# Protective effect of nigella sativa seeds against dimethoate induces histopathological and hematological changes in local layer chickens

A. Gh. Rhayf H. A. Naji M. Abdulrazzaq Coll. of Vet. Med./Unive. of AL-Qadissiya

#### Abstract

The objective of this work was designed to investigate some histopathological and hematological changes produced by dimethoate on the local layer chicken and the protective effect of nigella sativa seeds. Thirty local layer chickens were divided randomly in to (3) groups. The first group was untreated and served as control. The second group was orally given daily a dose of dimethoate at level (8) mg/kg B.W for 4weeks. The third group was orally supplemented with nigella sativa seeds 20mg/kg diet and then received dimethoate at same dose in second group.Hematological parameter were estimated after 4 weeks RBCs count, WBCs count and Hb concentration show significant (p<0.05) reduced compared with control group .Histopathologically dimethoate produce changes observed in the liver were swelling and vaculation of hepatocyte, atrophy, pycnosis, focal necrosis and infiltration of lymphocyte around portal vein.Kidney show some changes characterized by hemorrhage and congestion, degenerative changes of epithelial layer of tubules also there were several changes observed in brain like vaculation of glial cell, dilatation of small blood vessels of brain .where as coadministration of nigella sativa to the diet of dimethoate treated chickens ameliorated the hematological and histological impairment. There was significantly (P<0.05) reduced these changes in liver, kidney and brain also significantly (p<0.05) increase hematological parameter compared with dimethoate treated group.

#### Introduction

The control of insect pest relies heavily on the use of synthetic insecticides, but their wide spread use has lead to some serious problems including toxic residues on grass and toxicity to non- target organisms such as mammal, bride and fishes (1; 2 and 3). Among them, organophosphates (OPs) have been used for almost five decades in agriculture, veterinary medicine and industry as lubricants, plasticizers and flame-retardants. Their uncontrolled use in agriculture and public health operations has increased the scope of ecological imbalance non-target and many organisms consequently become victims (4). Dimethoate (DM), an important organophosphorus pesticide, is frequently used against a wide range of insects and mites and for indoor control of houseflies

(5). Generally, the majority of the population is chronically exposed to low doses of DM via food, contaminated drinking water, or by application of household insecticides containing DM (6, 7). Is primarily known as neurotoxin (8). It is also known to inhibit metabolic enzymes (9).In fact, the toxic effects of OP induce several pathologies like testes atrophy, chronic renal diseases. parathyroid hyperplasia, benign and malignant neoplasm of the endocrine organs, lymphatic system and liver disorders (10), earlier studies have shown that acute and sub chronic exposure to Dimethoute alter the antioxidant status and the histolology of liver and brain in rats (11; 5; 12; 13). Involvement of oxidative stress following exposure to dimethoate and to OPI in general has been reported (14;

15) and it has been demonstrated that lipid per oxidation mediated by free radicals is one of the molecular mechanisms involved in OPI-induced toxicity (16). The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress.Natural antioxidants from fruits and vegetables are reported provide to substantial protection that slow down the process of oxidative damage caused by reactive oxygen species (ROS) (17), among the promising medical plant.Negella sativa a dicotyledon of the ranunculacease family is an amazing herb with rich historical and religious background the seed of negella sativa are the source of active ingredients of this plant (18) .The antioxidant effect of

# **1-Materials:**

Dimethoute suprathwaite (100ml) was applied as commercial emulsifiable concentrate formation containing (40 %) active ingredient is dilated in water for the final concentration.Negella sativa seed were purchased from a local market with highly degree of quality assurance.

#### 2-Animals:-

Six mounts local layer chickens were supplied by local commercial producer and were divided into(3) group of (10) of each .They were housed in temperature  $(27\pm1c^{\circ})$ and light (14 hr /light : 10 hr/ dark) controlled room with the provision of grower feed and water ad libitum after one week of acclimatization.

# **Experimental design:**

The birds were randomly divided in to (3) equal group:-

1-First group: untreated group and served as control.

