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Protective effect of propolis on liver and kidney injury caused by methotrexate in chicks

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Abstract

The current study aimed to explain propolis's protective effect on the liver and kidney damage caused by methotrexate (MTX). A total of 80 chickens at one day old were used and divided into three groups; the first group was the control group, the second group received the propolis, the second group was treated with MTX, and the fourth group received both propolis and treated with MTX. After 15 days of experimental, all chickens were euthanized, and blood samples and liver and kidney tissue were collected. The result showed that the treated group with MTX showed an increase in serum levels of AST, ALT, AP, urea, creatinine, and uric acid in comparison with both the control and propolis group, while in the group treated with both Propolis and MTX showed serum level of AST, ALT, AP, urea, creatinine, and uric acid similar to that recorded in both in the control group and MTX group. The liver sections treated with methotrexate showed hyperplasia of fibrocytes in the portal area with infiltration of mononuclear inflammatory cells represented by macrophages, and coagulative necrosis in affected hepatocytes, clear vacuoles in the hepatocytes, massive infiltration of macrophages. Sections of the liver treated with methotrexate and propolis explain a marked decrease in the fatty degeneration, with few infiltrations of mononuclear inflammatory cells around portal areas. The liver section from propolis treated group and the control group showed typical hepatic tissue architecture. The kidney sections treated with methotrexate showed coagulative necrosis in the endothelial cells, glomeruli appearing irregular in shape, and hemorrhaging in the extracellular matrix. The sections of the kidney treated with methotrexate and propolis explain a marked rise in the renal tubules with the typical feature of a healthy one. The section of the kidney from the propolis treated group and control group showed typical architecture of renal tissue. In conclusion, propolis greatly protects against MTX's toxic effect in chicks' liver and kidney.

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Introduction

Bee glue (Propolis) is the common surname certain to the resinous artifact of intricate sonata calm by the honey bee since assorted plant supply (1). It encloses extra 300 workings. Phenolic amalgam, like flavonoids, is our foremost apparatus and chiefly liable for the natural action of propolis. It is established to do many natal tricks to treat numerous microbes, suppress the immune system, prevent inflammatory antioxidants, and treat multiple forms of cancers. Furthermore, it is testimony to cut the harmful consequences of some chemotherapeutic managers such as tamoxifen (2) and irinotecan. The undesirable effects on the kidney are the consequence of irinotecan was detailed to be abridged by the action of propolis (3). Methotrexate, the rival of folic acid, is one of the remedies for tumor treatment. It is

worn out in handling some evil-minded diseases of the immune system. Noxious properties are imperative on loads of organs like the bone marrow, liver, and kidney. 90% of MTX or extra than it is vacant through the urinary system. Hence, the hurt to the kidney caused by MTX holdup itis ending. Ensuing persistent and grand plasma expression results in a manifest augmentation of other MTX's toxicities (4). Thus, a nephroprotective manager's verdict is fixed for the harmless sketch on this imperative preparation. The latent it spawns free radicals and may cause swift oxidative stress, particularly in the existence of scarce internal enzymes that prevent oxidative stress enclosed by the natural structure (5). The broadly applied anticancer preparation methotrexate's proven value is narrowly suitable because of its allied toxic effect on the liver. The liver is an imperative appendage that preserves homeostasis and manages vital body roles (6). Unlike common flavonoids that embrace galangin, caffeic acid phenethyl ester, quercetin, and rutin in Bee glue have defended consequences on chemotherapy-caused poisonous effects and trimmed down the wrong things on normal tissues (7-9). consequently, the current reading of flavonoids loaded bee glue next to methotrexate-caused hepatotoxicity and nephrotoxicity in chicks.

Materials and methods

A total of 80 chicks weighing 35±5 grams were used at one day old. They were divided into four groups (20 chicks in each group). Group one is considered the control group. The second group was treated with propolis. The third group was treated with MTX. The fourth group was treated with Propolis and MTX. The propolis was dissolved in distal water. On the first day of the tentative practice, groups 2 and 4 received 100 mg/kg propolis for 15 days. On the tenth day of the experiment, groups 3 and 4 received MTX at 40 mg/kg (intraperitoneal injections) for five days (10,11). After 15 days of experimental, the animals in all groups were euthanized to collect tissue samples from liver and kidneys and blood for the biochemical test such as AST, ALT, AP, urea, creatinine, and uric acid concentration in blood serum (12,13).

