate ٩d). A recovery condition was observed after ٢٤ hour of initial administration.

A considerable increase in the activity of α –GPDH enzyme was observed in the proximal and distal convoluted tubules of kidney in rats receiving ١٠ and ٢٠ppm Cr for two and three months (plate ٩e). A significant increase in the activity of this enzyme was noted in the macula densa of rats receiving ٢٠ppm Cr for ٣ months (plate ٩f).

LIVER

Histochemical findings of SDH and α –GPDH enzymes reaction in the liver of rat subjected to Al and Cr for acute and chronic effects were as follows:

٤-٤-٣ SDH ENZYME ACTIVITY IN LIVERS:

The liver of control rats showed strong activity of SDH (plate ١٠a). Formazan granules were almost evenly distributed within the lobules which means that the intensity of the reaction in all zones of the lobules were equal (plate ١٠b).

٤-٤-٣-١ ALUMINUM EFFECT

A distinct reduction of SDH activity was demonstrated in the liver of animal after ١ hour of administration (plate ١١a). A gradual increase in the activity of SDH was noted after ٦ hours (plate ١١b). After ١٢ hours of treatment the liver showed the same manner of enzyme reaction of control rats (plate ١١c).

No differences in the activity of SDH were seen in the liver of rats received ١٢٥ and ٢٥٠ ppm Al during the whole exposure period, as well as rats received ٥٠٠ ppm Al for first month of exposure in comparison with liver of control. A slight decrease in the enzyme activity occurred in the liver of rats exposed to ٥٠٠ppm Al for ٢ months (plate ١١d). A drastic decrease in SDH activity was observed in the liver of animals exposed to ٥٠٠ppm for ٣ months (plate ١١e).

PLATE (٩): Light micrographs of the α -Glycerophosphate dehydrogenase activity in (a) cortex region after ١ hr, (b) cortex region after ٣ hrs, (c) cortex region after ٦ hrs, (d) medulla region after ٦ hrs, of rat kidney treated with single acute dose of Potassium dichromate, (e) kidney received ٢٠ppm Cr for ٢ months and (f) kidney received ٢٠ppm Cr for ٣ months.

PLATE (١٠): Light micrographs of the succinate dehydrogenase activity in the normal liver of Sprague-Dawely rat
pa= portal area
cv =central vein.

PLATE (١١): Light micrographs of the succinate dehydrogenase activity in (a) the liver after I hr, (b) the liver after ٦ hrs, (c) the liver after ١٢ hrs, of rat treated with single acute dose of Aluminum chloride, (d) liver of rat received ٥٠٠ppm Al for ٢ months, and (e) liver of rat received ٥٠٠ppm Al for ٣ months.

٤-٤-٣-٢ CHROMIUM EFFECT

A remarkable decrease in the SDH activity was observed in the intermediate zone of the liver lobules after ١ hour of treatment (plate ١٢a). A gradual increase in the activity of enzyme occurred after ١٢ hour (plate ١٢b). A recovery of normal enzyme activity (strong reaction) was noted in the pericentral and periportal zones after ٢٤ hour of treatment (plate ١٢c).

No difference in the activity of SDH was shown in the liver of rats exposed to ٥ppm Cr during the whole period of exposure (plate ١٢d). A considerable decrease in the activity of SDH was observed in rats receiving ١٠ and ٢٠ppm Cr along the period of exposure (plate ١٢e).

٤-٤-٤ α- GPDH ACTIVITY IN LIVERS:

A moderate to fairly strong (plate ١٣a) activity of α- GPDH was seen in the liver of the control rats. The intensity of reaction was equally distributed in the all zones of lobule.

٤-٤-٤-١ ALUMINUM EFFECT

Increased activity of α–GPDH enzyme was observed after ١ hour of treatment (plate ١٣b). Liver regain normal activity of this enzyme after ١٢ hour of treatment (plate ١٣c).

A strong activity of α–GPDH was observed in all zones of liver of rats receiving ١٢٥ ppm $Al^{+٣}$ for ٣ months period (plate ١٣d). Increased activity of this enzyme occurred in the liver of rats exposed to ٢٥٠ and ٥٠٠ ppm during ١st, ٢nd, and ٣rd as a strong reaction in all zones of lobules especially in hepatocytes near the periportal (plate ١٣e) and pericentral (plate ١٣f) regions.

٤-٤-٤-٢ CHROMIUM EFFECT

Increase in the activity of α–GPDH enzyme was observed in the liver of animals after ١ hour of administration (plate ١٤a). After ١٢ hours of treatment, liver showed a normal moderate reaction of α–GPDH (plate ١٤b).

