Wasan Najim Abdel-Sada /J. Pharm. Sci. & Res. Vol. 10(12), 2018, 3336-3339

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Protective Effect of Crude Oil of *Nigella sativa* on Adrenal gland in Male Albino Mice Treated with Low Toxic Dose of Paracetamol

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Abstract

Paracetamol is one of the most common nonprescription drugs in the world, it can be used by pregnant women as it is relatively safe. However, overdose may have significant side effects on the functions and tissues of different body organs. On the other hand, the black seeds have beneficial effects in various central nervous system disorders including epilepsy, neurotoxicity and memory impairment. *Nigella sativa* oil can be consumed by patients with peptic ulcer as it protected gastric mucosa from the ulcerative effect of alcohol. Also, the aqueous extract of *Nigella sativa* seeds has a protective effect against damage of pancreatic beta cells and the ethanolic extract of *Nigella sativa* helps alleviate stress and increases anoxia tolerance in mice. Therefore, the aim of current study was to investigate the possible protective effects of crude oil of black seeds against low toxic dose paracetamol-induced histomorphometry alterations in the adrenal glands of male mice. Methods: The animals were divided into one control group and three experimental groups, the former were given 0.3 ml from 0.9% of NaCl only, whereas the mice in experimental groups were given single sub-lethal dose of paracetamol (300 mg/kg body weight) and *Nigella sativa* oil (0.3 ml) alone and together. Results: the study showed a significant ($P<0.05$) reduction in the adrenal gland weight of the fourth group recorded and wasn't significant for the third group. In addition, the results revealed a significant increase ($P<0.05$) in average thickness of cortex and its three zones while the diameter of medulla significantly decreased ($P<0.05$) in the second group, in the third group. The administrated animals in the crude oil of *Nigella sativa* significantly decreased ($P<0.05$) in the diameter of the adrenal gland and thickness of zona fasciculata and average diameter of medulla as compared with control group. Therefore, the administration of low toxic dose of paracetamol induced stress-related changes in the adrenal gland. However, these changes can be antagonized by oral administration of crude oil of *Nigella sativa* seeds.

Keywords: *Nigella sativa*, Paracetamol, Adrenal gland, Adrenal cortex, Adrenal medulla, ACTH, Corticosteroids, Stress..

INTRODUCTION

Paracetamol or acetaminophen and its chemical name N-acetyl-para-aminophenol is one of the most common nonprescription drugs in the world, it can be used by pregnant women as it is relatively safe. However, overdose may have significant side effects on the functions and tissues of different body organs. When the dose of paracetamol is greater than the therapeutic dose (150 mg-12 gm), the formation of large amounts of toxic metabolites leads to the depletion of liver antioxidant glutathione (GSH) causing liver necrosis, nausea and vomiting within several hours [1]. Overdose administration with 350 mg/kg/day of acetaminophen for a week to pregnant rats leads to a significant reduction in seminal vesicle weight and testosterone in fetal testis which is contributed to subsequent male reproductive disorders [2]. Various studies had investigated use of medicinal herbs, such as black seed, to prevent the toxic effects of paracetamol. Giving rats an overdose of paracetamol caused a significant increase in liver enzymes and total bilirubin. However, these effects were significantly prevented administration of ethanolic extract of black seeds [3]. It was observed that 750 mg/kg/day of paracetamol given to rats once a week resulted in a marked increase in diastolic and systolic blood pressures, renal cortex necrosis and increase in plasma renin activity. However, these changes were partially normalized with administration of *Nigella sativa* oil by half an hour before paracetamol treatment [4].

