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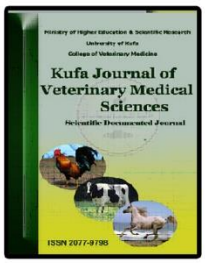

The Ameliorating Effect of *Nigella Sativa* Seeds against Chlorpyrifos Poisoning in Local Layer Hens

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Summary:

The use of pesticides has beneficial control of pests but it causing the damaging pollution to the environment. There are increasing successful efforts to minimize the destroying influence of these chemical on the life of laying hens. Here, the study was intended to explore the proposed effect of *Nigella sativa* (*NS*) seeds on local laying hens that were experimentally exposed to chlorpyrifos (CPF) poisoning in Al-Diwaniyah province. A population of 40 hens was randomly recruited as 10 birds per group. T1 group was treated daily (3.42) mg/kg B.W orally with CPF only, T2 group was treated together with 20 mg/kg *NS* seeds by diet and CPF (3.42) mg/kg B.W orally, T3 group was previously treated with20mg/kg *NS* by diet for 14 day followed together for 4 weeks by the oral use of CPF and *NS* by diet at same dose , C the control group no treatment received. The experiment continued for 30 days and followed by submitting blood and tissue samples to biochemical analyses and histopathological examination. In a comparison to the C, the result reveals that T1 had significant increases in Aspartate transaminase (AST) and Alanine Transaminase (ALT)enzymes ($P<0.05$) with no significant differences in Alkaline phosphatase (AIP) level ($P>0.05$). T2 and T3 didn't show any significant differences in the levels of all the liver enzymes ($P>0.05$) when compared to C. According to the comparison with C, T1 shows high significant differences in the level of Uric Acid, Creatinine, Triglyceride, and Total Protein while T2 reveals significant increases in the levels of Creatinine and Total Protein only ($P<0.05$). Interestingly, T3 doesn't show any statistical differences when compared to C. Histopathological changes in T1 include necrotic areas and deposition of hemosiderin in the spleen, destruction of the intestinal mucosa, edema and hemorrhage in the lung, degeneration of ciliated columnar epithelium, and proventricular infiltration of inflammatory cells between follicles. T2 shows mild depletion of white pulp and proliferation of red pulp in the spleen, extended and thinner intestinal villi with mild desquamation of epithelial cells with little hemorrhage, and congested with some hemorrhage plus mild thickening of interstitial tissue in the lung. T3 reveals splenic proliferation of white pulp with small and thickened arteriolar walls, proliferation of red pulp, normal extended intestinal villi, and mild congestion with mild alveolar emphysema in the lung. C reveals normal tissue without any changes. The study result comes to enhance the laying hen industries and provide future interests to supply *NS* as an additional or main part of the ration to prevent the poisoning effects of this chemical.

Keyword: Nigella sativa, CPF poisoning, local layer hens.

التأثير المحسن لحبة البركة ضد التسمم بالكلوربايروفوس في الدجاج المحلي البياض

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الخلاصة :

الهدف من دراستنا هو التحقيق في تاثير حبة البركة لتقليل السمية في الدجاج المحلي المصاب بالتسمم يمبيد الكلوربايروفوس في محافظة الديوانية . استخدمت في هذه الدراسة اربعون طيرا ,قسمت الى اربعة مجاميع كل مجموعة تتكون من عشرة طيور , مجموعة السيطرة لم تعامل باي شي , المجموعة الاولى عوملت باعطاء مبيد الكلوربايروفوس فمويا فقط مرة في اليوم بجرعة(3.42) ملغم/ كغم من وزن الجسم, المجموعة الثانية اعطيت سويا في نفس الوقت مبيد الكلوربايروفوس فمويا مرة في اليوم بجرعة(3.42) ملغم/ كغم من وزن الجسم و (20) ملغم/كغم من وزن الجسم حبة البركة بالعليقة, والمجموعة الثالثة كوقاية اعطيت مسبقا بالعليقة (20) ملغم/كغم من وزن الجسم مادة حبة البركة لمدة 14 يوم ومن بعدها لمدة اربع اسابيع اعطيت سويا مبيد الكلوربايروفوس فمويا مع حبة البركة بالعليقة بنفس الجرعة السابقة , استمرت الدراسة لمدة ثلاثون يوما وبعد انتهاءها كل العينات خضعت الى التحليل الكيموحيوي والفحص النسيجي المرضي لقراءة التغيرات في المصل والانسجة على التوالي اظهرت النتائج ان المجموعة الاولى اظهرت فرقا معنويا مع مجموعة السيطرة في مستوى انزيم الالينين ترانس امينيز AST و انزيم الانين امينوترانسفيريز ALT بينما لم تظهر اي فرق معنوي مع مستوى انزيم الفوسفاتيزالقاعدي AIP تحت مستوى احتمال($P<0.05$), المجموعة الثانية والثالثة لم تظهر اي تغيرات معنوية مع مستويات جميع الانزيمات فيما لو قورنت مع مجموعة السيطرة ($P<0.05$), المجموعة الاولى اظهرت فرقا معنويا في مستوى حامض اليوريك والكرياتينين والكلسيريدات الثلاثية ومستوى البروتين الكلي فيما لو قورنت مع مجموعة السيطرة تحت مستوى احتمال ($P<0.05$),المجموعة الثانية اظهرت فرقا معنويا وبزيادة في مستوى الكرايتينين والبروتين الكلي فقط اذا قورنت بمجموعة السيطرة تحت مستوى احتمال ($P<0.05$), المجموعة الثالثة لم تظهر اي فرق معنوي مع مجموعة السيطرة. التغيرات النسيجية في المجموعة الاولى تضمنت مناطق متنخرة وترسب مادة الهيموسدرين في الطحال, وتحطم في مخاطية الامعاء , الرئة اظهرت اوديما مع نزف وتنكس في الطبقة الطلائية العمودية المهدبة, في المعدة الحقيقية يوجد ترشح خلايا اللتهابية بين الحويصلات , اما في المجموعة الثانية فقد اظهرت تلاشي او ضمور طفيف في اللب الابيض وتكاثر خلوي في اللب الاحمر في الطحال , وجود زغابات ممتدة ونحيفة جدا في الامعاء , وزوال الكيراتين الطفيف في الخلايا الطلائية مع نزف قليل . اما في الرئة فقد اظهرت وجود احتقان مع نزف قليل مع تثخن طفيف في الانسجة الخلالية , المجموعة الثالثة اظهرت العديد من التغيرات في الطحال مثل وجود واسع وتكاثر خلوي في اللب الابيض مع وجود شرينات ثخينة وصغيرة محاطة بالتكاثر الخلوي في اللب الاحمر , في الامعاء اظهرت وجود زغابات طبيعية ممتدة , في الرئة اظهرت احتقان طفيف في انسجة الاوعية الدموية , بقية الحويصلات اظهرت نفاخ رئوي طفيف . في المعدة الحقيقية فقد اظهرت حويصلات طبيعية والتي تبطن بخلايا طلائية طبيعية , في مجموعة السيطرة اظهرت انسجة طبيعية بدون اي تغيرات . بالامكان الاعتبار ان حبة البركة لها دور ايجابي كبير على الدجاج البياض المحلي المصاب بالتسمم بواسطة الكلورباريفوس في ازالة السمية والمساعدة في حمايته من التسمم .