No remarkable difference in the activity of α–GPDH was observed in the liver of rats received ٥, ١٠ and ٢٠ ppm Cr for ١ month, and in the liver of rats receiving ٥ppm along ٣ months (plate ١٤c). Increased activity of this enzyme was observed in the liver of rats receiving ١٠ and ٢٠ ppm Cr in drinking water for ٢ and ٣ months (plate ١٤d).

PLATE (١٢): Light micrographs of the succinate dehydrogenase activity in (a) the liver after I hr, (b) the liver after ١٢ hrs, (c) the liver after ٢٤ hrs, of rat treated with single acute dose of potassium dichromate, (d) liver of rat received ٥ppm Cr for ٣ months, and (e) liver of rat received ٢٠ppm Cr for ٣ months.

PLATE (١٣): Light micrographs of the α -Glycerophosphate dehydrogenase activity in (a) the normal liver rat , (b) the liver after ١ hr, (c) the liver after ١٢ hrs, of rat treated with single acute dose of aluminum chloride and (d) liver of rat received ١٢٥ ppmAl for ٣ months,(e) liver of rat received ٢٥٠ppm AL for ٣ months and (f) liver of rat received ٥٠٠ppm AL for ٢ months.

PLATE (١٤): Light micrographs of the α -Glycerophosphate dehydrogenase activity in (a) The liver after ١ hr, (b) the liver after ١٢ hrs, of rat treated with single acute dose of Potassium dichromate, and (c) liver of rat received ٥ppm Cr for ٣ months and (d) liver of rat received ٢٠ppm Cr for ٣ months.

SPLEEN

The main histological parts (white and red pulp) of spleen showed different intensity in the activity of SDH and α–GPDH during successive time of treatment and a different level of doses.

٤-٤-٥ SDH ENZYME ACTIVITY IN SPLEENS:

Control animals showed a very strong activity of SDH was seen in different parts of white pulp (lymphatic nodules, endothelium of central artery and germinal center), and red pulp (splenic cords, and venous sinuses especially around the lymphatic nodules) (plate ١٥a).

٤-٤-٥-١ ALUMINUM EFFECTS

A weak reaction was detected in the experimented groups examined after ١, ٣, and ٦ hours of treatment (plate ١٥b). Spleen regains activity of this enzyme in the red pulp after ١٢ hours of treatment.

No remarkable difference in the activity of SDH was recorded in the spleen of rats receiving ١٢٥ppm Al for ٣ months in comparison with control. A considerable decrease in the activity of SDH, in all parts of spleen, were reported in the spleen of rats receiving ٢٥٠ and ٥٠٠ ppm Al during one, two, and three months of exposure period (plate ١٥c).

٤-٤-٥-٢ CHROMIUM EFFECT

A drastic reduction in the activity of SDH occurred after ١ hour of treatment (plate ١٥d). A very weak enzyme reaction was noted in the spleen examined after ٣and ٦ hours of treatment. A marked reaction occurred in the spleen after ٢٤ hours of treatment (plate ١٥e).

A reduction in the intensity of enzyme reaction occurred in the whole tissue along the period of subchronic exposure for all doses levels (plate ١٥f).

٤-٤-٦ α -GPDH ACTIVITY IN SPLEENS:

In the control rats, a moderate α –GPDH reaction was observed in the white and red pulp regions of spleen (plate ١٦a).

٤-٤-٦-١ ALUMINUM EFFECT:

Slight increase in the activity of α –GPDH was observed in the spleen after ١ hour of administration and the same intensity of reaction remained thereafter. Enzyme activity did not gain normal activity even after ٢٤ hours of administration (plate ١٦b).

The spleen of rats received ١٢٥ and ٢٥٠ ppm Al for two months period showed slight increase of enzyme activity compared to control (plate ١٦c). A marked increase in the activity of α –GPDH in capsule of spleen (plate ١٦d) was reported for rats receiving ٥٠٠ ppm Al for two months. Whole spleen tissue showed increase in the enzyme activity in rats receiving ٥٠٠ ppm Al for ٣ months (plate ١٦ e).

٤-٤-٦-٢ CHROMIUM EFFECT:

A strong enzyme reaction was detected in the capsules, trabeculae, endothelium of central arteries and endothelium of trabecular arteries after ١ hour of administration (plate ١٦f). A recovery reaction occurred after ٦ hours of treatment. Increase in the α–GPDH activity was reported in all parts of spleen of rats exposed to ٥, ١٠, and ٢٠ ppm Cr for ١, ٢, and ٣ months (plate ١٦g).