The black seed (*Nigella sativa* Linneaus) is known as black cumin, black caraway and "Habbat albaraka". It belongs to the family Ranunculaceae, which are small shrubs 20-90 cm height, has pale blue or purplish flowers and tapering green leaves. It grows in North Africa, Southern Europe and Southeast Asia. Also, it is cultivated in many countries like Middle Eastern, Mediterranean region, Alhijaz, Syria, India, Pakistan and Turkey. The mature black seeds and their oil have been used in the Middle East in traditional remedies to treat a variety of complaints including asthma, flatulence, headaches, influenza, rheumatism, cough, anti-inflammatory and fever. The seeds of Nigella sativa contain linolic acid 50-60%, oleic acid 20%, dihomolinoleic acid 10% and eicodadienoic acid 3% which are the main unsaturated fatty acids in seeds. Also, they contain saturated fatty acids including palmitic acid and stearic acid as well as proteins, alkaloids, saponins, vitamins, minerals, carbohydrates and about 30-48% of essential oil thymoquinone which is the bioactive compound in black seeds [5,6]. It acts as antioxidant and thus protects organs from the oxidation damage caused by the generation of free radicals [7]. It has been shown that *Nigella sativa* seeds reduce the level of liver enzymes and elevate the activity of antioxidant defence system in carbon tetrachloride treated rats and thus prevent hepatotoxicity [8]. The black seeds have beneficial effects in various central nervous system disorders including epilepsy, neurotoxicity and memory impairment [9]. *Nigella sativa* oil can be consumed by patients with peptic ulcer as it was found that, in rats, it protected gastric mucosa from the ulcerative effect of alcohol [10]. It also reduces risk of cardiovascular disease; a study found that black seed oil reduces lead-induced cardio toxicity in rats [11]. The aqueous extract of *Nigella sativa* seeds has a protective effect against damage of pancreatic beta cells caused by injection of alloxan in rats [12]. The ethanolic extract of *Nigella sativa* (200 mg/kg body weight) helps alleviate stress and increases anoxia tolerance in mice [13].

Adrenal gland is the most endocrine organ associated with chemically-induced lesions [14]. Adrenal glands are paired organs that lie near the superior poles of kidneys, they are flattened half-moon shaped structures. Each gland consists of a peripheral layer (the cortex) and a central layer (the medulla). The former is, histologically and functionally, sub-divided into three zones: zona glomerulosa (15%) that produces aldosterone, zona fasciculata (65%) that produces cortisol and corticosterone (predominantly produced in rodents) and zona reticularis (7%) that produces glucocorticoids and little amount of sex steroids namely estrogens, androgens and progestins. On the other hand, adrenal medulla secretes epinephrine and norepinephrine in response to direct stimulation from sympathetic nervous system [15].

Taken together, current study was carried out to investigate the effects of crude oil of *Nigella sativa* in protecting adrenal glands from side effects of a low toxic dose of paracetamol in adult male mice

MATERIALS AND METHODS

Paracetamol and crude oil of *Nigella sativa*

In current study, paracetamol, which is commercially named Hayamol, was made by Ibn Hayan company for Syrian

pharmaceutical industries and filled in ampoules each one contains 375 mg/5 ml, sub-lethal dose in present study was 300 mg/kg/body weight prepared by Dr. Azhar Abdul-Hafudh, Pharmacology Department, College of Dentistry/ University of Babylon, Iraq. The crude oil of black seed was bought from a traditional market.

Animals and experimental protocols

The present study was carried out on 20 adult male albino mice aged eight weeks and weighing 25-36 grams. They were obtained from the Iraqi Center for Drug Research (Baghdad), caged and maintained in the animal house at Faculty of Medicine/ University of Babylon under standard laboratory conditions (temperature 22+2ºC, 12h light/12h dark cycle). In addition, animals were fed pellet and water *ad libitum*. On the night before the experiment, the animals were fasted except fron water and treated with paracetamol by intraperitoneal injection.

The animals were randomly divided into four 5-mice groups as follows:

First group (Control group): Injected with 0.3 ml of normal (physiological) saline (0.9%), then administered with 0.3 ml of normal saline orally.

Second group: Injected with 300 mg/kg/bw of paracetamol, then administered with 0.3 ml of normal saline orally.

Third group: Injected with 0.3 ml of normal saline, then administered with 0.3 ml of black seed oil orally.

Fourth group: Injected with 300 mg/kg/bw of paracetamol, then administered with 0.3 ml of black seed oil orally.

