

Prophylactic Effect of Ethanolic Extract of *Saussurea Costus* Roots Against Hepato- Renal Toxicity Induced by Diazinon in Chickens

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Abstract

The study was performed to investigate the prophylactic role of *Saussurea costus* roots in the functional and histological changes caused by diazinon in the liver and kidneys in chickens. 18 chickens were used of Arbor Acres strain at 54 days of age and 1300-1500 gm average of body weight was used for this purpose. The birds were divided into 3 groups equally (6chicken in each groups), Group 1: served as a control negative received distilled water 1 ml. Group 2: served as a positive control group, received diazinon at a dose of 0.2 mg/kg/day. Group 3: were given an ethanolic extract of *Saussurea costus* at dose 300 mg/kg, then after one hour administrated diazinon at the dose of 0.2 mg/kg/day, this group served as a prophylactic group. The doses were given orally once daily for 4 weeks. The results of group 2 showed clinical signs such as ruffled feathers, salivation, diarrhea, breathing from the mouth, teary eyes, drooping of wings. The body weights of the chickens and weights of the liver and kidneys of group 2 significantly declined ($p \leq 0.05$) compared with groups 1 and 3. There was a significant decrease ($p \leq 0.05$) in WBC count, lymphocyte, total protein, albumin, GSH, SOD, CAT, and GPX levels, while a significant increase ($p \leq 0.05$) in heterophil, ALT, AST, creatinine, urea, uric acid, and MDA compared with group 1. The gross examination of the liver and kidney of group 2 were pale, easily crumbles and smaller than that of group 1. Histopathological changes of the liver of group 2 including congested and dilated central vein, vacuolated cytoplasm of hepatocytes, focal necrotic tissue filled with inflammatory cells, thickening of the bile duct, thickening wall of the portal artery. fibroblast in portal area, dilated sinusoid. Histopathological changes of the kidney including dilatation of renal tubule, hemorrhage, and atrophy in the glomerulus. we concluded that administration of ethanolic extract of *Saussurea costus* resulted in amelioration of the morphological changes in diazinon treated chickens, improved parameters and restored the parameters to near normal compared with group 1. These results revealed that *Saussurea costus* roots acts as an antioxidant substance and has a hepatic and renoprotective effect against toxicity induced by diazinon.

Keywords: *Saussurea costus* root, Diazinon, liver, kidney, chicken.

التأثير الوقائي للمستخلص الايثانولي لجذور القسط الهندي *Saussurea costus* ضد تسمم الكبد و الكلى التي يسببها الديازينون في الدجاج

الخلاصة

بحثت هذه الدراسة في الدور الوقائي لجذور القسط الهندي (*Saussurea costus*) في التغيرات الوظيفية والنسجية التي يسببها الديازينون في كبد وكلى الدجاج. 18 دجاجة من سلالة Arbor Acres بعمر 54 يوم و متوسط الوزن 1300-1500 غم استخدمت لهذا الدراسة , تم تقسيم الطيور إلى 3 مجموعات و 6 دجاجات لكل مجموعة ، المجموعة الاولى: اعتبرت مجموعة السيطرة السالبة تم تجريعها الماء المقطر 1 مل. المجموعة الثانية: اعتبرت مجموعة السيطرة الموجبة، تم تجريع الدجاج بالديازينون بجرعة 0.2 ملغم / كغم .

المجموعة الثالثة: تم تجريع الدجاج بالمستخلص الإيثانولي لجذور القسط الهندي بجرعة 300 ملغم / كغم ، ثم بعد ساعة واحدة تم إعطاء الديازينون بجرعة 0.2 ملغم / كغم ، اعتبرت هذه المجموعة كمجموعة وقائية. تم إعطاء الجرعات مرة واحدة يومياً عن طريق الفم لمدة 4 أسابيع. أظهرت نتائج المجموعة الثانية علامات سريرية على الدجاج مثل الريش المنفوش واللعب والإسهال والتنفس من الفم، العيون الدامعة وتدل الأجنحة. لوحظ انخفاض معنوي ($p \leq 0.05$) في أوزان جسم الدجاج وأوزان الكبد والكليتين مقارنة مع المجموعتين الأولى والثالثة. كان هناك انخفاض معنوي ($p \leq 0.05$) في عدد خلايا الدم البيضاء ، الخلايا الليمفاوية ، البروتين الكلي ، الألبومين ، GSH ، SOD ، CAT و GPX ، في حين كان هناك ارتفاع معنوي ($p \leq 0.05$) في العدلات ، ALT ، AST ، الكرياتينين ، اليوريا وحمض اليوريك و MDA مقارنة بالمجموعة الأولى. أظهر الفحص العياني للكبد والكلى في المجموعة الثانية اللون شاحباً وبتفتت بسهولة وأصغر من المجموعة الأولى. التغيرات النسيجية لكبد المجموعة الثانية أظهرت احتقان وتوسع الوريد المركزي، الخلايا الكبدية المتضخمة بالسابتوبلازم، تنكس في الخلايا الكبدية مع وجود ارتشاح للخلايا الالتهابية ، سماكة القناة الصفراوية وتثخن في جدار الشريان البابي. التغيرات النسيجية في الكلى لوحظ توسع النبيبات الكلوية والنزيف والضمور في الكبيبة. نستنتج من هذا ان إعطاء المستخلص الإيثانولي للقسط الهندي أدى إلى تحسين التغيرات العيانية والنسيجية في الدجاج المعالج بالديازينون ، وإعادة المعايير الدمية والكيموحيوية إلى ما يقرب من المعدل الطبيعي مقارنة بالمجموعة الأولى. وكشفت هذه النتائج ان جذور القسط الهندي *Saussurea costus* تعمل كمادة مضادة للأكسدة ولها تأثير وقائي لكبد والكلى ضد التسمم الناجم عن الديازينون.