الكلمات المفتاحية : حبة البركة , التسمم بالكلوربايريفوس , الدجاج المحلي البياض

Introduction:

The human food consumption relays on one important sector of industries which is the poultry industry. This field can be recognized as two main branches, which are egg production and meat production. Layer hens are the main players in the egg production (1). Globally, layer hens fill a large share of this industry by providing the amazing source of eggs (2). This important industry faces difficulties and obstacles that prevent much improvement. These problems could be declared as but not limited to diseases and chemical poisoning by some of the contaminants such as CPF, a chemical used to eradicates pests (3, 4). CPF is a poisonous organophosphorus pesticide which is widely used to control various indoor and outdoor pests (5,6,7). Studies and management routine tests have found that CPF substance and its derivatives are detected in meat, dairy products, and egg (8, 9). In addition, hen food exposure to these chemical contaminants leads to disturbing some important physiological functions such

as the cholinesterase enzyme system (10, 11) and cause cellular oxidative stress (5,6, 7). Our nature is so rich with unique compounds that are found in fruits and vegetables. These substances play very important roles as antioxidants to overcome the oxidative stress generated by the exposure to CPF (12). Flavonoids, garlic, tocopherol, fatty acids, and ascorbic acid are food components which apply very great and positive impact to decrease or prevent the harmful effects brought by the contamination of hen ration by these pesticides (13, 14,15,16).

NS also named black-caraway or black cumin is a plant that belongs to the Ranunculaceae family. With linear leafs, this precious plant grows in south and southwest of Asia. The improvement of health, immunostimulator, and antioxidant activity are some of the benefits that this plant could enhance to the body (17, 18). *NS* has been used with some substances such as CCl_4, doxorubicin, gentamicin, and methionine (19). For all those reasons discussed above, this study was initiated to explore the possible preventing effect of *nagilla sativa seeds* on some of the physiological and histopathological features when hens exposed to chloryrifos poisoning via food.

Materials and methods:

This study was performed in the Animal House, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

Materials:

A commercial product of CPF (48%) active ingredient was purchased from local retailers in Al-Diwaniyah province. The product had been further diluted with water to reach the administrable concentration. *NS* was also purchased from local retailers in a high quality product.

Animals:

A total of 40 local laying hens of 24 week old had been purchased from a local commercial producer. The hens then were housed under (27±1) Cº and 14 to 10 hours light to dark hour ratio in controlled room. The food and water were supplied and the experiment started after 7 days of the hen acclimation to the experimental environment.

Experimental Design:

The 40 hens were randomly assigned to 4 groups which are T1, T2, T3, and the control group (C) at 10 hens per group. The experiment continued for 30 days. The groups received the treatment that is shown in table (1)

Table (1): the groups and types of treatment.

The group	Treatment
C	**No treatment**
T1	**CPF (3.42 mg/kg B.W) orally (20)**
T2	***NS* (20 mg/kg diet) (21) + CPF (3.42mg/kg B.W) orally**
T3	***NS*(20 mg/kg diet) alone for 14 day as a protective dose followed by together *NS* (the seam dose) + CPF (3.42 mg/kg B.W) orally**

After the experiment had finished, the following processes were carried out

1-**Biochemical analyses:**

Non-heparinized vials were used to collect blood samples from all hens. The serum was separated using a centrifuge at 3000 rpm for 15 minutes. Then, the produced serum was used to perform the analyses of liver functional enzyme, ALT, AST, and ALP levels. These levels were

measured using a commercial kit manufactured by BioMerieux Company, France. The serum samples were also used to analyse uric acid using a commercial kit (Biomaghreb Company). Moreover, creatinine, total protein, and triglyceride were measured in the serum using commercial kits (Biolabo Company, France).

2-**Histopathological examination:**

For investigating of the histopathological changes, the hens were sacrificed and tissue samples were taken from spleen, lung, proventriculus, and intestine. The samples were dissected and kept in 10% formalin and dehydrated in ascending series of graded ethanol solutions. Then they were cleared in toluene and embedded in paraffin wax. After that, paraffin sections of 5-Mm thickness were produced and stained with H&E for light microscopic testing (22).

3-Statistical analyses:

Mean ± SE was used to display the current study data. One-way ANOVA test was used to compare and show differences between the group means. Significant differences were declared at the level of ($P<0.05$). The significance was sought by finding the least significant differences (LSD) (23).

Results:

1-Biochemical analyses:

Data shown in table (2) reveal a significant ($P<0.05$) increase in AST mean values in T1 (152.75±6.64) when compared with the values from T2 (121.2±3.21). At the levels of AST, T3 and C groups show no significant differences ($P>0.05$) (119.7±4.28) and (108.2±3.12) respectively. When testing the levels of ALP, no significant differences are noticed when comparing T1 (80.10±1.15), T2 (57.25±10.2), T3 (56.78±6.32), and C (59.66±9.66) groups. Significant elevation in the level of serum ALT is observed in the T1 group (11.21±1.15). No significant differences are present when comparing between T2 (7.2±2.1) , T3 (7.4±1.92), and C (6±3.1) groups.