After 24 hours of treatment, all animals were sacrificed and fresh adrenal glands were taken out, weighed separately by an electronic balance and fixed in Bouin's solution until the p-reparation of sections.

Histomorphometric study

Tissue sections were prepared according to the method described in [16]. In addition, the ocular micrometer was calibrated with stage micrometer to perform the required histomorphometry. Microscopic measurements included diameters of adrenal gland and that of adrenal medulla, and thickness of adrenal cortex and its constituent zones (glomerulosa, fasciculata, reticularis),.

Statistical analysis:

Data were analysed using computer-based software, the Statistical Package for Social Sciences (SPSS) version 20. Data were expressed as Mean±Standard Deviation (SD). Comparisons between means were conducted using One-Way Analysis of Variance (ANOVA). Statistical significance was set as ($P<0.05$).

3. Results and discussion

The results of current study showed that administration of black seed oil only had caused non-significant decrease (8.7±2.91mg) in mean weight of adrenal glands of third group mice and there was significant decrease (8.4±2.33mg; $P<0.05$) of adrenal glands weight of fourth group mice which were injected with low toxic dose of paracetamol and received 0.3 ml of *Nigella sativa* oil as compared with control mice and those in the second group. This reduction may be due to inhibition of adrenal glands' response to adrenocorticotropic hormone (ACTH) [17]. This assumption is supported by findings from a previous study where administration of 20ml/kg body weight aqueous extracts of black seeds for 21 days had resulted in decreased levels of corticosterone and ACTH in the serum of healthy and diabetic rats, respectively [20]

Since the reduction in adrenal glands' weight was almost similar in group 3 and groups 4 mice as compared to that observed in the other groups, it can be concluded that the administration of black seed oil had prevented Paracetamol-mediated stress-induced increase in the glands' weight (Table 1). Also, the results of current study revealed that administration of Paracetamol alone and black seed oil alone had produced opposed changes in the glands' weight (Table 1). A previous study revealed that administration of 100 and 200 mg/kg/bw of ethanolic extracts of *Nigella sativa* seeds had resulted in a noticeable reduction in plasma cortisol level, blood glucose and a marked decrease in adrenal glands weight of rats exposed to cold stress [13]. Thus the decrease in the weight of adrenal glands reported in current study may be due to lower production of adrenal steroids as a result of treatment with crude oil of black seed.

Data from current study showed significant increase in the mean thickness of adrenal gland cortex (496.2±98.53µm; $P<0.05$) while the mean diameter of adrenal medulla was significantly decreased (266.2±59.25µm; $P<0.05$) in mice injected with low toxic dose of paracetamol (300 mg/kg/bw) as compared to control group (Table 1). These findings might indicate an increase in adrenocortical cells content from cholesterol esters, the precursor of steroid hormones, hence increasing the activity of adrenocortical cells in the production and secretion of hormones.

Regarding to zona glomerulosa the production of aldosterone might be elevated as a result of increased concentration of extracellular potassium ions and the increased activity of renin-angiotensin system. These observations are in agreement with those reported by [4] where administration of rats with a toxic dose of paracetamol (750 mg/kg/day) orally for a week had resulted in an increased in plasma renin activity, hence, increasing the conversion of angiotensin I to angiotensin II in plasma. The latter acted directly on aldosterone-producing cells of zona glomerulosa. Another *in vitro* study revealed a direct effect of paracetamol on steroidogenesis and secretion of aldosterone by using human cortical cell line [18]. Moreover, previous studies found that paracetamol treatment elevated aldosterone secretion and increased arterial blood pressure in rats [19]and the concentrations of plasma aldosterone were elevated in patients with fulminant liver failure due to paracetamol overdose [20].