Introduction

Pesticides are toxic chemical compounds created to control pests, plant disease causative organisms, weeds, and other living organisms that reduce crop production in amount and quality (1). Pesticides are categorized into four major chemical composition classes, namely: organochlorines, organophosphorus, carbamates, pyrethrin, and pyrethroids. (2) Organophosphorous compounds (OPCs) are widely used in agriculture as effective insecticides. It is estimated that the use of pesticides such as OPCs has been increased by at least two to three times over the period 1995 to 2020 years (3). This will increase the occurrence of OPCs poisoning and its subsequent toxicity. By acetylcholinesterase (AChE) inhibition and increasing acetylcholine levels in the cholinergic synapse, they affect mainly the nervous system of the exposed organisms. OPs induce oxidative stress in addition to cholinergic effects(4), affect metabolic pathways (5), also cause multiple organ dysfunctions like hypoxia and insufficient tissue perfusion of the liver and heart (6).

Diazinon (DZN) is a widely used organophosphorous (OP) pesticide (diethoxy-((2-isopropyl-6-methyl-4-pyrimidinyl)oxy)-thioxophosphorane). DZN is a synthetic chemical

compound with activity in broad spectrum insecticide (7). poisonous effects of diazinon are due to the inhibiting activity of acetylcholinesterase, an enzyme that is essential for the proper functioning of the nervous system. DZN commonly used in agricultural and horticultural applications worldwide to control insects in crops, ornaments, lawns, fruits, vegetables, and other food products (8).

People and animals are usually exposure to Organophosphorous pesticides by consuming fresh and processed vegetables, contacting pesticide-contaminated surfaces, breathing the air in the nearby area of pesticide applications, and drinking pesticide-contaminated water, in addition to its harmful effects.(9)

Besides, several experiments about the biochemical and hematological effects of DZN on the experimental animal were performed. Severe structural and functional damages in liver and kidneys were assessed in diazinon-exposed animals (10-13)

Herbal medicine has played an active and important role in alternative and traditional medicine, which is used to treat many different diseases, it is safe, inexpensive, It can be easily obtained and its preparation method is

additionally trouble-free and overall, it fits individuals' societal and cultural needs (14)

Saussurea costus (synonym: *S.lappa* C.B. Clarke) is a well known local to India, Pakistan, and China, the region of the Himalayas where it rises to 2,500-3,500 meter above sea level (15, 16). It is an important medicinal plant commonly used in numerous native systems of medicine for the treatment of many diseases containing diverse active ingredients like flavonoids, steroids, terpenes, sesquiterpenes alkaloids, costunolide, lactone dehydrocostus, cynaropicrin, chlorogenic acid, phenols, gum and mucilage, glycosides, and saponins (17). It has been documented to have several biological activities like antifungal (18), anthelmintic (19), antidiabetic, antitumor (20), antimicrobial (21), immuno-modulator (22), antiulcer (23), anti-inflammatory (24) and antihepatotoxic (25).

Material and methods

Experimental Animals:

This study was conducted in the Poultry Research Unit of the pathology and Poultry diseases department / College of Veterinary Medicine - University of Basra. after the unit was created according to the conditions that followed in raising chickens such as cleaning, sterilization, and environmental conditions necessary for poultry raising such as temperature, ventilation, and lighting. Feed and water that was given to chickens *ad libitum*. In the experiment, 18 chickens were used that are clinically disease-free, of Arbor Acres strain, the weight ranged between 1300-1500 gm. All birds were acclimatized for two weeks before the start the experiment.

Chemical materials:

Diazinon 60% obtained from (Kafr El-Zayat, Egypt), *Saussurea costus* roots (Basra local market, Iraq), Malondialdehyde (BDH chemical Ltd., England), Glutathione (Sigma chemicals,

USA), Catalase Glutathione Peroxidase and Superoxidase dimutase kits (Elabscience Biotechnology Inc.China), Uric acid (Human, GmbH, Germany). Creatinine, urea, albumin, total protein, alanine, and aspartate aminotransferase were (Biolabo, France).

Plant material :

The alcoholic extract had been extracted from the root of *S. costus* that was used in our investigation. The root was specially picked from the local market and authenticated by a taxonomist, Prof. Dr. Iman Mohammed Abd Elzahraa, Department of Biology/ College of Sciences / University of Basra. It was turned to powder with the help of an electric grinder and kept in the dark container at 25°C.