Histopathological examination

The tissue samples from the liver and kidney were collected 15 days after treatment; these tissues were fixed in 10% neutral buffered formalin after 72 hours, representative samples from the liver and kidney from different groups were obtained for histopathological examination, and the samples were dehydrated with ethanol, cleared by xylene, infiltrated and embedded with hot paraffin wax at 58ºC (14). Paraffine cassettes were section at 5µm with a rotary microtome, then floated in a hot water bath at 38ºC, and lifted at clean histological glass slides; later, these slides were stained using Harris's hematoxylin and eosin stain, and examination under the light of microscope at 40, 100, and 400x (15).

Results

All groups treated with methotrexate 40 mg/kg showed significantly higher serum in AST, ALT, AP, urea, creatinine, and uric acid levels than the control cluster and other groups (Table 1).

Table 1: Explain the parameters of the current study

Parameters	Mean ± SE			
	Control	Propolis (P)	Methotrexate (M)	Both P+M
AST (IU/L)	171.16±20.00b	171.04±7.70b	198.88±10.20a	171.74±6.63b
ALT (IU/L)	38.48±1.72b	39.00±1.33b	43.00±2.35a	39.00±2.37b
AP(IU/L)	420.18±25.68b	421.22±17.98b	488.15±8.36a	421.03±13.71b
Urea (mg/dl)	7.55±0.56b	8.31±1.00b	12.51±1.73a	8.81±0.76b
Creatinine (mg/dl)	0.21±0.02b	0.20±0.02b	0.33±0.04a	0.20±0.02b
Uric acid (mg/dl)	9.31±2.06b	9.04±1.26b	14.70±1.38a	9.16±0.45cb

Vertical letters mean a significant difference between different groups at P<0.05.

Histopathological examination

The sections of the liver treated with methotrexate showed hyperplasia of fibrocytes in the portal area with infiltration of mononuclear inflammatory cells represented by macrophages, with the presence of fatty degeneration in affected hepatocytes as a clear vacuole that pushes the nucleus to the cell edge (Figure 1), in addition, the section showed the presence of coagulative necrosis in affected hepatocytes that appears as amorphous eosinophilic materials with association with distension of hepatic sinusoids (Figure 2), the clear vacuoles that represented fatty degeneration were predominant in the hepatocytes that did not suffer from copulative necrosis which appear as one giant or multiple small clear vacuoles in these hepatocytes (Figure 3). In contrast, the massive infiltration of macrophages was recorded frequently in hepatic tissues as a mass accumulation of inflammatory cells around the portal area (Figure 4). The liver sections treated with methotrexate and propolis explain a marked decrease in the fatty degeneration of affected hepatocytes in addition to fewer hepatocytes with

the necrotic response as a result of propolis treatment (Figure 5). At the same time, the infiltration of mononuclear inflammatory cells around portal areas showed an intense decrease in the number of infiltrated macrophages (Figure 6). The liver section from the propolis treated group and control group showed typical hepatic tissue architecture (Figure 7).

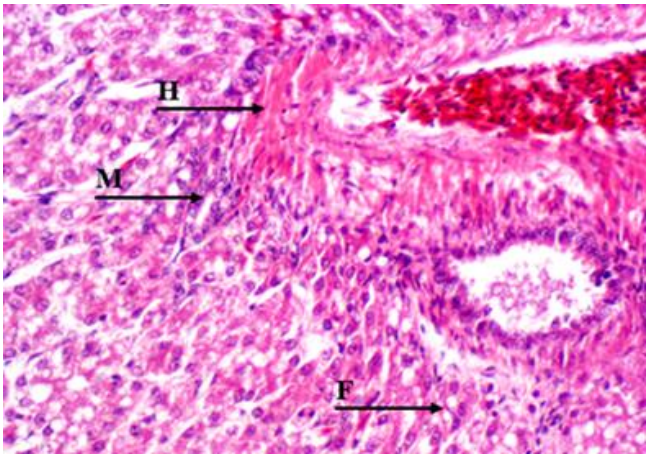

Figure 1: The chicken liver treated with methotrexate showed hyperplasia of fibrocytes in the portal area (H), infiltration of macrophages (M), and fatty degeneration (F). H&E, 400x.