Furthermore, current study showed a significant elevation ($P<0.05$) in the mean thickness of zona fasciculata and zona reticularis in the paracetamol alone treated group when compared with the control and other groups. These data might be attributed to hypertrophy in the adrenocortical cells of these zones and increased production of glucocorticoids, cortisol and androgens that are secreted from zona fasciculata and zona reticularis as a result of an increase in ACTH which is controlled by the hypothalamus-pituitary-adrenal axis (HPA) [17]. In addition, the stress resulting from injecting the animals with low toxic dose of paracetamol may be a reason for increasing cortisol secretion. It was found that the orally administered acetaminophen at a dose of 1gm/kg/bw once a day for a week had increased the weight of adrenal glands [21]. It was also observed that the treatment of human adrenocortical cells with paracetamol increased the secretion of progesterone and aldosterone while inhibiting the secretion of cortisol, androgens and other glucocorticoids. It was suggested that these findings due to absence of HPA mechanism which is regulated by negative feedback when studied *in vitro* [18].

It is well-known that increased cortisol secretion increases glycogen storage in the liver, but previous studies had shown that giving high dose of paracetamol caused depletion of liver content of glycogen and induced liver necrosis when glutathione (GSH) stores are depleted in cytoplasm and mitochondria of hepatocytes [22,23] where liver injury disrupts its function as a glycogen reservoir [17].

The significant decrease ($P<0.05$) in the mean diameter of adrenal medulla may be due to depletion of adrenomedullar cells from epinephrine and norepinephrine as a result of their increased rate of secretion in response to stimulation of sympathetic nervous system by paracetamol-mediated stress. The latter results in increasing basal metabolic rate in the body with subsequent enhancement of glycogenolysis in the liver and muscles, increasing glucose release into the blood and reducing insulin hormone [17]. It was found that the large dose of paracetamol

caused depletion of liver glycogen content which is associated with hyperglycaemia [24].due to release of stress hormones or due to the direct effect of acetaminophen or its metabolites on the liver and pancreas [25].

The administered dose of paracetamol in present study did not show a significant effect on overall adrenal glands' diameter. It appears from the results of present study that the significant increase in the mean cortical thickness, including its three zones, and the apparent decrease in the mean diameter of the medulla were almost proportional so that the overall mean diameter of the gland, apparently, did not change.

In addition, data obtained from current study (Table 1) showed that giving mice 0.3 ml of crude oil of black seed caused a significant decline in mean diameter of adrenal gland (586.2±72.92µm; P<0.05), the mean thickness of zona fasciculata (57.8±4.87µm) and the mean diameter of the medulla (351.6±58.69µm) in comparison with control group. However, a significant decline (P<0.05) was observed in the mean diameter of the adrenal gland, the mean thickness of its cortex (290.2±41.49µm), mean thickness of the glomerulosa (33.27±3.24µm), fasciculata and reticularis (45±7.45µm) zones as compared to the paracetamol alone group. This indicated that the levels of cortisol, epinephrine and norepinephrine had likely decreased compared with control group. On the other hand, when compared to the paracetamol alone group the administration of crude oil of black seed might have reduced levels of aldosterone, glucocorticoids, cortisol, corticosterone and androgens which are normally secreted by the cortex. Taken together, it can be concluded that the crude oil of black seed has preserved adrenal function to near normal. In a study on rats, a significant decrease in plasma levels of glucose, cholesterol and cortisol was observed when animals, exposed to stress, were administered the ethanolic extract of *Nigella sativa* [13]. Also, it was found that giving water extract of black seeds to rats for three weeks resulted in elevated glucagon, corticosterone and ACTH in plasma of healthy and alloxan-induced diabetic rats. These data suggest that the black seed has an effect on increasing the metabolism of glucose by increasing serum insulin concentration and inhibition of the (HPA) [12].