Preparation ethanolic extract from *S. costus*:

Fifty grams of powder with two hundred ml of ethanol (70%) were placed in the round bottle flask and extracted at 70°C for 12 h. The extract was filtered with the Whatman filter paper, then the extract was put in the Petri dish under the shade at room temperature, the extracts were collected and stored in a container that a tightly closed and saved for use(26).

Experimental design

Eighteen healthy chickens of Arbor Acres breed weighing between 1300 and 1500g, the chickens were classified into 3 groups each group containing six animal as follow:

Group 1: (Negative control) the chickens received distilled water.

Group 2: (positive control group), chickens administered DZN at dose 0.2 mg/kg/day orally for 4 weeks. (27)

Group3:(prophylactic group), chickens were given an ethanolic extract of *S.costus* at dose 300 mg/kg orally (28), then after one hour subjected to DZN at the dose of 0.2 mg/kg/day for 4 weeks.

Studying parameters:

Measurement of the Bodyweight:

The weight of each animal was recorded in the 0 days and the 28 days using an electronic balance.

Clinical signs:

Record clinical signs that observed on chickens during the experimental period

Specimens collection:

1. Blood Collection:

At the end of the experimental period, Blood samples had been obtained from the wings by using a 5cc sterile syringe, 1ml of blood obtained in EDTA tube for WBC count, Heterophil, lymphocyte analysis. The remainder of blood (6-7 ml) was dropped in tubes without anticoagulant and then serum samples were isolated from blood by centrifuge at 3000rpm for 15 minutes and separated in Eppendorf tubes, Serum preserved at -20°C until used for biochemical analysis (like CAT, GPx, SOD, GSH, MDA, ALT, AST, Total protein, Albumin, creatinine, uric acid, and urea).

2. Organs :

The chicken was slaughtered after drawing blood, internal organs including the liver and kidneys were removed and weighted with an electronic balance. The organs have been fixed for histological examination by using a 10% formalin.

Biochemical measurement:

1: Measurements of Serum ALT and AST:

Serum ALT and AST had been enzymatically measured by using a special chemical kit (Biolabo, France), (29).

2: Measurements of serum Total protein and Albumin:

Had been measured by using Biolabo, France (30).

3: Measurements of serum creatinine

Serum Creatinine was measured enzymatically by using a special chemical kit (Biolabo, France), (31).

4: Measurements of serum urea

urea level was estimated using a commercial kit (Biolabo, France), (32).

5: Measurements of serum uric acid

The method is used to determine uric acid by reaction with urease as follows (33, 34).

6: Measurements of Malondialdehyde (MDA)

The concentration of MDA in serum was determined according to Buege and Aust method (35).

7: Measurements of superoxidase dismutase (SOD): (Hydroxylamine Method) was analyzed by colorimetric methods using reagent kits obtained from Elabscience/ USA.

8: Measurements of Serum GSH concentration:

The serum thiol concentration was measured according to the Ellman method as follows (36).

9: Measurements of Catalase (CAT):

was analyzed by using colorimetric methods using reagent kits obtained from Elabscience/ USA.

10: Measurements of Glutathione Peroxidase (GPX):

was analyzed by using colorimetric methods of reagent kits obtained from Elabscience/ USA.

Histological techniques

The animals were dissected at the end of the experiment, and samples of the organs were liver and kidney. These organs were fixed in 10% formalin buffered, slowly dehydrated in increased ethanol concentrations, handled with xylene, and embedded in paraffin. sections of 5 microns thickness of paraffin-embedded tissue were placed on glass slides and stained with hematoxylin and eosin stain (37)

Statistical Analysis

Experimental data were expressed as mean \pm standard deviation, the results were statistically analyzed using ANOVA by SPSS programming

difference and were considered significant at $p \leq 0.05$. (38)

Results and Discussion

-Clinical Signs

During the experiment, no death was detected in any of the study groups. chickens treated with

a dose of 0.2 mg/kg of diazinon daily by oral for 4 weeks. Some chickens display symptoms such as Ruffled feathers, salivation, diarrhea, difficulty breathing (breathing from the mouth), teary eyes, drooping of wings. Some birds were couldn't stand and sit with curled toes on their hocks figure (1),(2). no other significant clinical manifestation was noticed following *Saussurea costus* administration.



Figure(1): Chicken breathing from the mouth



Figure(2): Chicken showing diarrhea.

Effect of *Saussurea costus* roots ethanolic extract roots on bodyweight and weight of liver and kidneys in chickens treated with diazinon.

For all the experimental animals the initial body weights were the same. The final body weights of the chickens of the positive control group declined significantly ($p \leq 0.05$) compared to the negative control group and the prophylactic group as it is shown in table (1). In group 3, the

body weights of the chickens were non-significant differences compared to the negative control group.