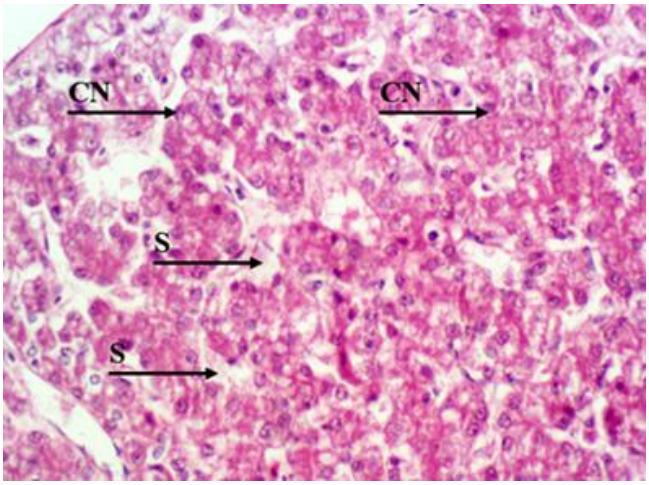

Figure 2: The chicken liver treated with methotrexate showed coagulative necrosis in affected hepatocytes (CN), and distension of hepatic sinusoids (S). H&E, 400x.

The kidney sections treated with methotrexate showed coagulative necrosis in the endothelial cells that line the renal tubule and appear as cellular debris in the lumen. This necrosis also involved the glomeruli, which appear irregular in shape with vacuolar degeneration in the endothelial cells (Figure 8), with hemorrhage in the extracellular matrix (Figure 9).

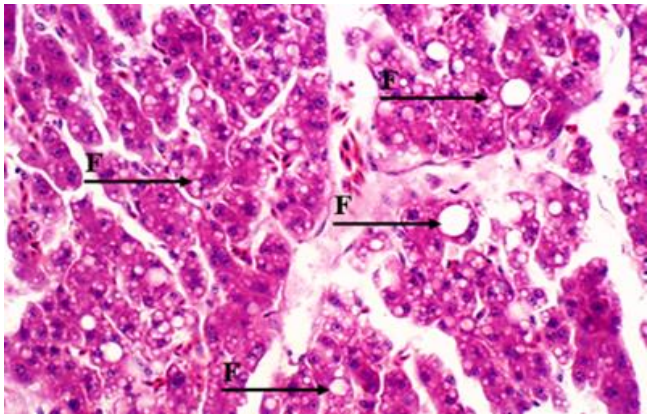

Figure 3: The chicken liver treated with methotrexate showed massive fatty hepatocyte degeneration (F). H&E, 400x.

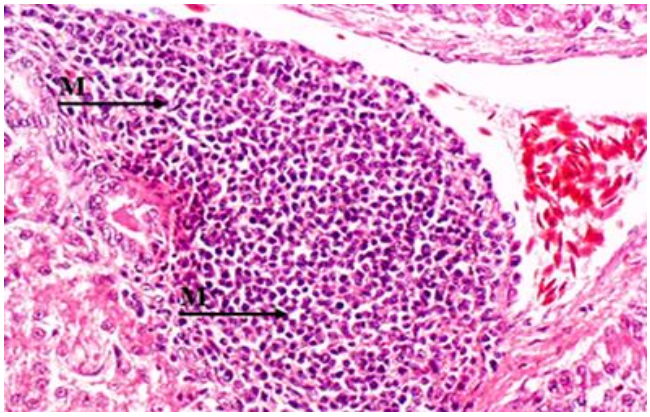

Figure 4: The chicken liver treated with methotrexate showed massive infiltration of mononuclear inflammatory cells (M). H&E, 400x.

