THE EFFECT OF ARTEMISIA HERBA ALBA ON CHICKENS FED RATION CONTAMINATED WITH AFLATOXIN-B1

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ABSTRACT

Ninety (90), one-day-old Baladi chicks (males and females) were divided into three equal groups and allotted in their cages. Group (1) was the negative control (fed on a standard balanced ration without mixing aflatoxin-B1). Chickens of Group (2) were fed daily on ration contaminated with prepared aflatoxin-B1 at a dose of 0.05 ppm (500μg/kg feed), and were not treated (positive control). Group (3) was fed daily on a ration contaminated with prepared aflatoxin-B1 (500μg/kg feed) and treated with crude aqueous extract (CAE) of Artemisia herba alba (0.39 g /kg B.wt. in the drinking water for 45 consecutive days, starting from 30 to 75 day of age). The chickens were weighed every 15 days till the age of 75 days, and the weight-gain was calculated. The values of haemoglobin, total erythrocytic and leukocytic counts, serum total proteins, albumin, globulin, serum urea, creatinine, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin were determined. Histopathological sections were prepared from the livers at the age of 75 day (end of the experiment).

The body weight and body-gain of chickens fed daily on ration contaminated with the prepared aflatoxin-B1 and treated with Artemisia herba alba crude aqueous extract (Group 3) was significantly increased when compared with positive control group (Group 2). The Erythrocytic (RBCs) and total leukocytic (WBCs) count besides the hemoglobin (Hb%) were significantly increased, while the total protein, albumin and globulin were significantly elevated in (Group 3) when compared with (Group 2). The histopathological examination revealed that the liver showed regenerated hepatocytes, mild fatty changes and necrosis. These results clearly indicate that the Artemisia herba alba was partially protected the liver against the aflatoxin-toxicosis, resulting in improvement of the growth performance of chickens (body weight and weight-gain). It ameliorated the toxic effect of aflatoxin. This was elucidated by the augmented gain in the body weight and increase in blood proteins.

الملخص العربى

تأثـــير نبـــات الشـــيح على الدجـــاج المغـــذى على علـــف به أفـــلاتـــوكســـين-ب١

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أستخدمت فى هذه الدراسة ٩٠ كتكوت بلدى (سلالة محلية) عمر يوم، وقسمت هذه الكتاكيت إلى ثلاثة مجموعات متساوية (٣٠ كتكوت لكل مجموعة)، أعتبرت المجموعة الأولى مجموعة ضابطة سلبية (مغذاة على علف متزن وغير محتوى على الأفلاتوكسين - ب١)، عند عمر شهر تم تغذية كتاكيت المجموعتين الثانية والثالثة على علف متزن ومحتوى على ٥ر٠ جزء فى المليون (٥٠٠ ميكروجرام / كجم علف) من الأفلاتوكسين - ب١ ولمدة ٤٥ يوماً إبتداء من عمر ٣٠ يوم وحتى عمر ٧٥ يوم، وتركت المجموعة الثانية كمجموعة ضابطة إيجابية (مغذاه على علف به أفلاتوكسين - ب١ وغير معالجة)، ومع بداية التغذية على العلف المحتوى على الأفلاتوكسين - ب١ "عمر شهر" تم علاج المجموعة الثالثة بالمستخلص المائى الخام لنبات الشيح (٣٩ر٠جم / كجم وزن حى)، وكان العلاج فى مياه الشرب ولمدة ٤٥ يوم متتالية، وبعد شهر من التغذية على العلف المحتوى على الأفلاتوكسين - ب١ تم وصف الأعراض المرضية وكذلك الصفة التشريحية للكتاكيت النافقة، وقد تم وزن الفراخ كل ١٥ يوم وحساب الوزن المكتسب لكل طائر، كما أخذت عينتين دم من كل مجموعة عند عمر ٧٥ يوم (فى نهاية التجربة)، الأولى على مانع للتجلط وذلك لعد كرات الدم الحمراء والبيضاء وقياس نسبة الهيموجلوبين، والعينة الثانية على غير مانع للتجلط لعمل السيرم اللازم لقياس بعض وظائف الكبد (ALT,AST) والبليروبين الكلى، وبعض وظائف الكلى (اليوريا والكرياتينين)، والبروتين الكلى والألبيومين والجلوبيولين. كما تم أخذ عينات نسيجية من الكبد لدراستها هستوباثولوچيا.