Treatment with a single toxic dose of 300 mg/kg body weight of paracetamol together with 0.3 ml of crude oil of black seed showed significant reduction (P<0.05) in the mean diameter of adrenal gland (509±28.75µm) and the mean thickness of its cortex (238.6±48.18µm) as well as a significant decrease (P<0.05) in the mean thickness of zona fasciculata (54.2±4.15µm). However, there was non-significant decrease in the thickness of zona glomerulosa and zona reticularis and in the diameter of medulla as compared to control group. While the decline was significant (P<0.05) in all of these variables, the mean diameter of medulla was constant as compared with paracetamol alone group. These results might be attributed to the anti-stress effect of *Nigella sativa* oil. Moreover, the results of current study showed that the treatment with *Nigella sativa* oil alone caused a significant decline in the diameter of adrenal gland and the thickness of its cortex, including the glomerulosa, fasciculata and reticularis zones as compared with group of mice injected only with paracetamol which showed a marked increase in these variables compared to the control group. Consequently, there is a decrease in the production and secretion of adrenal hormones in the fourth group that decreased energy production required to resist stress [17]. These findings are in agreement with one of the studies carried out on rats that had shown that giving the water extract of black seed reduced the concentration of corticosterone and ACTH, but increased the level of insulin and reduced glucagon in plasma [12]. Also, the ethanol extraction of the same plant reduced the level of plasma cortisol and reduced blood glucose [13]. These observations indicated that *Nigella sativa* oil has enhanced resistance to the stressful effect of low toxic dose of paracetamol.

From the results of current study it can concluded that the crude oil of *Nigella sativa* has an effect on the adrenal gland function against the stress produced by injection of low toxic dose of paracetamol. The latter stimulated adrenal glands as was noticed from the significant increase in the adrenal cortex layers and the depletion of medulla content that caused the significantly decrease in its diameter. The crude oil of *Nigella sativa* had inhibited paracetamol-induced effects in mice treated with crude oil of *Nigella sativa* 0.3 ml orally with or without the injection of the sub-lethal dose of paracetamol drug. In addition, the adrenal glands weight and its diameter has decreased as well as a decrease in the thickness of glomerulosa, fasciculata and reticularis zones while preserving the diameter of medulla to near normal level. These effects of crude oil of *Nigella sativa* could be interpreted in light of its possible regulatory effects on the hypothalamus-pituitary-adrenal axis. Further studies are recommended to measure the levels of stress hormones; aldosterone, corticosterone, epinephrine, norepinphrine and ACTH to enhance results of the current study.

Table 1 Effects of administration of a sub-lethal dose of paracetamol (300mg/kg/bw) alone and in combination with crude oil of *Nigella sativa* (0.3ml) on adrenal glands

Variables	Treatments				LSD Value
	Normal saline (Control)	Paracetamol 300mg/kg/bw	Crude oil of *Nigella sativa* (0.3ml)	Paracetamol (300mg/kg) and Crude oil of *Nigella sativa* (0.3ml)	
Mean adrenal glands weight (mg)	15.18±5.83	13.96±7.32	8.7±2.91	8.4±2.33 **a**	6.754
Mean adrenal gland diameter (µm) (4x)	1022.8±94.4	986.8±154	586.2±72.92 **ab**	509±28.75 **ab**	132
Mean cortical thickness (µm) (10x)	360.8±119.9	496.2±98.53 a	290.2±41.49 **b**	238.6±48.18 **ab**	112.43
Mean zona glomerulosa thickness (µm) (40x)	36.61±2.59	91.52±4.65 **a**	33.27±3.24 **b**	33.15±4.14 **b**	5.016
Mean zona fasciculata thickness (µm) (40x)	86.8±6.83	161.2±23.04 **a**	57.8±4.87 **ab**	54.2±4.15 **ab**	16.67
Mean zona reticularis thickness (µm) (40x)	52.6±5.03	88±24.26 **a**	45±7.45 **b**	43.4±7.8 **b**	18.115
Mean medulla diameter (µm) (10x)	453.8±68.03	266.2±59.25 **a**	351.6±58.69 **a**	365.8±105.93	101.24

Values are expressed as mean ± standard deviation.

a: Significantly different (P<0.05) from control group in the same row. ;

b: Significantly different (P<0.05) from paracetamol group (300 mg/kg) in the same row.

; LSD: Least Significant Dose.

Ethical Clearance: It was obtained from the Scientific Research Committee at College of Dentistry/ University of Babylon, Iraq.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

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