Table (3) reveals significant increases in the levels of uric acid in T1 (21.66±1.88) when comparing with T2 (16.9±1.84), T3 (15.82±2.43) and C (16.2±1.4) groups. A significant increase in the creatinine level of T1 (1.78±0.32) is shown when comparing with T3 and C groups.

On the other hand, the result points out insignificant differences ($P>0.05$) between T1 and T2 groups. Interestingly, significant differences in the levels of triglyceride between T1 (73.18±4.21), T2 (79.58±3.32), T3 (81.01±2.98), and C (81.12±2.58) groups are presented; however, insignificant differences between the T2 and T3 groups when comparing with C group are not noticed. Total protein (TP) levels show significant elevations in T1 (3.92±0.92) when comparing with T3 (6.03±2.08) and C (6.23±1.22) groups, but no significant differences are revealed when comparing T1 and T2 groups ($P>0.05$).

Table (2): Effect of administered CPF and *NS* seeds on liver enzymes the in local laying hens.

Test / Treatment	AST IU/L	AIP IU/L	ALT IU/L
C group	108.2±3.12a	59.66±9.66a	6±3.1a
T1 group	152.75±6.64b	80.10±1.15a	11.21±1.15b
T2 group	121.2±3.21a	57.25±10.2a	7.2±2.1a
T3 group	119.7±4.28a	56.78±6.32a	7.4±1.92a

-Results expressed as Mean ±S.E.

- Different letters refer to significant differences at level (P< 0.05).

-Similar letters refer to no differences at level (P>0.05).

Table (3): Effect of administered CPF and *NS* seeds on various biochemicals in local laying hens.

Test / Treatment	Uric Acid mg/dl	Creatinine g/dl	Triglyceride mg/dl	Total Protein gm/dl
C group	16.2±1.4a	0.61±0.18a	81.12±2.58a	6.23±1.22a
T1 group	21.66±1.88b	1.78±0.32b	73.18±4.21b	3.92±0.92b
T2 group	16.9±1.84a	1.52±0.78b	79.58±3.32a	5.58±0.8a
T3 group	15.82±2.43a	0.72±0.91a	81.01±2.98a	6.03±2.08a

-Results expressed as Mean ±S.E.
- Different letters refer to significant differences at level (P< 0.05).
-Similar letters refer to no differences at level (P>0.05).

2-Histopathology

Using light microscope, the following different histopathological changes have been identified in the different tissue types that were collected from the experiment hens.

1- T1

In the Spleen, reveals the presence of necrotic areas and deposition of hemosiderin substance within the lymphoid tissue. Moreover, there is complete depletion of the white pulp with the proliferation of red pulp (figure 1). Plus, there is haemorrhage in the lymphoid tissue (figure 2).

For the intestine, Chlorpyifos caused severe congestion and haemorrhage in the mucosa, degeneration of epithelial cells that line the villi, high infiltration of inflammatory cells figure (3), damaged intestinal mucosa, desquamation of epithelial cells which line the villi within the lumen, and thickened muscularis figure (4).

In the case of lung, there are alveolar accumulation of edematous fluids, interstitial haemorrhage and congestion, alveolar cell lining degeneration figure(5), ciliated columnar epithelial lining degeneration of bronchi, and haemorrhage with congestion of blood vessels figure(6).

In the proventiculus, there are inflammatory cells Infiltration between follicles and follicular degeneration and destruction of columnar and epithelial liner cells figure(7) and figure (8) respectively.

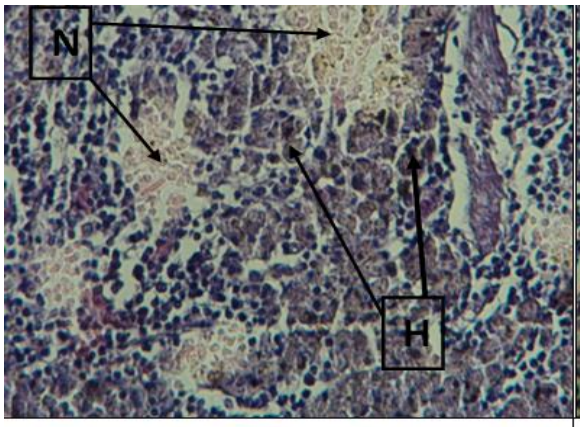

Fig. (1) Group (T1): Histopathological section of spleen reveals necrotic areas (N) and deposition of hemosidrin (H) within the lymphoid tissue. Also there is complete depletion of white pulp with proliferation of red pulp. 40X, H&E stain.

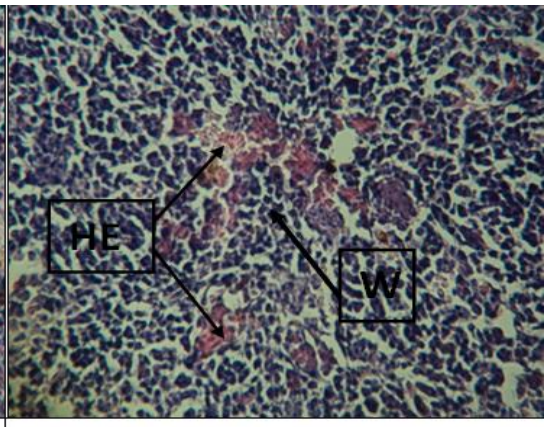

Fig. (2) Group (T1): Histopathological section of spleen reveals depletion of white pulp (W) with proliferation of red pulp also there is hemorrhage (HE) in the lymphoid tissue.40X, H&E stain.

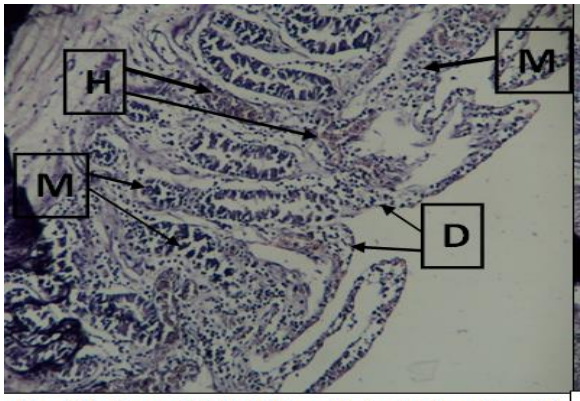

Fig. (3) Group (T1): Histopathological section of intestine reveal severe congestion and hemorrhage ©in the mucosa of intestine, also there is degeneration of epithelial cells which lining the villi (D), high infiltration of inflammatory cells (M). 10X, H&E stain.