The liver and kidney weights were significantly reduced as a result of toxicity with diazinon, whereas the findings showed non-significant differences ($p > 0.05$) were observed in weights of liver and kidneys in the prophylactic group compared to the negative control group.

Table(1):Effect of ethanolic extract of *Saussurea costus* roots on body weight and weights of liver and kidneys in chickens treated with diazinon.

Groups	Initial body weight (g)	Final body weight (g)	Liver weight (g)	Kidneys weight (g)
Negative control group (G1)	1458.3 ± 171.51 A	1591.7 ± 90.36 A	48.01 ± 2.01 A	16.55 ± 1.46 A
Positive control group (G2)	1462.5 ± 276.36 A	1033.3 ± 51.63 B	40.33 ± 0.37 B	10.80 ± 0.97 B
Prophylactic group (G3)	1454.2 ± 102.97 A	1675.0 ± 352.84 A	45.61 ± 3.68 A	15.00 ± 1.54 A

Values expressed as Mean ±SD (n=6), Different capital letters denote significant differences (P≤0.05) between experimental groups.

Effect of *Saussurea costus* roots ethanolic extract on WBC count, Lymphocyte, and Heterophil in chickens treated with diazinon.

There had been a significant decrease in the WBC count and the lymphocyte percentage in the positive group compared to the negative and prophylactic groups. While no significant differences (p≤0.05) were observed in the WBC

count and lymphocyte of chickens in a prophylactic group compared to the negative control. The results of heterophil observed a significant increased (p≤ 0.05) in the positive control group compared to the negative group. while the results of heterophil observed no significant changes (p≤0.05) between the prophylactic and negative groups (table 2).

Table (2): Effect of ethanol extract of *Saussurea costus* on WBC count, Lymphocyte, and Heterophil in chickens treated with diazinon.

Groups	WBC(10 ³ /ml)	Lymphocyte (%)	Heterophil (%)
Negative control group (G1)	8.13 ± 0.16 A	75.96 ± 0.24 A	20.15 ± 0.32 B
Positive control group (G2)	5.94 ± 0.99 B	55.88± 3.59 B	29.00 ± 1.54 A
Prophylactic group (G3)	8.24 ± 0.90 A	74.11 ± 5.70 A	21.00± 0.63 B

Values expressed as Mean ±SD (n=6), Different capital letters denote significant differences (P≤0.05) between experimental groups.

Effect of *Saussurea costus* roots ethanolic extract on ALT, AST, Albumin, and Total protein concentration in chickens treated with diazinon.

The findings showed that the use of diazinon for 4 weeks resulted in a significant increase (p≤ 0.05) in serum ALT and AST concentration as

compared with the negative group as it's shown in table (3). The serum ALT and AST concentration findings appeared nosignificant changes (p≤0.05) in the prophylactic group compare with the negative group.

The obtained results in a table (3) observed a significant decrease (p≤ 0.05) in serum total protein and albumin in the positive group. While

the results showed non-significant changes ($p \leq 0.05$) in serum total protein and albumin of the

prophylactic group compare with the negative group.

Table (3): Effect of ethanolic extract of *Saussurea costus* on ALT, AST, Albumin, and Total Protein concentration in chickens treated with diazinon.

Groups	ALT (U/L)	AST (U/L)	Albumin (g/L)	Total Protein (g/L)
Negative control group (G1)	3.90 \pm 0.36 B	187.00 \pm 8.76 B	7.75 \pm 0.53 A	23.38 \pm 0.52 A
Positive control group (G2)	14.16 \pm 0.51 A	320.00 \pm 59.21 A	5.15 \pm 0.51 B	19.51 \pm 0.77 B
Prophylactic group (G3)	4.16 \pm 0.87 B	209.00 \pm 49.41 B	7.20 \pm 0.46 A	22.50 \pm 0.94 A

Values expressed as Mean \pm SD (n=6), Different capital letters denote significant differences ($P \leq 0.05$) between experimental groups.

Effect of *Saussurea costus* roots ethanolic extract on serum creatinine, urea, and uric acid concentration in chickens treated with diazinon.

Table(4) indicates that diazinon induced a significant elevation ($p \leq 0.05$) in serum

creatinine, urea, and uric acid concentration of chickens in the positive control group. No significant changes at ($p \leq 0.05$) in creatinine, urea, and uric acid were observed in prophylactic and negative control groups.

Table (4): Effect of ethanolic extract of *Saussurea costus* on serum creatinine, urea, and uric acid concentration in chickens treated with diazinon.

Groups	creatinine (mg/dL)	urea (mg/dL)	uric Acid (mg/dL)
Negative control group (G1)	0.24 \pm 0.03 B	2.66 \pm 0.65 B	2.04 \pm 0.03 B
Positive control group (G2)	1.23 \pm 0.46 A	4.56 \pm 0.53 A	6.97 \pm 1.14 A
Prophylactic group (G3)	0.27 \pm 0.05 B	2.90 \pm 0.62 B	2.26 \pm 0.11 B

Values expressed as Mean \pm SD (n=6), Different capital letters denote significant differences ($P \leq 0.05$) between experimental groups.