PLATE (١٥): Light micrographs of the Succinate dehydrogenase activity (a) in the normal spleen, (b) in the spleen after I hr of treatment with single acute dose of Aluminum chloride, (c) in the spleen of rat received ٥٠٠ppm Al for ٣ months,(d) in the spleen after I hr of treatment with single acute dose of Potassium dichromate,(e) in the spleen after ٢٤ hrs of treatment with single acute dose of Potassium dichromate, and (f) in the spleen of rat received ٢٠ ppm Cr(VI) for ٢ months.

PLATE (١٦): Light micrograph of the α -Glycerophosphate dehydrogenase activity (a) in the normal spleen, (b) in the spleen after ١ hr of treatment with single acute dose of Aluminum chloride,(c) in the spleen of rat received ٢٥٠ppm Al for ٢ months, (d) in the spleen of rat received ٥٠٠ppm Al for ٢ months, (e) in the spleen of rat received ٥٠٠ppm Al for ٣ months, (f) in the spleen after ١ hr of treatment with single acute dose of Potassium dichromate and (g) in the spleen of rat received ٢٠ ppm Cr(VI) for ٣ months .

MUSCLES.

Fiber typed-related SDH and α-GPDH enzymes activities of normal and treated rats were described for sartorius thigh muscle and abdominal muscles.

According to peter (١٩٧٢) the histochemical assay distinguishes three types of fibers:

١-Red, small-diameter, intensely stained, aerobic, slow-oxidative fibers (SO),

٢-White, large-diameter, poorly stained, anaerobic, fast-glycolytic fibers (FG).

٣-Intermediate, fast-oxidative glycolytic fibers (FOG).

٤-٤-٧SDH ENZYME ACTIVITY IN MUSCLES:

In control rats, SO fibers, predominated in the sartorius muscle (plate ١٧a), had the greatest activity of SDH. FG fibers predominated in the abdominal muscles (plate ١٧b) and showed little SDH activity, whereas FOG fibers showed intermediate SDH activity in both sartorius and abdominal muscles.

٤-٤-٧-١ ALUMINUM EFFECTS:

Marked decrease in the activity of SDH was observed in both SO and FOG fibers of Sartorius muscle of rats after 1 hour of chemical administration (plate ١٧c). Sartorius muscle fibers showed the same intensity of enzyme reaction after 3, 6, 12 hours of chemical administration. However, a slight increase in the activity of SDH was noticed in FOG fibers after 24 hours of treatment (plate ١٧d). A recovery state initiated after 24h of treatment.

No difference in the activity of SDH was recorded in the first ٣ hours of treatment in fibers of abdominal muscles in comparison to control. A drastic decreases in the SDH activity was noted in both SO and FOG fibers of abdominal muscles after ٦ hours of treatment (plate ١٧e). A gradual increase in the activity of this enzyme was observed in fibres of muscle after ١٢ hours (plate ١٧f) and ٢٤ hours (plate ١٧g) of chemical administration.

No remarkable difference in enzyme activity, in comparison with control was observed in sartorius and abdominal muscles of rats receiving ١٢٥ ppm Al along the three months period of exposure.

A considerable decrease in the activity of SDH in SO fibres of sartorius was seen in rats receiving ٢٥٠ppm Al for ٣ months (plate ١٨a), and in rats receiving ٥٠٠ppm Al for ٢ months (plate ١٨b) and ٣ months (plate ١٨c). This effect can lead to differentiate only two intensities of the enzyme activity, one (moderate intensity) was observed in SO and FOG fibres and the other (weak intensity) in FG fibres.

A marked reduction in the activity of SDH in FOG fibres was observed in abdominal muscles of rats receiving ٥٠٠ppm Al for ١ month, whereas a strong reaction of this enzyme was noted in SO fibers (plate ١٨d). All groups of rats receiving ٢٥٠ ppm Al (plate ١٨e) and ٥٠٠ ppm Al (plate ١٨f) for ٢ and ٣ months showed a weak activity of SDH in SO and FOG fibres respectively.

٤-٤-٧-٢ CHROMIUM EFFECT

SDH activity was decreased in SO fibers and for less extent in FOG fibers of sartorius muscle after ١ hour of treatment (plate ١٩a). Slight increase in the activity of SDH, an indication of starting of recovery condition occurred after ١٢ hour of chemical administration (plate ١٩b). A normal SDH activity was recorded after ٢٤ hours of chemical administration.