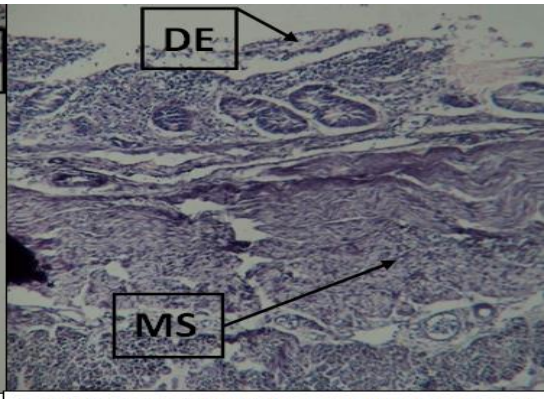

Fig. (4) Group (T1): Histopathological section of intestine reveal destruction of intestinal mucosa, degeneration and desquamation (DE) of epithelial cells which lining villi within the lumen, thickening of muscularis (MS). 4X, H&E stain.

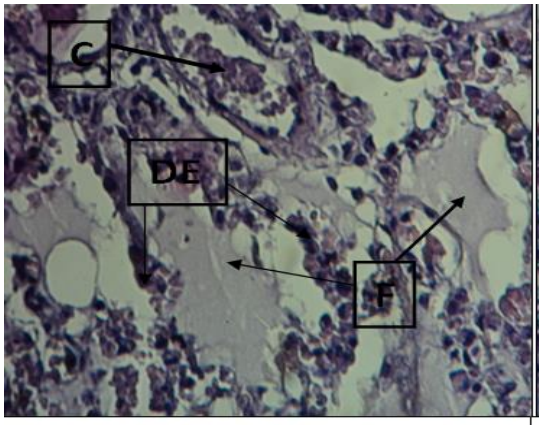

Fig. (5): Histopathological section of lung reveal Accumulation of edematous fluids(F) within the alveoli, also there is hemorrhage and congestion(C) in the interstitial tissue, degeneration of alveolar cells which lining the alveoli(DE). 40X, H&E stain.

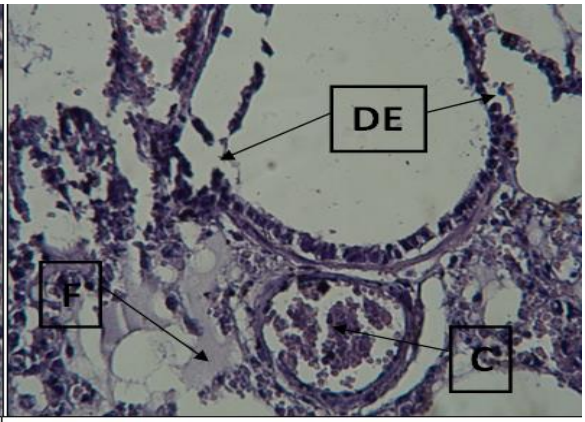

Fig. (6): Histopathological section of lung reveals Degeneraton of ciliated columnar epithelium (DE) which lining bronchus, hemorrhage& congestion of blood vessels(C), edematous fluid within the alveoli (F). 40X, H&E stain.

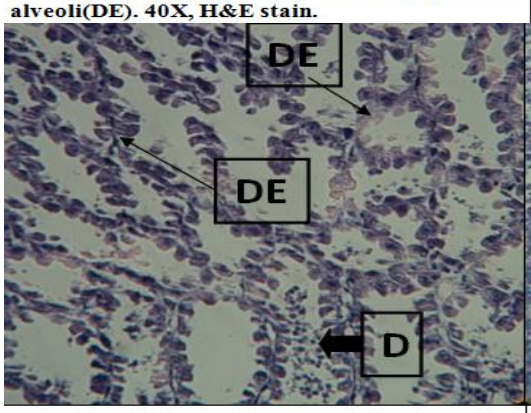

Fig. (7): Histopathological section of proventiculus reveal Infiltration of inflammatory cells between follicles (M), destruction and degeneration of columnar cells which lining the follicles of proventiculus (DE). 40X, H&E stain.

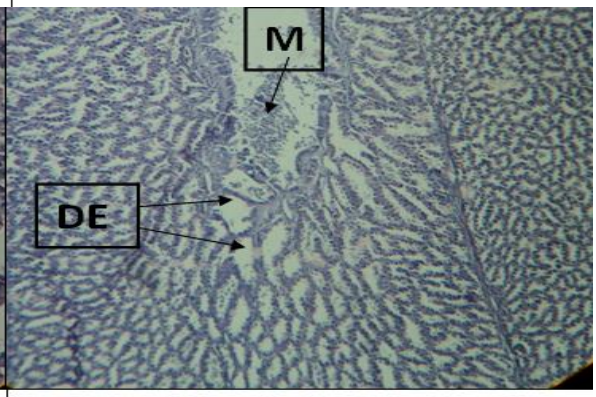

Fig. (8): Histopathological section of proventiculus reveal High infiltration of inflammatory cells (M), degeneration of epithelial cells which lining the proventiculus follicles (DE). 10X, H&E stain.

2- T2

In the spleen, There have been mild depletion of white pulp and proliferation of red pulp, mild haemorrhage within the lymphoid tissue figure (9) with thick-wall arteriole and proliferation of lymphocytes figure (10).

For the intestine, the result shows extended and thin villi with mild desquamation of epithelial liner cells figures (11) and mild infiltration of inflammatory cells in the intestinal mucosa with little haemorrhage figures (12).

In case of the lung, there are congestion and little haemorrhage, mild thickening of interstitial tissue, mild infiltration of inflammatory cells, and some alveolar emphysema figure (13).