**2-** Second group: administered orally with dimethoate (8) mg/kg B.W daily for four weeks. The dose correspond (20%) of the oral LD50 (20).

negella sativa has been examined using different hepatic and urinary toxicity vivo murrain models induced by (CCL 4) ,doxorubicin ,gentamicin ,methionine (18) .However ,there are not a single report is available at present on dimethoute toxic pathology on liver ,kidney ,brain in birds .In general literature on dimethoute toxicity in birds is meager (19) also in view of this it is much interest and particle importance to study the influence of plant products on induce oxidative stress by pesticide exposure Therefore the present study was primarily aimed to investigate the possible and hematological pathology changes induce by dimethoute on kidney ,liver and brain of chicken.

# Materials and methods

**3-** Third group: were orally supplemented with nigella sativa seeds sativa (20) mg/kg diet (21) with dimethoute at dose (8) mg/kg B.W.

# 3-Blood test:-

On the last day the birds were sacrificed, blood of each was collected by cardiac puncture method. The blood samples were maintained in EDTA Bulb and plain tube for assay of blood RBC, WBC, Hb.

# 4-Histopathological examination:-

Sample from kidney, liver and brain was intended for histopathological examination by light microscopy, were immediately fixed in (10%) of formalin and processed in series of graded ethanol solution. They were embedded in paraffin, serially section at (5Mm) and stain with H&E (Luna; 1968).the lesion were generally scored as no change (-), mild (+), moderate (++), and sever (+++).

#### 5-Statistical analysis:-

The result were expressed as means  $\pm$  SEM , Histopathology scores were analyzed by (ANOVA) and T-test to know significant differentiated between the values on a level (p<0.05).

#### -Hematological studies:

Data of hematological parameter after (30) days are shown in table (1). Oral administration of dimethoate at 8mg /kg B.W. (20% LD50) in local layer chickens produce significant (p< 0.05) reduction in WBCs count, RBCs count, Hb concentration compared untreated control group (group 1). While co- administration nigella sativa with dimethoate (group3) significantly (p< 0.05) restored these parameters when compared with dimethoate- alone treated group (group 2).

- **Histopathological studies:** Section from the liver, kidney and brain from treated birds were examined under light microscope.

-The liver: Dimethoate intoxication exhibited severe changes such as swelling of hepatocyte with cytoplasm vaculation (figure1). These changes are severe (2.500± 0.223) in second group (dimethoate group) compared with third group (nigella sativa co- administration with dimethoate) (figure2) the change is mild or not found (1.166±0.477) and control group (group 1)  $(0.000\pm0.000)$ . There were lymphocytes infiltrations were observed around portal vein with focal necrosis (figure 3). These changes observed in the liver and these injury was significantly increase (2.666±0.210)in the second group compared with third group $(1.333\pm0.421)$  in which there were mild changes(figure control 4) or  $group(0.000\pm0,000)$ ( table 2). Also there were

#### Results

dilation of hepatic sinusoid due to atrophy of hepatic cells and dark stained nuclei indicating cell pycnosis.(figure5) it was more severe in second group( $2.666\pm0.333$ ) compared with third group ( $1.333\pm0.333$ ) and control group ( $0.000\pm0.000$ ) as in table 2.

-Kidney: Histological studies have shown many abnormalities in kidney characterized by severe and congestion hemorrhage (figure 6) (2.666±0.216) in dimethoate group compared with (dimethoate co- administration with nigella sativa group)  $(1.500 \pm 0.223)$  and control group  $(0.000\pm0.000)$  as in table 3. Also there were severe degenerative changes of tubular epithelial layer characterized by sloughing of epithelial cells from basement tubular membrane (figure 7) in second group (dimethoate group) (2.833±0.1666).These changes were moderate (figure 8) $(1.333\pm0.210)$ in third group (dimethoate co- administration with nigella sativa) and control group  $(0.000\pm0.000)$  as table 3.