Effect of *Saussurea costus* roots ethanolic extract on serum MDA, GSH, SOD, CAT, and GPX in chickens treated with diazinon.

The results showed a significant increase ($p \leq 0.05$) of the MDA value in the positive control group compared with the negative and prophylactic groups, but no significant differences ($p \leq 0.05$) among both prophylactic

and negative control groups table (5).

In the table (5) diazinon induced a significant low ($p \leq 0.05$) in levels of GSH, SOD, CAT, and GPX compare with the negative group, and nosignificant differences ($p \leq 0.05$) among both prophylactic and negative control groups were observed in GSH, SOD, CAT and GPX levels.

Table (5): Effect of ethanolic extract of *Saussurea costus* roots on serum MDA, GSH, SOD, CAT, and GPX in chickens treated with diazinon.

Groups	MDA ($\mu\text{mol/l}$)	GSH ($\mu\text{mol/l}$)	SOD (U/ml)	CAT (U/ml)	GPX (U)
Negative control group	0.80 ± 0.65 B	255.12 ± 8.27 A	151.01 ± 8.35 A	20.08 ± 2.61 A	162.97 ± 21.47 A
Positive control group	3.66 ± 0.46 A	134.33 ± 1.50 B	77.90 ± 5.18 B	7.40 ± 0.08 B	76.33 ± 1.96 B
Prophylactic group	0.92 ± 0.41 B	244.17 ± 44.88 A	147.92 ± 1.67 A	17.05 ± 7.93 A	156.34 ± 11.26 A

Values expressed as Mean \pm SD (n=6), Different capital letters denote significant differences ($P \leq 0.05$) between experimental groups.

Morphological Examination of liver and kidney.

The morphological changes observed on the organs of chickens include:-The liver of negative control and the prophylactic group displayed normal size, firmness, has prominent sharply defined edges and reddish-brown colored as seen in Figure (3),(5). Whereas a positive control group liver was pale, crumbles easily, and smaller

from those of a negative control group as seen in Figure (4).

Kidneys of negative control and the prophylactic group were long reddish-brown, smooth, as seen in Figure (6),(8), but kidneys of the positive control group were pale, smaller from those of a negative group as shown in figure (7).



Figure (3) : A liver of negative control chicken. showing firm, and has prominent sharply defined edges and reddish-brown colored.

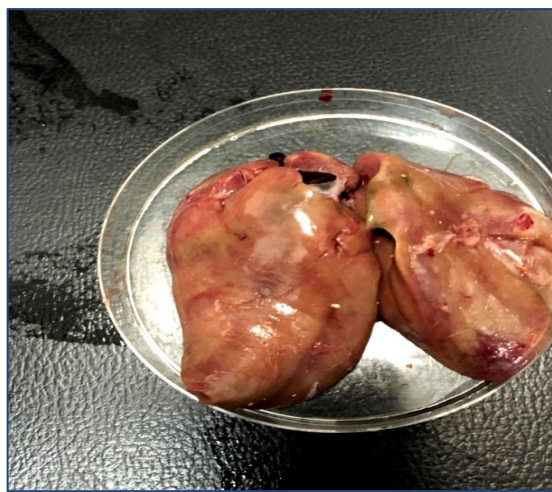


Figure (4) : A liver of positive control chicken showing less firm, pale, easily crumbles and smaller than that of normal.

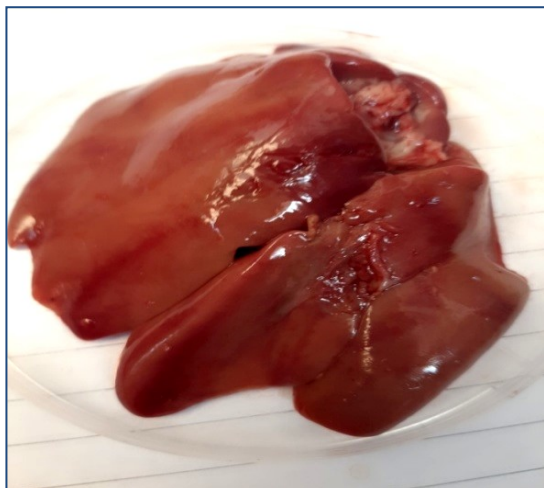


Figure (5) : A liver of prophylactic chicken showing firm, and has prominent sharply defined edges and near to the normal status.



Figure (6): Kidneys of negative control chicken. showing reddish-brown in color, smooth.

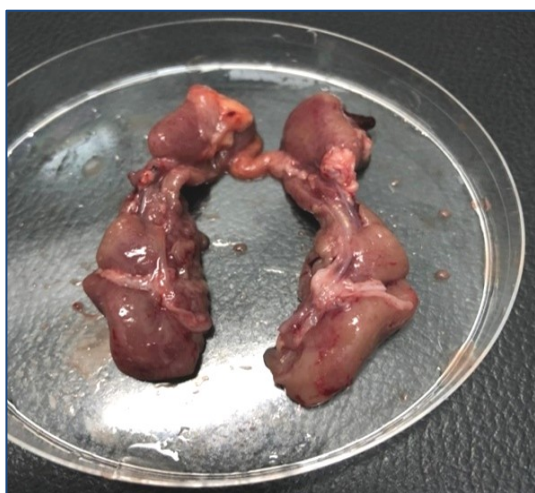


Figure (7): Kidneys of positive control chicken are pale and smaller than that of negative.