In the proventiculus, The results show mild degeneration and desquamations of columnar liner epithelium, thick fibrous trabiculae between the follicles figure (14)**,** mild degeneration of epithelial cells, and vacuolation of other cells figure (15)**.**

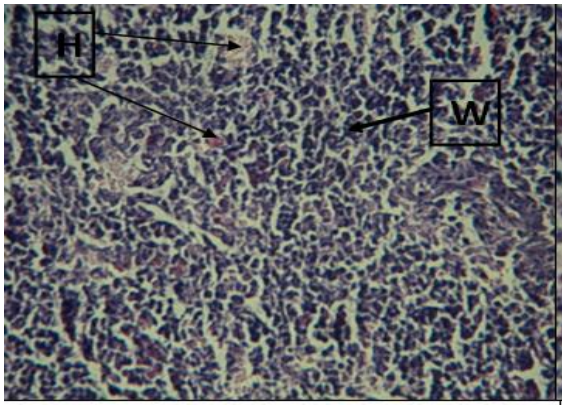

Fig. (9) Group (T2): Histopathological section of spleen reveals Mild depletion of white pulp (W) and proliferation of red pulp, mild hemorrhage within the lymphoid tissue (H). 40X, H&E stain.

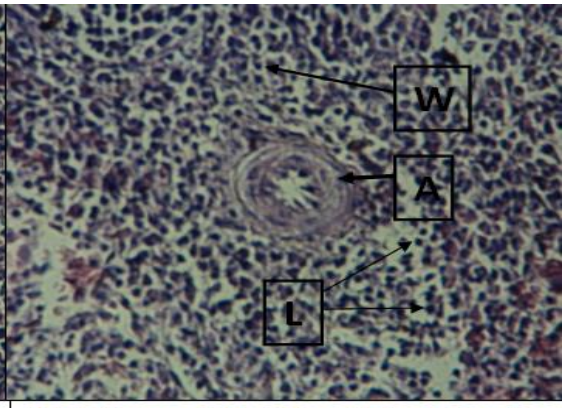

Fig. (10) group (T2): Histopathological section of spleen reveal Mild depletion of white pulp (W) with presence of thick-wall arteriole (A), proliferation of red pulp, and proliferation of lymphocytes in the lymphoid tissue (L). 40X, H&E stain.

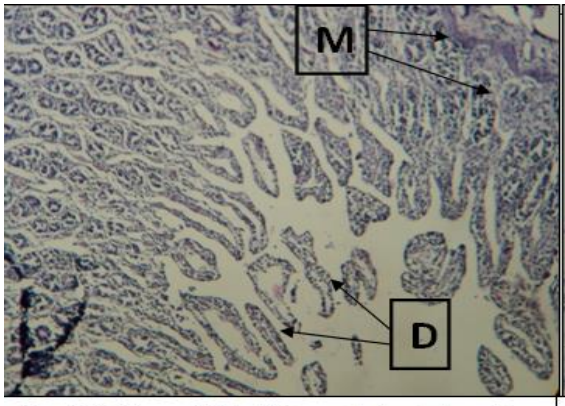

Fig. (11) Group (T2): Histopathological section of intestine reveal Presence of extended and thin villi, mild desquamation of epithelial cells which lining villi (D) and mild infiltration of inflammatory cells (M). 10X, H&E stain.

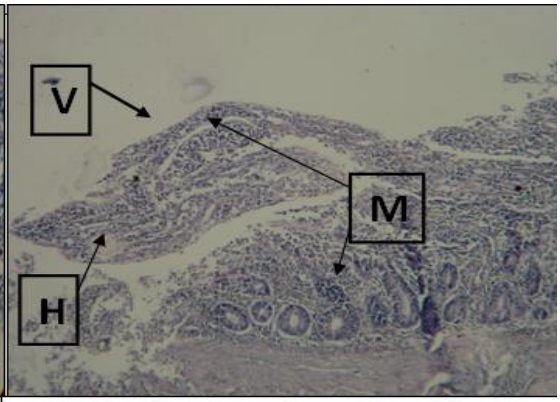

Fig. (12) group (T2): Histopathological section of intestine reveal Extended and elongated villi (V), mild infiltration of inflammatory cells in the intestinal mucosa (M), and few hemorrhage (H). 10X, H&E stain.

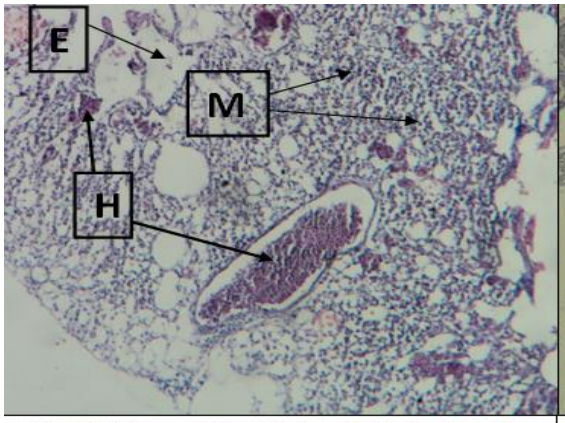

Fig. (13) Group (T2): Histopathological section of lung reveals Presence of congestion and little hemorrhage (H) with mild thickening of interstitial tissue with presence of few inflammatory cells infiltration (M). Other villi showed pulmonary emphysema (E). 10X, H&E

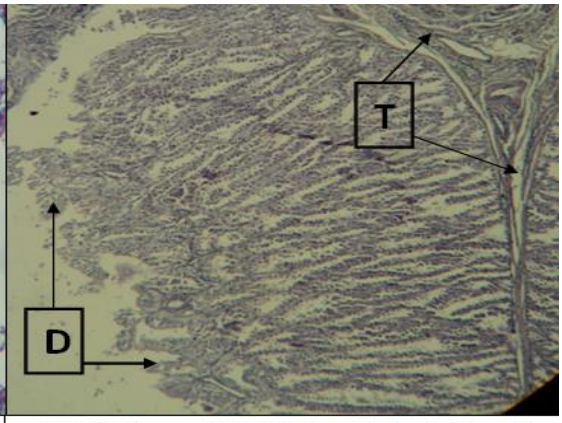

Fig. (14) Group (T2): Histopathological section of proventiculus reveals Mild degeneration and desquamations of columnar epithelium which lining the proventiculus (D), also there is thick fibrous trabiculae between the follicles (T). 10X, H&E stain.