<u>-Brain</u>: Histological section showed abnormalities characterized vaculated spaces in glial cells, gliosis and finely branching small blood vessels (figure 9 and 10) these changes were observe in second group(dimethoate group) ( $2.500\pm0.223$ ) compared with third group (dimethoate co-demonstration with nigella sativa) it was mild ( $1.333\pm0.421$ ) and control group ( $0.000\pm0.000$ )( table 4).

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Groups	Hb(mg/dl)	RBCs*106	WBCs*10 <sup>3</sup>	
Control	15.333±0.6666 <sup>a</sup>	3.400±0.1238 <sup>a</sup>	15.000±0.7745 <sup>a</sup>	
Dimethoate group	9.500±0.4281 <sup>b</sup>	1.900±0.0730 <sup>b</sup>	8.666±0.7264 <sup>b</sup>	
Nigella sativa gr goup+dimethoate	12.333±0.5577 °	2.533±0.1358 <sup>c</sup>	11.166±0.5426 <sup>c</sup>	

Table 1:- Effect of oral administration of dimethoate on hematological parameters in local layer chicken and protective effect of nigella sativa seeds.

-Figures represent mean  $\pm$  stander error.

-Different letters represent significant difference between groups horizantally at (p < 0.05).

Groups Lesions score	Control	Dimethoate	Nigella S sativa +dimethoate	
-Swelling and va vaculation of he hepatocyte.	$0.000 \pm 0.000^{a}$	2.500±0.223 <sup>b</sup>	1.1666±0.477 «	
Hepatocyte a atrophy and p piknosis	0.000±0.000 <sup>a</sup>	2.666±0.333 <sup>b</sup>	1.333±0.333 °	
Focal necrosis and in infiltration of ly lymphocyte.	0.000±0.000 <sup>a</sup>	2 2.666±0.210 <sup>b</sup>	1.333±0.421°	

Table 2:- Mean of pathological lesion in liver.

-Figures represent mean  $\pm$  stander error.

-Different letters represent significant difference between groups vertically at (p < 0.05)

Table 3: Mean of pathological lesion in kidney.

Groups Lesion score	control	dimethoate	Nigella S sativa +dimethoate
Hemorrhage and co congestion	0.000±0.000 <sup>a</sup>	2.666±0.216 <sup>b</sup>	1.500± 0.223 °
degeneration of tubular epithelial cells	$0.000 \pm 0.000$ <sup>a</sup>	2.833±0.1666 <sup>b</sup>	1.333±0.210 °

-Figures represent mean  $\pm$  stander error.

-Different letters represent significant difference between groups vertically at (p < 0.05).

<u>Table 4</u>:- Mean of pathological lesion in brain.

		0	
Groups Lesion score	control	dimethoate	Nigella sativa+dimethoate
-vac vaculation of glial cells, and small blood vessels dilitation in brain	0.000±0.000 <sup>a</sup>	2.500±0.223 <sup>b</sup>	1.333±0.421 <sup>c</sup>

-Figures represent mean  $\pm$  stander error.

-Different letters represent significant difference between groups vertically at (p < 0.05)



Figure (1): Histopathological section of liver treated with dimethoate show swelling of hepatocyte (arrow) with vaculation of cytoplasm (double arrow). (X 100H&E).



Figure (2): histopathological section of liver co-treated nigella sativa with dimethoate.show mild swelling and not found vaculation of cytoplasm. (X50 H&E)



Figure (3): Histopathological section of liver treated with dimethoate show infiltration of lymphocyte (arrow) with focal necrosis of hepatocyte (Double arrow). (X100 H& E).



Figure (4): Histopathological section of liver treated with dimethoate show dilated of hepatic cord due to atrophy of hepatocyte (arrow) and in some cells show piknosis (double arrow)(X100H&E)



Figure (5): Histopathological section of liver co-treated nigella sativa with dimethoate show mild degenerative change (X100H&E)



Figure (6): Histopathological section of kidney treated with dimethoate show congestion and hemorrhage (arrow). (X100H&E).



Figure (7): Histopathological section of kidney treated with dimethoate show necrosis (arrow) and sloughining of epithelial cells of tubules (double arrow) (X100H&E).