Figure (8): Kidneys of prophylactic chicken showing long reddish-brown in color, near to normal status.

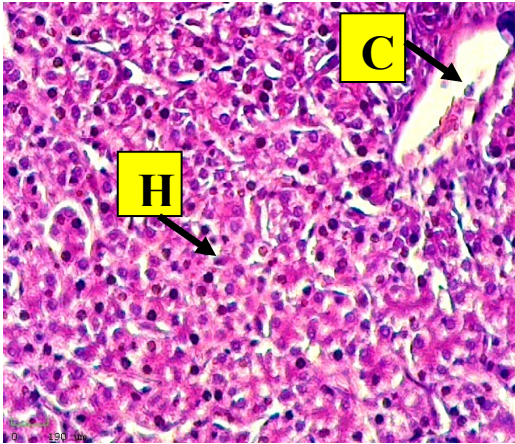
Histopathological Examination:

Negative group liver of chicken observed normal portal area, central vein, and hepatocytes as seen in figure (9). Whereas the chickens showed histopathological alterations in the positive control group, including congested and dilated central vein, vacuolated cytoplasm of

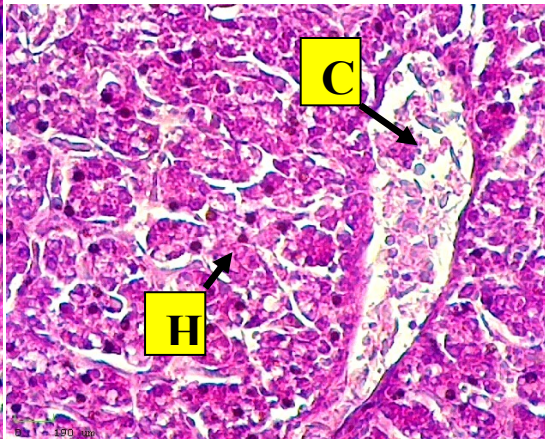
hepatocytes, focal necrotic tissue filled with inflammatory cells, thickening of the bile duct, thickening wall of the portal artery. Fibroblast in portal area, dilated sinusoid as seen in figure (10). The liver of chickens in the prophylactic group revealed the central vein, sinusoid, and hepatocytes are normal as seen in figure (11).

In the chicken negative control group, the kidney appeared normal renal tubules and glomerulus as shown in figure (12), When the diazinon was used it induced dilatation of renal tubule, hemorrhage, and atrophy in glomerulus as shown in figure

(13). In Figure (14) kidney of chickens in the prophylactic group observed renal tubules and glomerulus are normal, some of the epithelial cells are swelling.



Figure(9): Histological section of the chicken liver of negative control group showing normal central vein (C) and normal hepatocyte (H). stained with H&E,(400X)



Figure(10):Histological section of the chicken liver of positive control group showing congested and dilated central vein (C), focal necrotic tissue filled with inflammatory cells, vacuolated cytoplasm of hepatocytes (H), stained with H&E,(400X) .

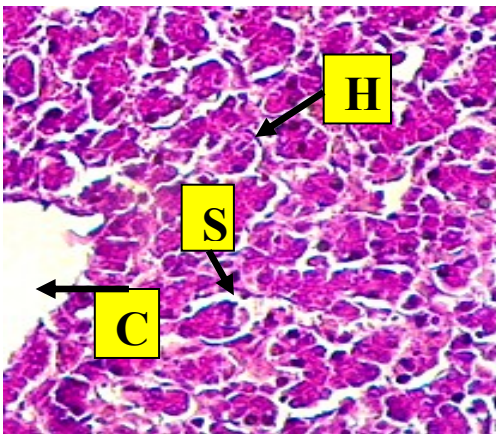


Figure (11):Histological section of the chicken liver of prophylactic group showing normal central vein (C), normal sinusoid(S) and normal hepatocytes (H), stained with H&E,(400X) .

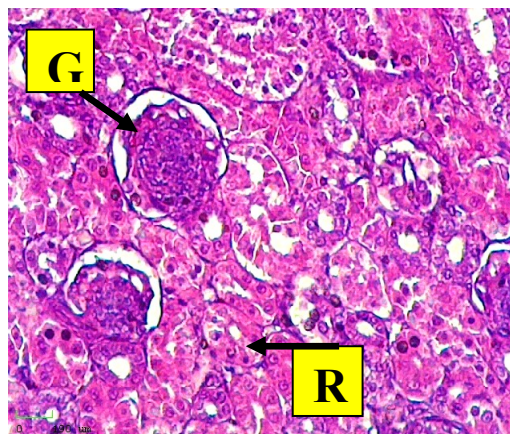


Figure (12):Histological section of the chicken kidney of negative control group showing normal renal tubules (R) and glomerulus(G), stained with H&E,(400X)