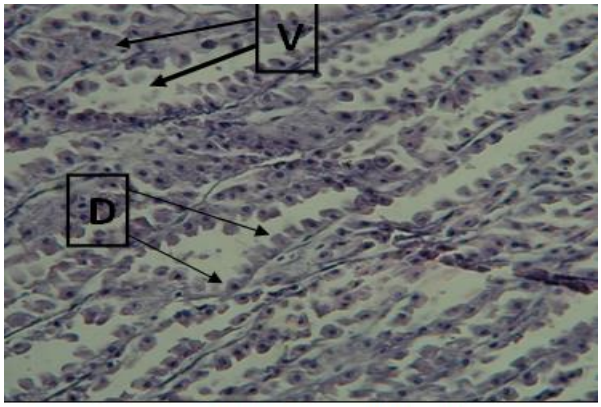

Fig. (15) Group (T2): Histopathological section of proventiculus reveals Mild degeneration of epithelial cells of proventiculus follicles (D) and vacuolation of other cells (V). 40X, H&E stain.

3- T3

In the spleen, there are wide proliferated white pulp, small and thickened-wall arterioles, and proliferating red pulp figure (16). In the case of intestine, the result shows normal extended villi with high intestinal glands and slight thickened muscularis figure (17).

For the lung, there are mild congestion of blood vessels, thickened and normal interstitial tissue, and alveolar mild emphysema figure (18).

In the proventiculus, the findings indicate normal follicles and normal liner epithelial cells figure (19).

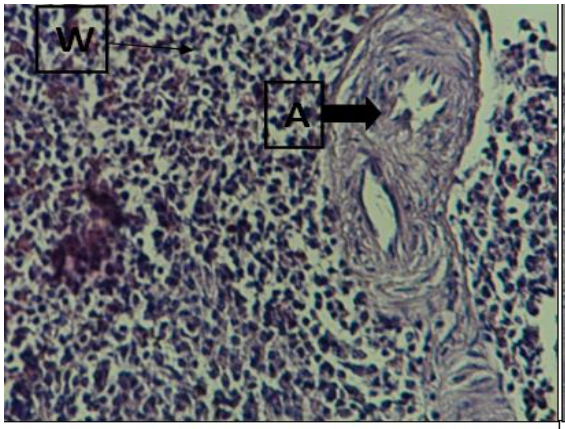

Fig. (16)) group (T3): Histopathological section of spleen reveal Presence of wide and proliferating white pulp (W) with small and thickened wall arterioles (A), surrounding with proliferating red pulp. 40X, H&E stain.

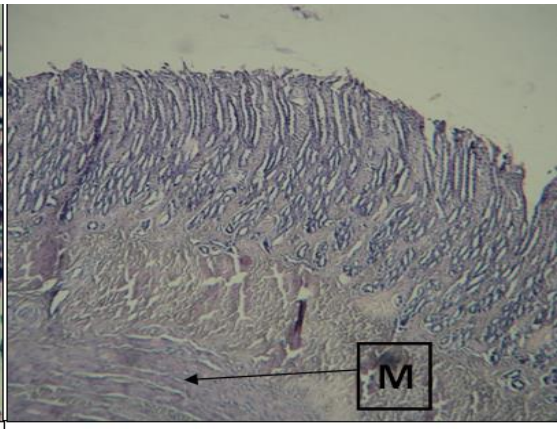

Fig. (17) Group (T3): Histopathological section of intestine reveal there is normal extended intestinal villi (V), with high intestinal gland (G), but there is slightly thickening of muscularis (M). 40X, H&E stain.

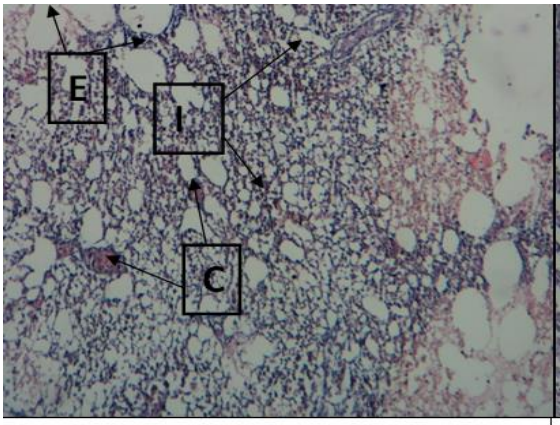

Fig. (18) Group (T3): Histopathological section of lung reveal Mild congestion of blood vessels (C), thickening and normal interstitial tissue (I). Other alveoli showed mild pulmonary emphysema (E). 10X, H&E stain.

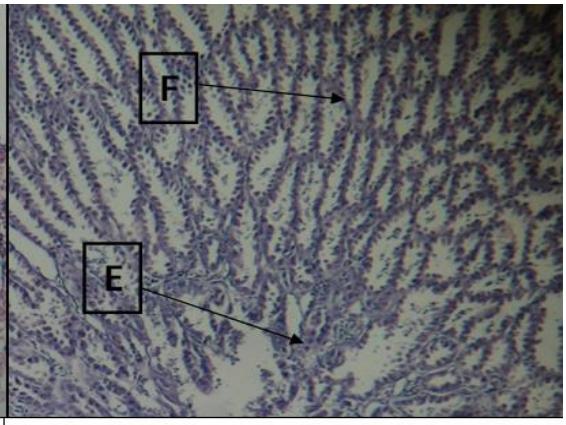

Fig. (19) Group (T3): Histopathological section of proventiculus reveals there is normal proventiculus follicles (F) which lining with normal epithelial cells (E). 10X, H&E stain.

4- Control group (C)

No changes are seen in the intestine figure 20, lung figure 22, proventiculus figure 23, and spleen figure 24.

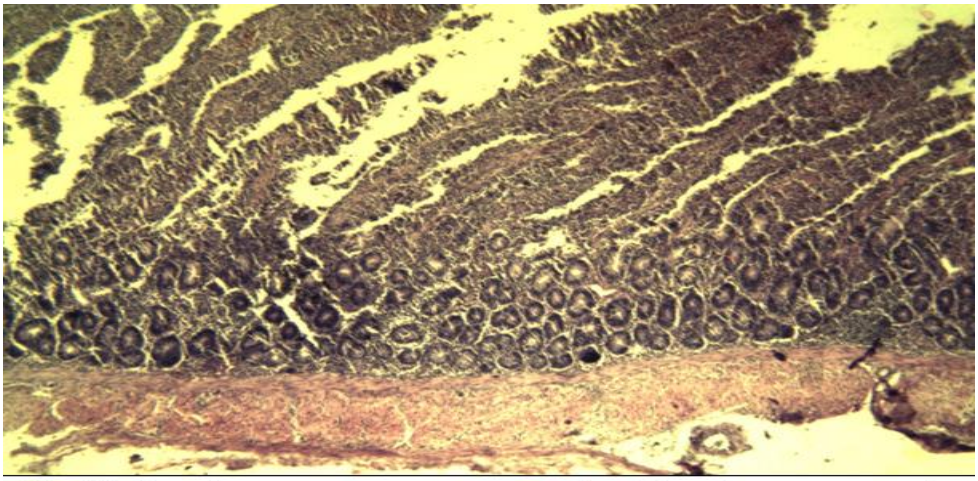

Fig. (20) Control group: Histopathological section of Intestine reveal normal tissue without any changes. 20X H&E.