Figure (8): Histopathological section of kidney co-treated nigella sativa with dimethoate show moderate degenerative changes in epithelial tubules(X100H&E).



Figure (9): Histopathological section of brain treated with dimethoate show vaculation (arrow) and dilatation of blood vessels (double arrow). (X100 H&E).



Figure (10): Histopathological section of brain treated with dimethoate show vaculation (arrow) (X100H&E).

#### Discussion

Dimethoate is an organophosphate insecticide known to produce oxidative stress in human and animal cells. As a lipophilic molecule, it can easily pass through the cell membrane into the cytoplasm. Once inside the cell, dimethoate can induce a high level of damages in several tissues (23). In the present study, exposure of birds to (DM) orally resulted in a significant decrease of hematological parameter (RBCs) count and (Hb) compared to control group and nigella sativa coadministration with dimethoate these results were agreed with (24) showed, that the R.B.C. count and Hb level significantly

decreased in the blood of Dimethoute and Diazinon treated rabbits compared to control. The decrease in Hb along with the decrease in RBC might be due the effect of pesticides on blood forming organ (bone marrow and liver) and inhibition of many hem biosynthesis.Also (25) step of suggested that the decrease in RBC count is either indicative of excessive damage to erythrocytes or inhibition of erythrocyte formation. Moreover, the hepatic hem biosynthesis has already been reported to be affected by insecticidal exposure, which also contributes to decreased RBC count and hemoglobin concentration (26). Also our

study show significantly decrease in WBCs count in second group compared to control group and nigella sativa co- administration with dimethoate group. These result agreed with (27) they report that the reduction in WBCs of the birds exposed to chloropyrifos and methidathion may be due to depression of production of WBCs by haemabiotic system.Histopathologically our result shown that oral administration of dimethoateinduced hepatotoxicity characterized by hepatocyte enlargement with cytoplasmic vaculation, infiltration of lymphocyte, focal necrosis, dilatation of sinusoids due to shrinkage of hepatocyte and some cell had dark stain nuclei. Our results are in agreement with similar data reported in different experimental models of rats exposed to dimethoate and other pesticides (15, 11) and confirm the pathogenic role of oxidative stress in the liver. Also our results is agreed with (28) they report dilatation of hepatic moderate sinioside, vacular degeneration in chicken administration of chlorpyrifose also there are several study reported that Dimethoate intoxication exhibited severe histopathological changes in the liver such as mononuclear cells infiltration, congestion, enlargement of the hepatic sinusoids and enlargement of the portal central and the veins and hepatocellular damage in rats (29) and mice (30). That occurs due to dimethoate intoxication can cause oxidative stress by the generation of free radicals and induce hepatic lipid per oxidation in mice (15) and rats (5). The kidney is another organ sensitive to external factors which induce histopathological changes. The main changes reported in this study were hemorrhage and congestion in the glomeruli and proximal tubules and degenerative changes characterized by necrosis and sloughing in epithelial layer of tubules. This result is agreed with (31) they reported in kidneys, necrosis of tubular epithelial cells, cytoplasmic vaculation, cellular infiltration

and atrophy of glomeruli were observed. Sub-arachnoids space was much dilated in CY-treated broiler chicks and another investigation on the histological changes in kidney were done by (32) which show a narrowed Bowman's space, degenerative of tubular epithelial cells and widened tubular lumen seen in the kidney of mothers rats and their pups treated with DM .one possible mechanism for tubular lesions observed was the direct toxic effect on the cell function. Other possible mechanism for the tubular lesions may involve reactive free radical or oxidative stress or both (33).In our results histological section of brain show cytoplasmic vaculation of glial cells and dilatation of small blood vessels in brain these changes were agreed with (34) they report severe distortion in cellular architecture were observed in cerebral cortex of dimethoate- treated rats. And (28) report that brain of chicken administration chlorpyrifose showed mild neuronal degeneration and vaculation in purkinje cells. This can be due to reactive oxygen species which may contribute, as reported by (35). However, the lipophilic nature of OPs facilitates their interaction with the cell membrane and leads to perturbations in the phospholipids bilayer structure, enhancing the production of reactive oxygen species (ROS), which in turn generate oxidative stress in different tissues (36).Our results obtained from the present study showed that the Nigella sativa seeds could mostly protect blood, liver, kidney and brain from severe alterations induced by exposure to dimethoate and this agreed with (37) they report that when. N. sativa oil, given to rats, prevented the formation of unwanted free radicals and protected them against acute dimethoate exposure. and (38) report that Nigella sativa seeds can be considered as a promising therapeutic agent against hematotoxicity,