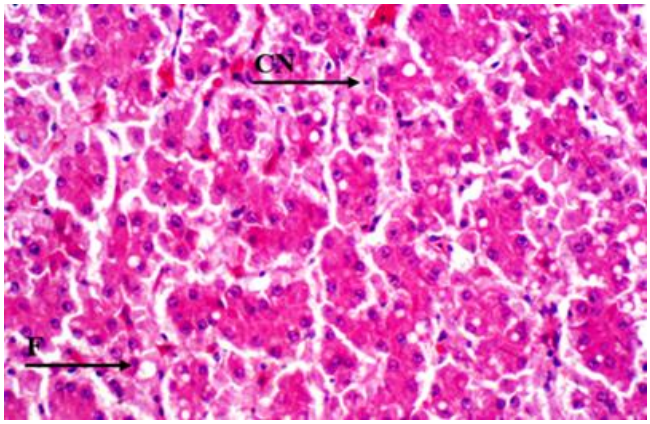

Figure 5: The chicken liver treated with methotrexate and propolis showed decreases in the coagulative necrosis in hepatocytes (CN), with few hepatocytes affected with fatty degeneration (F). H&E, 400x.

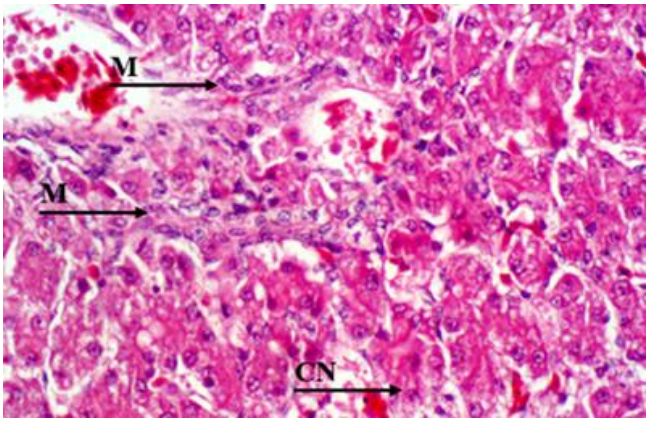

Figure 6: The chicken liver treated with methotrexate and propolis showed decreased coagulative necrosis in hepatocytes (CN) and a few macrophages around the portal area (M). H&E, 400x.

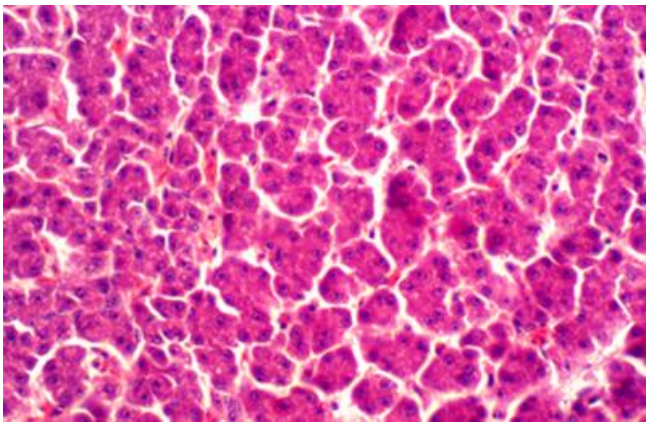

Figure 7: The chicken liver treated with propolis showed typical histological features of hepatic tissue. H&E, 400x.

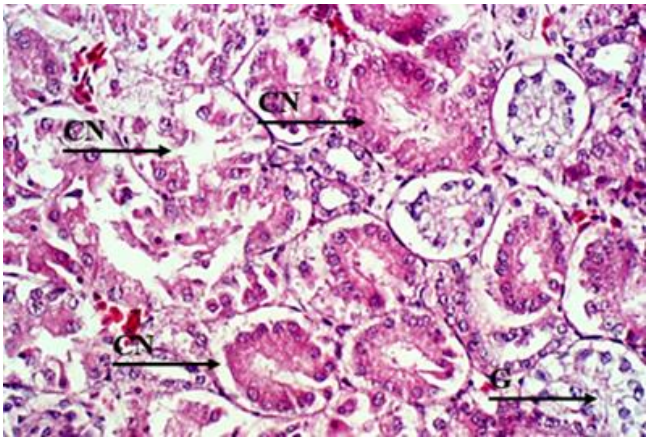

Figure 8: The chicken kidney treated with methotrexate showed coagulative necrosis in renal tubules (CN), with necrosis in the glomeruli (G). H&E, 400x.