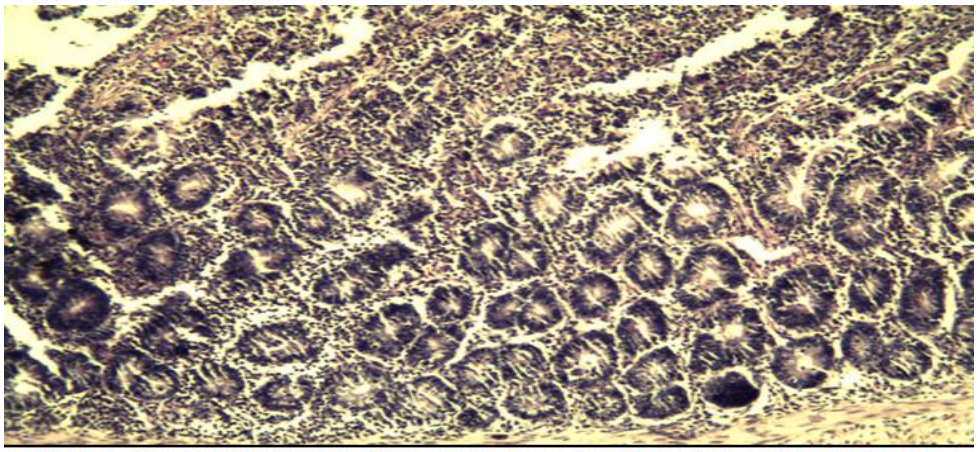

Fig. (21) Control group: Histopathological section of Intestine reveal normal tissue without any changes. 20X H&E.

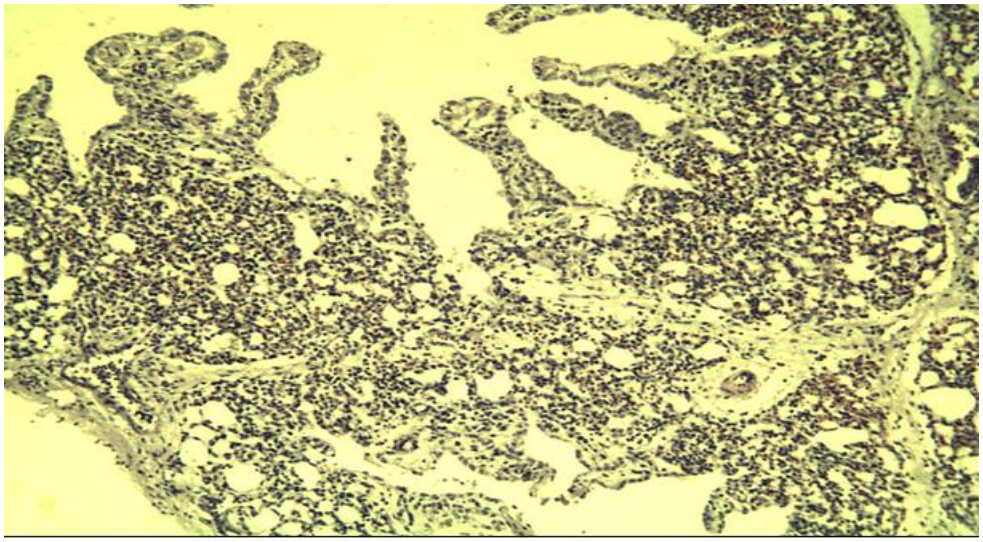

Fig. (22) Control group: Histopathological section of Lung reveal normal tissue without any changes. 20X H&E.

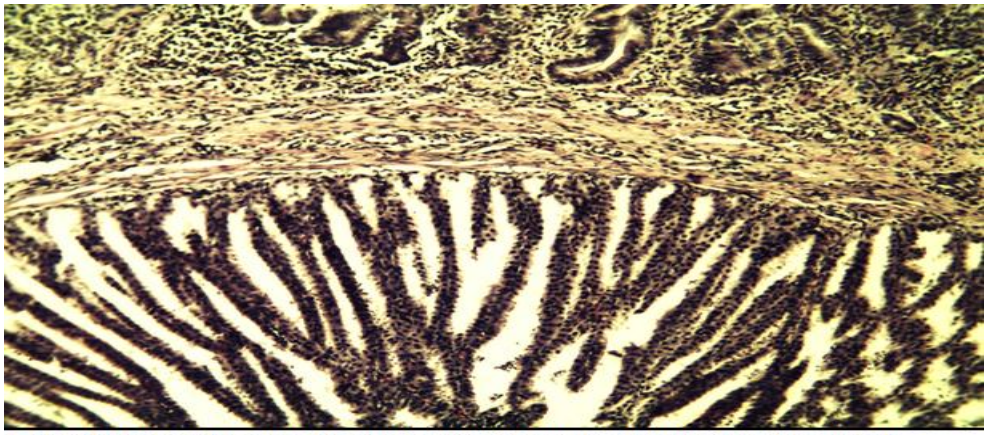

Fig. (23) Control group: Histopathological section of proventiculus reveal normal tissue without any changes. 20X H&E.