Slow oxidative (SO) fibers of abdomen muscles showed no enzyme activity differences in comparison to control after ١ hour of treatment, while intermediate fibers (FOG) showed marked decrease in the enzyme activity (plate ١٩c). After ٦ hours of administration, SO and FOG fibers, showed the same intensity of SDH activity (plate ١٩d). A recovery condition occurred after ٢٤ hours of treatment.

A slight decrease in SDH activity in SO and FOG fibers of sartorius muscle was recorded in rats received ٥ and ١٠ ppm Cr for ٢ months and ٢٠ ppm for ١ month (plate ٢٠a). A marked reduction in the enzyme activity was reported in both SO and FOG fibers of sartorius muscle of rats received ٢٠ppm for ٣ months (plate ٢٠b).

In abdomen muscles, a slight decrease in the SDH activity in SO and FOG fibers was noted in rats exposed to ٥ppm Cr for ١, ٢, and ٣ months (plate ٢٠c). A marked reduction in SDH activity was noted in whole fibers of muscle of rats received ١٠ppm for ٢ and ٣ months (plate ٢٠d). Very weak reaction was recorded in the abdomen muscle fibers of rats exposed to ٢٠ppm for ٣ months (plate ٢٠e).

PLATE (١٧): Light micrograph of the Succinate dehydrogenase activity (a) in the normal Sartorius muscle, (b) in the normal abdominal muscle, (c) in the Sartorius muscle after ١ hr, (d) in the Sartorius muscle after ٢٤ hrs, (e) in the abdominal muscle after ٦ hrs,(f) in the abdominal muscle after ١٢ hrs and (g) in the abdominal muscle after ٢٤ hrs of treatment with single acute dose of Aluminum chloride.

fg = fast glycolytic fibres
fog= fast oxidative glycolytic fibres
so= slow oxidative fibres

PLATE (١٨): Light micrograph of the Succinate dehydrogenase activity in sartorius muscle of rat received, (a) ٢٥٠ppm Al for ٣ months, (b) ٥٠٠ppm Al for ٢ months (c) ٥٠٠ppm Al for ٣ months, and in abdominal muscle of rat received, (d) ٥٠٠ppm Al for ١ month, (e) ٢٥٠ppm Al for ٢ months, (f) ٢٥٠ppm Al for ٣ months.

PLATE (١٩): Light micrograph of the Succinate dehydrogenase activity in the Sartorius muscle (a) after ١ hr, (b) after ١٢ hrs, and in the abdominal muscle, (c) after ٦ hrs, and (d) after ٦ hrs of treatment with single acute dose of potassium dichromate.

PLATE (٢٠): Light micrograph of the Succinate dehydrogenase activity in sartorius muscle of rat received, (a) ٢٠ppm Cr(VI) for ١ month, (b) ٢٠ppm Cr(VI) for ٣ months, and in the abdominal muscle of rat received, (c) ٥ppm Cr(VI) for ١ months, (d) ١٠ppm Cr(VI) for ٣ months, (e) ٢٠ppm Cr(VI) for ٣ months.

٤-٤-٨ SDH ENZYME ACTIVITY IN LUNGS:

The activity of SDH enzyme was differently distributed through the lung histological areas in rats exposed to acute and sub chronic toxicity of Aluminum and Chromium.

In control rats, a strong reaction of SDH enzyme was seen in epithelial layer lining the intrapulmonary bronchus, terminal bronchioles, and respiratory bronchioles and to less extent in pulmonary alveoli (plate ٢١a).

٤-٤-٨-١ ALUMINUM EFFECTS:

No remarkable differences in the activity of SDH were recorded in the lungs of rats exposed to different doses of Aluminum in comparison to those described in control animals.

٤-٤-٨-٢ CHROMIUM EFFECTS:

A remarkable increase in the SDH activity was observed in the histological components of lung after ١ hour of treatment (plate ٢١b).

A recovery reaction occurred in most lung elements after ٢٤ hours of treatment (plate ٢١c)

No remarkable differences in the activity of SDH were observed in the lung of rats received ٥, ١٠ and ٢٠ ppmCr for ١ month, and in the lung of rats received ٥ppm for ٣ months. Increase activity of this enzyme was observed in the lung of rats received ١٠ and ٢٠ ppm Cr in drinking water for ٢ and ٣ months (plate ٢١d).

٤-٥ HISTOPATHOLOGICAL EFFECTS

Haematoxylin and eosin (HE) stained sections of kidney, liver, spleen, lung, muscle, and testis of control animals showed that the morphological picture of all the investigated organs were normal.