The sections of the kidney treated with methotrexate and propolis explain a marked rise in the renal tubules have the typical feature of a healthy one with the presence of few tubules showing coagulative necrosis in one or more endothelial cells that line this tubule with the increase in cellularity of glomeruli and gain their standard histological features (Figures 10 and 11). The section of the kidney from the propolis treated group and control group showed typical architecture of renal tissue (Figure 12 and 13).

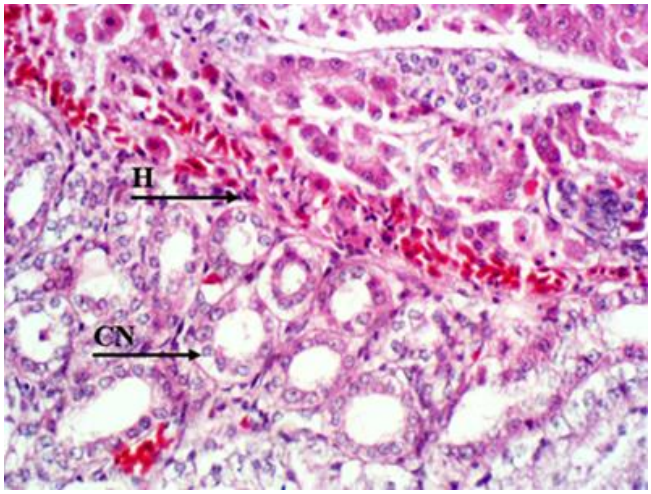

Figure 9: The chicken kidney treated with methotrexate showed coagulative necrosis in renal tubules (CN), with hemorrhage (H). H&E, 400x.

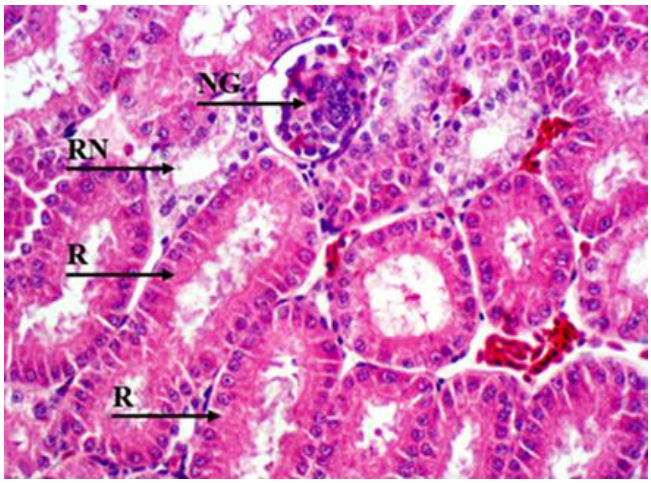

Figure 10: The chicken kidney treated with methotrexate and propolis showed typical renal tubules architecture (R), few tubules with necrosis (RN), and normal glomeruli (NG). H&E, 400x.

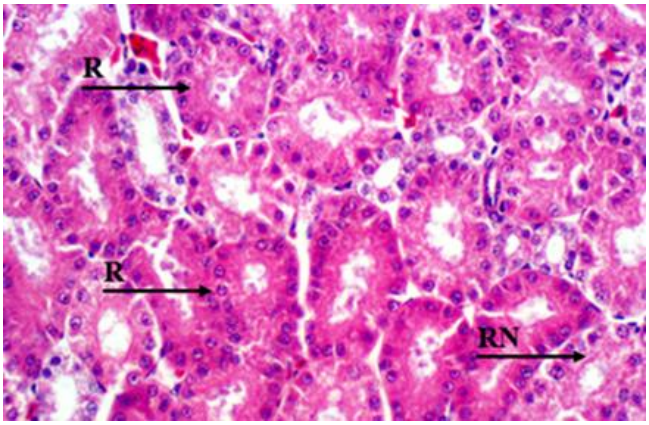

Figure 11: The chicken kidney treated with methotrexate and propolis showed typical renal tubule architecture (R), and few tubules with necrosis (RN). H&E, 400x.