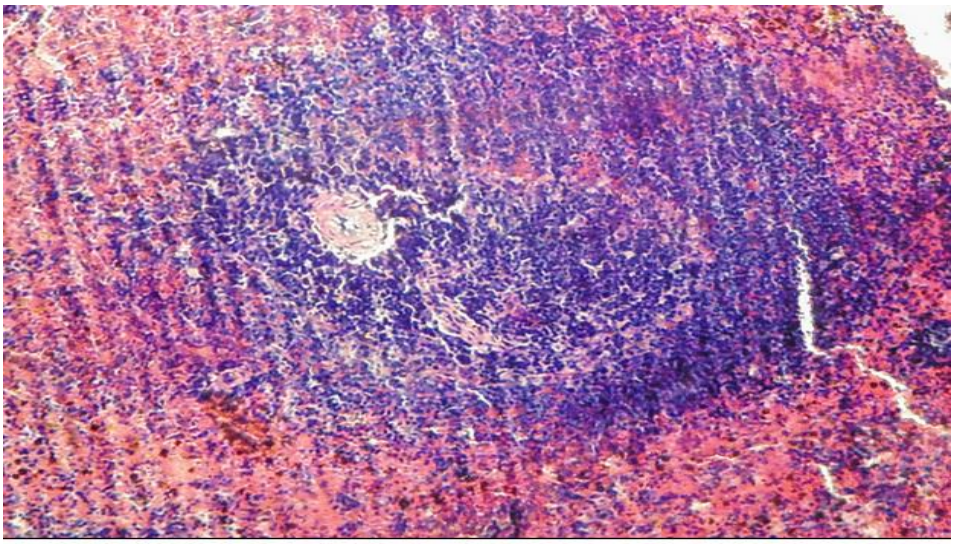

Fig. (24) Control group: Histopathological section of spleen reveal normal tissue without any changes. 20X H&E.

Discussion:

1-Biochemical analysis:

A- Liver enzyme levels:

Using pesticides, CPF for example, to control wide range of harmful pests could lead to bad consequences of getting the environment polluted with these poisonous chemicals, and contaminated food of humans and animals is a best example for this problem (24, 25). Here, the results declare that T1 group had significant increases compared to C group in the levels of AST and ALT enzymes. Moreover, T1 showed no significant difference in AIP levels (very mild increase) when comparing with C group, table (2). These results agree with (26, 27, 28) who recognized damages in the liver manifested with high levels of AST and ALT in rats. The enzymes are increased during pesticide poisoning is linked to the damages that these chemicals induce (29), and it was found that CPF had changed the hepatic enzyme levels in rats (30).

T2 and T3 groups didn't show any significant difference in all enzyme levels when compared to C group (P>0.05), table (2). These results are in agreement with (31) who noticed normal liver enzyme when had used *NS* (32). *NS* induces some healing and repairing processes through the activation of some pathways such as thymoquinone and glutathione, total thiol increasing, getting rid of radicals, quinone reductase, catalase reaction, superoxide dismutase and glutathione transferase (32, 33).

The current study results show that *NS* prevented increasing of liver enzyme in T2 and T3, and this agrees with (34) who discovered that *NS* had stopped the increase in these enzymes.

B- Biochemical parameters:

Here, T1 indicates significant increases in Uric Acid, Creatinine, while triglyceride and total Protein show significant decrease when comparing with C group (P<0.05), table (3). These results come in agreement with (35,36,37,38,39) who showed increases in the blood urea and creatinine when rats had exposed to CPF. Moreover, the present study results match with (36, 40,41 ,42) who recognized decreases in the total protein and amino acids after poisoning with CPF in rat and fish.

For the T2 group, it has a significant increase in creatinine level when compared with C group, and this agrees with (20). This increase may have been due to the longer time needed for the renal tissue to get healed (20). Decreasing the levels of triglycerides and increasing the levels of cratinine after exposure to CPF were improved when adding 1% of *NS* (43, 44). In our study, adding *NS* to the diet for long time had significantly reduced (p<0.05) creatinine when compared to C group, and this agrees with (45) who showed restoring close to normal levels of these parameters in rats.

2- Histopathlogical examination:

T1 group shows histopathological changes that are characterized by the presence of necrotic areas and deposition of hemosiderin in the spleen, destruction of the intestinal mucosa, edematous and haemorrhagic lung, and degeneration of ciliated columnar epithelium in the proventriculus with infiltration of inflammatory cells between follicles. T2 reveals mild splenic depletion of white pulp with proliferation of red pulp, intestinal extended and thinning of the villi with mild desquamation of epithelial cells and little haemorrhage, pulmonary congestion and little haemorrhage with mild thickened interstitial tissue. T3 informs splenic proliferated white pulp with small and thickened-wall arterioles surrounded by proliferated red pulp, intestinal normal extended villi, mild pulmonary congestion with mild alveolar emphysema, and normal proventriculus. In the control group reveals normal tissue without any changes.

For T1 in spleen, our results match (46, 47) who showed abnormal distribution of inflammatory cells in white pulp with reducing the numbers of these cells, edema, hemorrhage, and haemosiderosis deposition. Moreover in the intestine, it had been shown (47) that CPF poisoning induced less long villi with necrosis and mucosal and muscular layer edema which agree with our result. In the case of lung, similar changes of tissue destruction with abnormal alveoli, inflammatory cell infiltration, severe

emphysema, hemorrhage of the interstitial tissue, 75% hepatization lung tissue, and fibrinous **pleuritis were noticed by (48, 49) which match our study results.**

In the proventriculus, it had been revealed by (50) the occurrence of liner epithelial hyperplasia, necrosis and infiltration of inflammatory cells, and formation of diphtheritic membrane which agree with our results.

For T2, Our results are supported and agree with (51) who revealed disappearance of inflammation and necrosis when *NS* had been used.

In the case of T3, *NS* improved the healthy status of the affected tissues by CPF.

These healing effects of *NS* could be linked to certain active ingredients that the seeds contain. The seed extract contains 32 volatile terpenes, 8 fatty acids, and, terpene and diterpenes alkaloids. Those terpenes include some of dithymoquinone, thymoquinone, carvone, p-cymene, trans-anethol, and limonine (52, 53). Dithymoquinone and thymoquinone have tumor cytotoxic and healing activities (53).

There are many illness conditions and diseases that could be treated with *NS* such as bronchial asthma, blood hypertension, bacterial infections, fungal infections, pain, inflammation, and immunity weakness (54, 55).

It is concluded from the obtained results that chlorpyrifos is a dangerous chemical which causes contamination to the environment materials such as food. Thus, *Nigella sativa* usage increases the hopes to prevent and treat the health damages caused by the exposure to chlorpyrifos.

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