٤-٥-١ Kidney:

No pathomorphological changes were visible in the kidney of rat exposed to Aluminum and Chromium except that kidney of rats received ٥٠٠ppm Al. for ٣ months. The only obvious histopathological findings in kidney were intracytoplasmic vacuolization, interstitial edema and degeneration of the tubular epithelial cells (plate ٢٢a,b).

٤-٥-٢ Liver:

Livers of control rats had a normal histological structure with a characteristic pattern of hexagonal lobules. In the liver of all treated groups, no major morphological changes were observed compared to the control group.

٤-٥-٣ Spleen:

The spleen in control animals displayed the typical architecture, where a lymphoid follicle was clearly evident. No pathomorphological alterations were recorded in the spleen of rats exposed for both Aluminum and Chromium.

٤-٥-٤ Lung:

In comparison to normal lungs (plate ٢٢c), no pathological changes were reported in lung of rats exposed to Aluminum sub chronic toxicity. The following observations were seen in lung of rats exposed to ١٠ and ٢٠ppm Cr for ٣ months (plate ٢٢d): diffuse thickening of alveolar wall, the bronchial epithelium showed marked degeneration surrounded by heavy chronic inflammatory cells infiltration (i.e. Chronic Bronchitis & Interstitial pneumonitis).

٤-٥-٥Testis:

Morphological changes of testis were noted in the animals exposed to Al & Cr for ٣ months even in lower doses (plate ٢٢e). The morphological alterations were confined to thickening of the seminferous tubules (S.T) wall with marked vascular congestion and hyalinization and evident of early fibrosis. No Sertoli or Lydig cells hyperplasia.

٤-٦ Cr IMMUNOTOXICITY

Rice, Muskmelon and Dukhen lectins suppressed the *in vivo* T cells mitogenic activity in rat footpad reaction (plate ٢٣). However, that of rice lectin showed marked suppression as compared to other lectins (Table ١٠).

PLATE (٢١): Light micrograph of the Succinate dehydrogenase activity (a) in the normal lung, in the lung, (b) after ١ hour, (c) after ٢٤ hrs, of treatment with single acute dose of potassium dichromate and (d) in the lung of rat received ٢٠ppm Cr(VI) for ٣ months.

Plate (٢٢); Light micrograph of (a) normal kidney, (b) kidney showed intracytoplasmic vacuolization and interstitial edema from rat received ٥٠٠ppm Al in drinking water for ٣ months, (c) normal lung, (d) lung showed Pneumonitis from rat received ١٠ppm Cr (VI) in drinking water for ٣ months, and (e) testis showed interstitial fibrosis from rat received ٢٠ppm Cr (VI) in drinking water for ٣ months. (Haematoxilin and eosin stain.)

Arrow head referred to pathological sign

Plate (٢٣): Normal (a) and Lectin injected footpad (b) of rat receiving a single oral acute dose of potassium dichromate. Arrow referred to right foot.

TABLE (١٠): Indurations of pad skin of chromium treated and control Sprague-Dawley rats injected with plant lectins.

LECTIN TYPE	PAD	CONTROL THICKNESS * VALUE (+S.D) RANGE	TREATED THICKNESS VALUE (+S.D) RANGE
R	LEFT	١.٧٤٢(٠.٠١٤) ١.٧٢٠-١.٧٦٠	١.٠٢٢(٠.٠٥٣) ٠.٩٧٠-١.١١٠
	RIGHT	١.٦١٠(٠.٧٤٠) ١.٥١٠-١.٧٢٠	٠.٦٤٠(٠.٠٣٦) ٠.٦٠٠-٠.٦٩٠
B	LEFT	٠.٨٦٠(٠.٠٣٩) ٠.٨٠٠-٠.٩٠٠	٠.٨٥٥(٠.٠٣٩) ٠.٨٠٠-٠.٩١٠
	RIGHT	٠.٥٣٥(٠.٠٥٦) ٠.٤٦٠-٠.٥٩٠	٠.٦٩٥(٠.٠٦٧) ٠.٦١٠-٠.٧٩٠
D	LEFT	٠.٨٩٢(٠.١١٤) ٠.٧٢٠-١.٠٠	٠.٨٦٠(٠.٠٤٥) ٠.٨١٠-٠.٩١٠
	RIGHT	٠.٨٣٥(٠.١٢٥) ٠.٦٢٠-٠.٩٣٠	٠.٦٨٠(٠.٠٣٧) ٠.٦٢٠-٠.٧٢٠

*Thickness of animal skin before inject mean ٠.٥

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