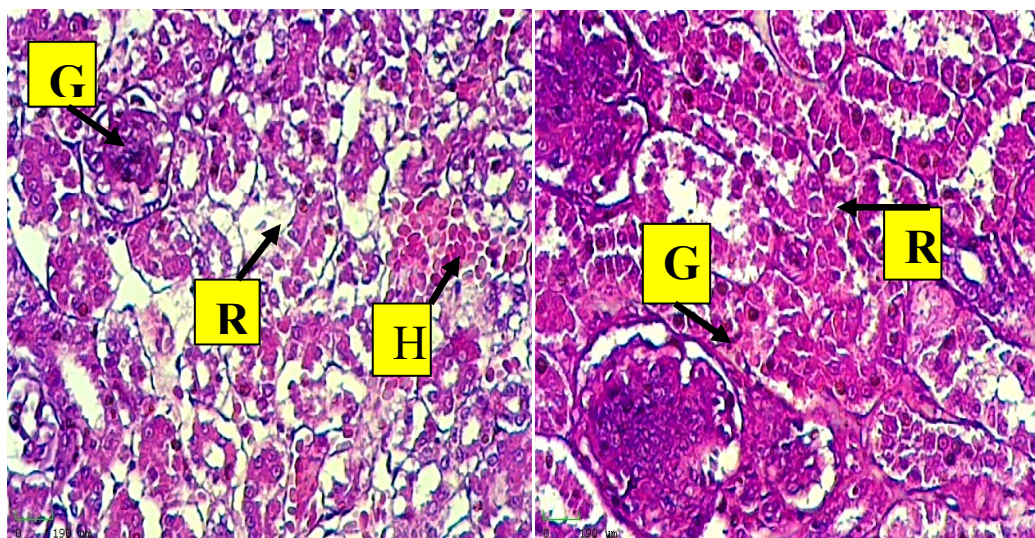


Figure (13):Histological section of the chicken kidney of positive control group showing dilatation of renal tubule (R), hemorrhage(H) and atrophy in glomerulus (G), stained with H&E,(400X)

Figure (14):Histological section of the chicken kidney of prophylactic group showing normal renal tubules (R) and glomerulus (G), some of the epithelial cells are swelling, stained with H&E,(400X)

To the best of knowledge, it was the first research to examine *S.costus* as protection against hepatorenal toxicity caused by diazinon. The clinical signs confirm the effectiveness of organic phosphorous pesticides on the nervous system by inhibiting the AChE enzyme leading to imbalance and disruption in the physiological function of this important organ. In this study, the ethanolic extract of *S. costus* administration with DZN ameliorating the abnormal signs of toxicity compared to control positive group. (39, 40)

Diazinon administration led to a significant decrease ($p \leq 0.05$) in B.W of chickens compare with the negative group.. In the study(13) documented the most important signs of toxicity in exposed animals to DZN were decreased bodyweight, decreased food intake, and diarrhea. Defined reduced body weight might occur from increased dose and raised diazinon aggregation in the blood of exposed animals, resulted from a loss of appetite, lowered intake of food, and/or metabolic disturbance. It suggested that a reduction in body weight in DZN intoxicated

experimental animals subjected may cause increased lipid and protein degradation by the consequences of the OPC toxicity (41). On the other hand, hepatic and renal toxicity can cause the reduced size of the liver and kidney due to acute or chronic hepatic or kidney damage resulting in cell loss (42). our findings observed that the administration of the ethanolic extract of *S. costus* induced improvement of bodyweight of chickens and liver and kidney weights, through its contents which were flavonoids, proteins, and carbohydrates, which are needed for growth, body repair, and maintenance.

The total WBCs count and lymphocytes percentage decreased significantly and Heterophils percentage increased significantly in DZN treated chickens as compare with the negative control group. These results in an agreement to the previous studies (43, 44). Total WBCs are decreased in groups treated with DZN, indicating dysfunction in hematological tissues such as bone marrow, spleen, and thymus, leading to an indication of deficiency in the DZN-induced immune system (45). These findings were in

agreement with the observation of (46) who recorded that diazinon treatments may deplete the lymphocyte supply in the body and indicated that continued exposure of rats to a sublethal dose of DZN showed lower leukocyte counts. On the other hand, the elevation of Heterophils percentage found in this study coincides with those of (47) Who has noticed that Fry Rainbow Trout has neutrophilia after exposure to DZN. The possible cause of neutrophilia in host defense may be due to the phagocytic cells.

Our result observed the administration of *S. costus* a significantly increased total WBCs count as a compared to the positive control group and restore count near to negative control group may be due to active materials known as dehydrocostus lactone and costunolide in *S. costus* (48) that refers to *S. costus* improved the immunity of DZN- treated chickens.