immunotoxicity, hepatoxicity, nephrotxicity and cardio toxicity induced by Diazinon and may be against other chemical pollutants, environmental contaminants and pathogenic factors.

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# التأثير الواقي لبذور الحبة السوداء لتقليل التغيرات الدموية والنسيجية في الدجاج التأثير الواقي لبذور الحبة المحلي البياض.

اطياف غانم رهيف النائلي هالة عباس ناجي محاسن عبد الرزاق كلية الطب البيطري /جامعة القادسية

#### الخلاصة

صممت هذه الدراسة لمعرفة التغيرات الدموية والنسيجية المرضية الناتجة من التاثير السمي للدايمثويت في الدجاج ومعرفة تاثير الحبة السوداء لتقليل السمية . استخدمت في هذه التجربة (٣٠) دجاجة قسمت عشوانيا الى ثلاث مجاميع . المجموعة الأولى عوملت على انها مجموعة ضابطة واعطيت المجموعة الثانية(٨) ملغم/ كغم من وزن الجسم دايمثويت فمويا مرة في اليوم لمدة (٣٠) يوما اما المجموعة الثالثة فقط اعطيت(٢٠) ملغم/كغم من وزن الجسم حبة سوداء مع العليقة ثم اعطيت دايمثويت فمويا نفس الجرعة في المجموعة الثانية. وفي نهاية التجربة فحصت المؤشرات الدموية التالية: عدد خلايا الجم الحمراء، عدد خلايا الدم البيضاء والهيموكلوبين وكذلك التغيرات النسيجية في الكبد ، الكلية والدماغ. أظهرت المؤشرات الدموية التالية: عدد خلايا الجم الحمراء، عدد خلايا الدم البيضاء والهيموكلوبين وكذلك التغيرات النسيجية في الكبد ، الكلية والدماغ. أظهرت المؤشرات الدموية انخاضا معنويا (٥٣) في ماطنية الثانية مقارنة معرموعة السيطرة وقد شملت التغيرات النسيجية في الكبد زيادة في عدد الخلايا اللمفاوية خاصة في منطقة الوريد الاوسط وموت بعض المجموعة المعرورة وقد شملت التغيرات النسيجية في الكبد زيادة في عدد الخلايا المفاوية خاصة في منطقة الوريد الاوسط وموت بعض الخلايا مما دى الى ظهور بعض الفجوات كذلك ظهر توسع في الحبال الكبدية نتيجة ضمور في الخلايا الكبدية كما ظهرت بعض الخلايا ما دى الى ظهور بعض الفجوات كذلك ظهر توسع في الحبال الكبدية نتيجة ضمور في الخلايا الكبية كما ظهرت بعض الخلايا ما دى الى ظهور بعض الفجوات كذلك ظهر توسع في الحبال الكبدية نتيجة ضمور في الخلايا الكبدية كما ظهرت بعض الخلايا ما دى الى ظهور بعض الفجوات كذلك ظهر توسع في الحبال الكبدية نتيجة ضمور في الحلايا الكبدية كما ظهرت بعض الدماغ كذلك وجود توسع في الاوعية الدموية الصغيرة في نسيج الدماغ اظهرت الدراسة ان اضافة الحبة السوداء للعليقة في المجموعة الثالثة اظهر انخفاض معنوي (p<0.05) في التغيرات النسيجية في الكبد والكلية والدماغ الناتجة في المجموعة الثانية وكذلك اظهرت ارتفاع معنوي(p<0.05) في المؤشرات الدموية في المجموعة الثالثة مقارنة بمجموعة السيطرة .