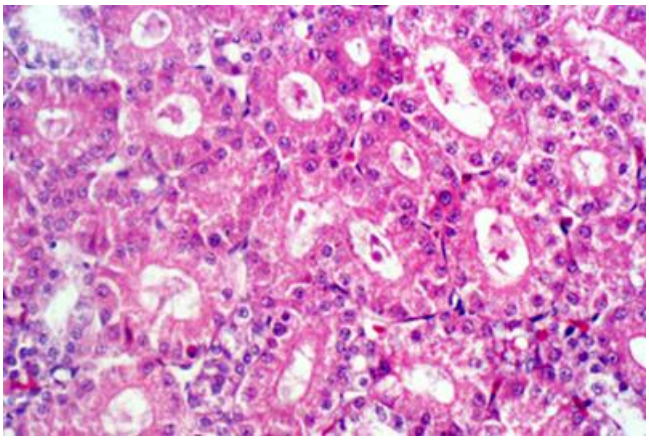

Figure 12: The chicken kidney treated with propolis showed typical histological features of renal tissue. H&E, 400x.

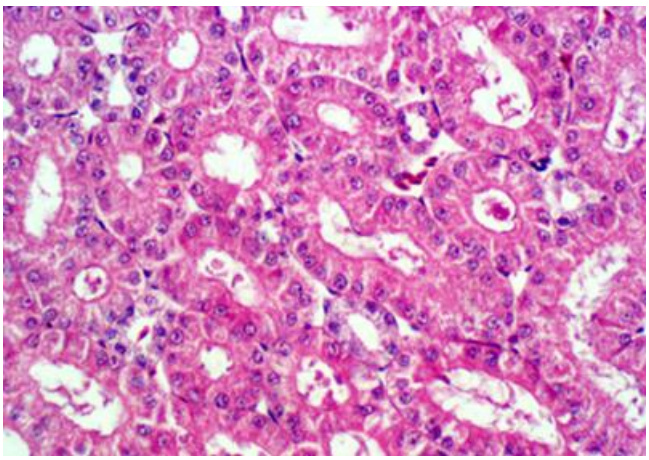

Figure 13: The chicken kidney obtained from the control group showed typical histological features of renal tissue. H&E, 400x.

Discussion

The result of the current study showed that the methotrexate treated groups showed serum significantly rise in AST, AP, ALT, Creatinine, Urea, and uric acid levels compared with the control group and other groups (16) due to MTX being famous for origin hurt effect of the liver and kidney by reminding oxidative hassle and dwindling the antioxidant guard (17). while all groups treated with propolis showed insignificant differences compared with the control group, this agrees with (16), as bee glue is old as an immune system tonic mediator because of its skill as a free radical's searcher (18). a few investigators plot by propolis would be to grant healthier fallout in scheming the undesirable possessions of cancer cure (16,19). The result of the current study showed that the methotrexate treated groups showed intense hepatic and renal injury represented by coagulative necrosis, fatty changes, and hemorrhage, while the treatment with propolis the methotrexate will enhance the health and decrease the effect so these lesions, in contrast, the impact of propolis alone were showed an improvement to the histological feature of the liver and kidney tissues in comparison with the control group. Methotrexate is widely considered a competitor to folic acid in dealing with different grades of lymphoblastic leukemia with other inflammatory diseases such as rheumatoid arthritis (20). Methotrexate induces hepatic and renal injury by its action and antioxidant agent by reducing the production of catalase and glutathione in addition to superoxide dismutase; in the total effect, this will cause a reduction in the production of s-adenosyl methionine that causes an increase in the reactive oxygen species within the cells, this reverse correlation between the decrease in defense antioxidant output and growth in toxic reactive oxygen radicals will lead to cellular pathological status termed as oxidative stress which is measured as the primary origin of these pathological changes that recorded in liver and kidney of chickens (21). In addition, the ATP binding cassette was bound to methotrexate and led to accumulating it in the cell in the form of glutamate (poly), which will lead to shut down the production of pyrimidine and purine by inhibiting the function of dihydrofolate reductase and AICAR transformylase within renal endothelial cells which considered the leading cause of the renal injury (22). On the other hand, the propolis will exert their effects such as antioxidant and anti-inflammatory roles by increasing the production and releasing of an antioxidant such as malondialdehyde, glutathione, and superoxide dismutase that will cause a reduction in the action of oxidative stress and will enhance the biological activities and lipid peroxidation in the cell that will overcome the increased level of reactive oxygen species (23,24).