وقد أوضحت النتائج أن المستخلص المائى الخام لنبات الشيح قد أدى إلى تحسين آداء الطيور فى نهاية التجربة (بعد ٧٥ يوم) وذلك بزيادة أوزان الفراخ وكذلك الأوزان المكتسبة زيادة معنوية فى الطيور المغذاه على علف به أفلاتوكسين عند مقارنته بالمجموعة الضابطة الإيجابية (مغذاه على علف به أفلاتوكسين - ب١، وغير معالجة)، كما أنه أدى إلى زيادة عدد كرات الدم الحمراء والبيضاء ونسبة الهيموجلوبين، كما أنه أدى إلى إرتفاع فى مستوى البروتين الكلى والألبيومين والجلوبيولين معنوياً عند مقارنته بالمجموعة الضابطة الإيجابية (مغذاه على علف به أفلاتوكسين -ب١ وغير معالجة)، كما أنه أدى إلى تقليل مستوى والبليروبين الكلى واليوريا والكرياتينين معنوياً عند مقارنته بالمجموعة الضابطة الإيجابية، وقد أوضحت النتائج الهستوباثولوچية وجود تنكرز واضح فى خلايا الكبد للدجاج المغذى على عليقة بها سم الأفلاتوكسين - ب١، بينما تحسنت هذه الصورة الباثولوچية نسبياً فى خلايا الكبد للدجاج المعالج بالمستخلص المائى الخام لنبات الشيح، وخلاصة هذه الدراسة : يعتبر نبات الشيح محسن جيد لآداء الطيور ولوظائف الكبد.

INTRODUCTION

Artemisia herba alba (Wormwood) has been widely used in folkloric medicine for remedy of liver diseases such as hepatitis, jaundice and fatty liver in the traditional oriental medicine **(Huang et al, 2004)**. Artemisia herba alba shoots and Ammi visnaga seeds were fed to 7 day old Bovans chicks at the rate of 2% and 10% of the diet for 9 weeks. The chicks; fed diets contained 2% Ammi visnaga seeds or 2% Artemisia herba alba shoots; showed a less marked depression in the growth rate and a less damage to the vital organs, compared with those fed 10% Ammi visnaga or 10% Artemisia herba alba **(Ibrahim et al, 2004)**. The essential oil of the Artemisia herba alba was active against some Gram-positive and Gram-negative bacteria **(Yashphe et al, 1979)**. The antifungal activity of Artemisia herba alba was found to be associated with two major volatile compounds (Carvone and piperitone) which were isolated from the fresh leaves of the plant **(Saleh et al, 2006)**.

The hepatoprotetive activity of the extracts of Artemisia was investigated against substances which induced hepatitis in mice. The water extract of Artemisia campestris scavenged the free radicals formed by carbon tetrachloride treatment resulting in protection against carbon tetrachloride-induced liver toxicosis **(Aniya et al, 2000)**. Artemisia rupestris had significantly protective effect against the chemical liver injury and could treat the immunological induced hepatitis **(Israpil et al, 2002)**. The extract of Artemisia apiacea possessed not only the anti-oxidant effect, but also the protective activities in the carbon tetrachloride-intoxicated rats **(Kim et al, 2003)**. The aqueous extract of Artemisia capillaris Thunb inhibited the expression of the inflammatory proteins **(Hong et al, 2004)**. Artemisia capillaries and three other herbs were widely used in Asia to prevent and treat the neonatal jaundice **(Huang et al, 2004)**. Treatment of mice with different doses of Artemisia vulgaris (aqueous-methanol extract, 150-600 mg (sol)/ kg) reduced the toxin-induced rise in plasma ALT and AST **(Gilani et al, 2005)**.

The hematological and biochemical alterations observed in duckling and chickens fed on ration contaminated with 0.5 ppm aflatoxin were anaemia, hypoproteinaemia and increased aspartate aminotransferase activity "AST" **(Brown and Abrams, 1965)**. Feeding turkey poults aflatoxin-B1 in a dose of 0.5 ppm caused a decrease in the total serum protein and albumin levels **(Richard et al, 1973)**.

Histopathologically, fatty change, hyperplasia of the bile duct epithelium and hepatocytes, nuclear enlargement, margination of the chromatin and nuclear dissolution were detected in poults given o.25 and 0.5 ppm aflatoxin-B1 **(Pier and Heddleston, 1970)**. Liver fibrosis, hydropic degeneration of hepatocytes and hyerplasia of the bile ducts were seen in chickens fed ration contaning 0.45 ppm aflatoxin-B1 **(Shawky, 1989)**. The histopathological findings in chickens fed diet contaning 0.5 ppm aflatoxin-B1 for one month were dialated hepatic blood vessels, vacuolar degeneration of the hepatocytes, local necrosis in the liver parenchyma and bile duct hyperplasia with aggregation of inflamatory cells **(Marcel, 1994)**. Artemisia herba alba improved the liver architecture, decreased the cellular swelling and apoptosis cells. This scientifically vali-

dated the traditional use of Artemisia vulgaris for various liver disorders **(Gilani et al, 2005)**. Microscopically, there was a mild hydropic degeneration in the hepatocytes and proximal convoluted renal tubules **(Marrif et al, 1995)**.