Our findings showed a significant raised in the serum level of ALT, AST, and a significant decline in albumin and total protein in the positive group in comparison with the negative group. (49-52) have reported similar results. The observed rise in levels of ALT and AST, and also the decreased total protein and albumin levels, are the main diagnostic symptoms of hepatic illness (53). Studies have indicated that of these biomarkers are released into the bloodstream when the parenchymal hepatic cells are exposed to toxic pesticides such as diazinon (50)

Diazinon has a direct toxicity effect on the metabolisms of carbohydrates, lipids, and proteins. Many reporters suggesting the cause of hypoproteinemia may result from a severely decreased protein synthesis or due to any renal failure, liver injury, or urinary protein elimination (50). (54) have been reported that tissue destruction induced by diazinon and apoptosis of hepatocytes may be the main causes for reducing total protein synthesis and liver immunoglobulin.

The plasma levels of ALT and AST in pre treated and post treated groups of mice by *S. costus* were significantly decreased than that of the positive group (55). The costunolide isolated

from *S. Costus* caused a decrease in plasma AST and ALT activity compared with the diabetic group and consequently in liver damage induced by STZ-induced diabetes in rats (56). In the current study, the ethanolic extract of *S. costus* administration enhanced the hepatotoxicity induced by diazinon, the result observed decreased liver enzymes (AST and ALT) and increased total protein and albumin in the prophylactic group in comparison with the positive control group may be due to phytochemical compounds such as flavonoids and chlorogenic acid that serve as antioxidant material help to inhibit free radicals that caused lipid peroxidation and prevents diazinon poisoning, these results in agreement with (57, 58).

The insecticide, diazinon significantly raised in the creatinine, urea levels, and changes in relative weights of the kidney. The significant elevation in serum creatinine and urea may be associated with weakness of glomerular activity and tubular injury in the kidneys (53). Also, increasing the uric acid concentration in the sera of treated chickens in comparison to a negative control group could result from degradation of purines and pyrimidines or an increased uric acid levels due to either overproduction or the inability to excretion (59) Renal damage induced by diazinon can be due to the oxidative stress resulted by the production of free radicals. DZN intoxication significantly raised markers of serum kidney injury: creatinine, urea, and uric acid (60, 61) which match our findings. Administration of *S. costus* extracts to diazinon-intoxicated groups, successfully altered creatinine urea and uric acid levels to almost negative group values that indicate nephroprotective activities of *S. costus* on the toxic effect of diazinon, due to the high concentration of flavonoids and alkaloids they act as an antioxidant and/or free-radical scavenging activities. The *S. costus* extract is abundant in bioactive molecules, like dehydrocostus lactone, costunolide, cynaropicrin, monoterpenes, sesquiterpenoids, flavonoids, lignans, triterpenes,

steroids, and glycosides they produce as an antioxidant and/or free-radical scavenging activities these results in agreement with (28, 62).

OP pesticides are among the most aspects of lipid peroxidation formation and can rise lipid peroxidation by altering the plasma membrane. Decomposition of diazinon caused the formation of oxygen free radicals mostly superoxide anions and strong hydroxyl radicals and then, to an elevation in the level of MDA, that had been demonstrated by (63). Organophosphorus are oxidants that weaken enzymatic antioxidant defenses, involving superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) (5). While treatment with the ethanolic extract of *S. costus* has caused a decline of the MDA and elevation in levels of GSH, SOD, CAT, and GPX of the prophylactic group in comparison to the positive group, therefore, these findings might be because of *S. costus* roots possessing the phytochemical components as flavonoids that prevent toxic metabolite activities and so stabilization of the cell membranes of the intracellular proteins and another materials (64).

The liver of chickens in the positive control group observed histopathological damages as congested and dilated central vein, vacuolated cytoplasm of hepatocytes, focal necrotic tissue filled with inflammatory cells, thickening of the bile duct, thickening wall of the portal artery. Fibroblast in portal area, dilated sinusoid. Similar to (65) who recorded diazinon treated animals for 4 weeks resulted in many histopathological changes involving infiltration of focal inflammatory cells, severe congestion of the blood vessels, central and portal veins, and degeneration of bile ducts. The sinusoidal spaces had been dilated and filled with red blood cells. Cytoplasmic vacuolation occurred in the hepatocytes.

While the kidneys of the diazinon group observed dilatation of renal tubule, hemorrhage, and atrophy in the glomerulus. These findings in agreement with (13) who reported a diazinon-

treated rabbit kidney for four weeks displaying glomerulus atrophy with the lining epithelial cells degeneration of renal tubules and congestion between renal tubules. After four weeks, the renal tubules suffered severe damage and their cells exhibited cytoplasmic vacuolation and some glomeruli atrophy. Congested blood capillaries between the degenerated tubules.

The findings of this study indicated that *S. costus* extract inhibited hepatotoxicity and nephrotoxicity induced by diazinon in chickens. The inhibited effects of *S. costus* extract might be attributed to its antioxidant and anti-inflammatory activities. Thus, *S. costus* extract can be regarded as an inhibited agent to free radical-induced liver and kidney damage caused by diazinon and the extract could reduce the harmful aspects of diazinon in chickens. These findings agree with that of (28, 55, 66)

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