Conclusion

In conclusion, propolis greatly protects against MTX's toxic effect in both liver and kidney in chicks.

Acknowledgment

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Conflict of interest

The author declares no Conflict of interest

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التأثير الوقائي للعكبر ضد الأذى المحدث بالميثوتركسيت على الكبد والكلية

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الخلاصة

هدفت الدراسة الحالية الى التعرف على التأثيرات الوقائية للعكبر على أذى الكبد والكلية في أفراخ الدجاج. تم استخدام ٨٠ فرخا بعمر يوم واحد قسمت عشوائيا الى أربعة مجاميع بواقع عشرون فرخا في المجموعة الواحدة. المجموعة الأولى اعتبرت مجموعة سيطرة. المجموعة الثالثة أعطيت مادة العكبر. المجموعة الثالثة عوملت بالميتوتركسيت. المجموعة الرابعة أعطيت العكبر بالإضافة الى الميتوتركسيت. وبعد مرور خمسة عشر يوما على بدء التجربة تم قتل جميع الأفراخ وجمع الدم وعينات من نسيج الكبد والكلية. أشارت نتائج الدراسة الحالية بعد مرور خمسة عشر يوما الى ارتفاع معنوي في مستوى ناقلة أمين الالنين وناقلة أمين الاسبارتيت والفوسفتاز القاعدي وحامض اليوريا والكرياتينين في المجموعة المعاملة بالميتوتركسيت

لوحده بالمقارنة مع مجموعة السيطرة ومجموعة العكبر. أما المجموعة التي أعطيت العكبر ثم الميتوتركسيت فقد أظهرت نتائج هذه الإنزيمات انخفاضا معنويا وكانت مقارنة لتركيزها في مجموعة السيطرة ومجموعة العكبر. أظهر نسيج الكبد المعالجة بالميثوتريكسات تضخما في الخلايا الليفية في الباحة البابية مع ارتشاح الخلايا الالتهابية وحيدة النواة، ووجود نخر تجلطي في خلايا الكبد، وظهور فجوات رائقة في خلايا الكبد. أما نسيج الكبد المعالجة بالميثوتريكسات والعكبر فقد اظهر انخفاضا ملحوظا في التنكس الدهني، مع القليل من الارتشاح للخلايا الالتهابية وحيدة النواة في الباحة البابية. أظهر مقاطع الكبد من المجموعة المعالجة بالعكبر والسيطرة بنية نموذجية لنسيج الكبد. أظهرت مقاطع الكلية المعالجة بالميثوتريكسات نخر تجلطي في الخلايا البطانية، وظهرت الكبيبات بشكل غير منتظم مع النزيف الخلوي. وفي نسيج كلية المجموعة المعاملة بالميثوتريكسات والعكبر فقد التركيب السوي للنبيبات والنسيج الكلوي. أظهرت مقاطع الكلية في المجموعة المعاملة بالعكبر والسيطرة البنية النموذجية لنسيج الكلية. وفي الخلاصة، فان استخدام العكبر له تأثيرات وقائية ضد التسمم بالميثوتريكسات المحدث في كب وكلية الأفراخ.