The exposure of the chickens to as low as 0.2-1 ppm. aflatoxin-B1 in the diet led to poor growth rates, reduced feed efficiency, marked drop in egg production, liver damage, bile duct proliferation and a decreased resistance to the common infectious diseases (**Smith and Hamilton, 1970 and Newberne, 1973**). The repeated exposure of chickens to low doses of aflatoxin-B1 (0.5 ppm) contaminating their rations caused abnormal signs, hematological, serum biochemical and histopathological findings **(Marcel, 1994)**.

The aim of this study was to investigate the effect of Artemisia herba alba extract on the Baladi chickens; fed daily on ration contaminated with prepared aflatoxin-B1 (500μg/kg feed); through blood picture, liver and kidney functions besides the histopathological findings and the growth performance.

MATERIALS AND METHODS

Ninety, one day old Baladi chicks (males and females) were purchased at the age of one day and kept in a confined parasite free environment until starting the experiment at the age of 30 days. The chickens were divided into three equal groups (30 chicks per each). They were allotted in their separate units in metal wire-floored batteries; after arranging them using the ranking method **(Gardiner and Wehr, 1950)**.

According to the method of **Davis et al (1966)**, the aflatoxin was produced in this work from Aspergillius flavus (provided by the Botany Department, Faculty of Science , Benha University) which was grown on a liquid yeast extract sucrose (YES) medium. The determination of the aflatoxin; produced in the liquid medium; after extraction was done by thin layer chromatography (TLC) as described by **Peterson and Ciegher (1967)**. The prepared aflatoxin-B1 was mixed with the rations to provide a final concentration of 0.5 ppm (500μg /kg feed) according to Gouda et al (1984).

The commercial starter ration was obtained from the Islamic Centre Company. The ration was balanced (containing crude protein not less than 21%, crude fat not less than 2.7%, crude fibers not more than 2.7% and metabolizing energy not less than 2950 Kcal/ kg ration). Group (1) was fed on balanced ration without mixing aflatoxin-B1. While Groups (2&3) were daily fed on the contaminated rations with aflatoxin-B1 for 45 consecutive days during the age of 30 to 75 days. The rations and water were given to the chickens ad-libitum.

The aqueous extract of Artemesia herba alba shoots (leaves and stems) was prepared according to **Marrif et al (1995)** through soaking the shoots in the distilled water for over night, then sieved and offered to the birds after thirsty for 2-3 hr. at least.

Table (1) shows the experimental design during the age of 30-75 days. Group (1) was considered as a negative control (fed on balanced ration without mixing aflatoxin-B1) and

Group (2) was not treated and regarded as positive control group. While Group (3) was treated with crude aqueous extract (CAE) of Artemisia herba alba in a dose of 0.39 g /kg B.wt. in the drinking water during the age of 30 to 75 days according to **Marrif et al (1995)**.

After two weeks from dosing, the signs were described besides the post-mortem lesions in the dead birds. The chickens were weighed every two weeks and the weight-gain was calculated. The sacrificed chickens were necropsied and the lesions were recorded .

Two blood samples were collected at the age of 75 days (end of the experiment). The 1st sample was collected on EDTA for hematological studies, and the 2nd one was collected to prepare serum for biochemical studies. The values of the hemoglobin and total erythrocytic and leukocytic counts were determined according to the standard techniques described by **Jain (1986)**. The serum biochemical parameters were evaluated. The serum total proteins **(Sonnenwirth and Jarett (1980)**, albumin **(Beng and Lim, 1973)** and globulin values were calculated according to the results of albumin and globulin. The serum urea **(Patton and Crouch, 1977)** and creatinine **(Husdan and Rapaport (1968)**, besides the activity of the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) **(Reitman and Frankel, 1957)**, together with the total bilirubin **(Jendrassiki, 1938)** were determined.

Specimens from the liver were collected at necropsy (at the age of 75 days) and fixed in neutral buffered formalin. Five micron thick paraffin thickness sections were prepared, stained with H&E and examined microscopically **(Drury et al, 1976)**.

The differences among treatments were tested at $P \leq 0.05$ by the analysis of variance; ANOVA; according to **Duncan (1955) and Snedecor and Cochran (1969)** using the computer software program called **SPSS, Ver., 11, (2001)**.

RESULTS

Clinical sings and findings were noted in the chickens fed daily on the contaminated rations with aflatoxin-B1 (500μg/kg feed) for 45 consecutive days (starting from 30 to 75 days of age). The observed signs were depression, dullness, loss of appetite, ruffled feathers, emaciation and unthriftness after two weeks post-treatment. The necropsy of the dead and sacrificed chickens showed congested and enlarged liver, emaciation and septicemic carcasses.

Table (2) shows the mean body weights of the chickens fed daily on the rations contaminated with aflatoxin-B1 and treated with Artemisia herba alba crude aqueous extract. The mean body weight of chickens fed on the rations contaminated with aflatoxin-B1 and treated with Artemisia herba alba crude aqueous extract (Group 3) was significantly increased at the age of 75 days when compared with Group (2) at $P \leq 0.05$.

Table (3) shows the mean body weight-gain of the chickens. The mean body-weight gain of Group (3) was significantly increased at the age of 75 days when compared with Group (2) at $P \leq 0.05$.

Table (4) shows the values of some hematological parameters at the age of 75 days. The values of the erythrocytes (RBCs), total leukocytes (WBCs) and hemoglobin (Hb%) were significantly increased in Group (3) when compared with Group (2) at $P \leq 0.05$.

Table (5) shows the values of some biochemical parameters at the age of 75 days. The values of the total protein, albumin and globulin were significantly elevated whereas the ALT, AST, total bilirubin, serum urea and creatinine were significantly lowered in Group (3) when compared with Group (2) at $P \leq 0.05$.

Microscopically, Fig. (1) shows the normal histological structure of the liver cells (hepatocytes). The chickens fed ration contained 0.5 ppm aflatoxin-B1 for 45 consecutive days and not treated (Group 2) shows fatty and vacuolar degeneration of the hepatocytes besides focal necrosis in the liver parenchyma with aggregations of inflammatory cells (Fig,2). The liver of chickens fed ration contained aflatoxin-B1 and treated with Artemisia crude aqueous extract (Group 3) shows an ameliorated fatty changes and vacuolar degeneration of the hepatocytes. Morever, mild hydropic degeneration was encountered (Fig,3).

Table (1): Experimental design during the age of 30-75 days.

Groups	Design	No of chickens	Treatments		Necropsy and blood sampling at the age of 75 days
			Aflatoxin-B1 (500 µgm / kg ration)	Artemisia herba alba extract (0.39 gm / kg B.wt)	
Group (1)	Negative control	30	--	--	+
Group (2)	Positive control	30	+	--	+
Group (3)	Experimental	30	+	+	+

Table (2): Mean body weight of Chickens fed ration contaminated with aflatoxin-B1 and Treated with Artemisia herba alba aqueous extract.

Age in days	Groups			LSD at P≤ 0.05
	Group (1) Chickens fed standard ration without aflatoxin-B1 (-Ve Control)	Chickens fed ration contaminated with aflatoxin-B1 (500µg /kg ration)		
		Group (1) Not treated (+Ve Control)	Group (1) Treated with Artemisia herba alba extract	
1	28.04[a] ±0.43	27.72[a] ±0.48	27.64[a] ±0.51	----
15	100.01[a] ±1.27	99.93[a] ±1.16	99.96[a] ±1.28	----
30	260.36[a] ±1.37	162.84[b] ±1.75	173.08[b] ±1.33	82.9300[*]
45	351.43[a] ±2.67	245.30[b] ±2.94	251.59[b] ±1.91	9.5000[*]
60	539.16[a] ±3.44	387.11[b] ±2.46	399.47[b] ±3.09	48.4400[*]
75	768.14[a] ±3.87	411.54[c] ±3.83	600.81[b] ±3.85	17.1300[*]
Improvement % of body weight	100	53.58	78.22	----

• ([*]): Significance at P≤0.05. •n=30 •Data were analysed by One Way ANOVA.
•LSD: Least significance difference among means at P≤0.05
•Means with different alphabetical superscripts in the same row are significantly different.

Table (3): Mean body weight-gain of Chickens fed ration contaminated with aflatoxin-B1 and Treated with Artemisia herba alba aqueous extract.

Age in days	Groups			LSD at P≤ 0.05
	Group (1) Chickens fed standard ration without aflatoxin-B1 (-Ve Control)	Chickens fed ration contaminated with aflatoxin-B1 (500µg /kg ration)		
		Group (2) Not treated (+Ve Control)	Group (3) Treated with Artemisia herba alba extract	
15	71.16[a] ±1.47	70.96[a] ±1.99	72.36[a] ±1.29	----
30	154.27[a] ±2.37	153.56[a] ±2.71	155.18[a] ±2.51	81.480[*]
45	97.18[a] ±2.79	38.03[c] ±4.12	52.34[b] ±3.92	12.500[*]
60	190.12[a] ±4.31	35.77[c] ±3.31	67.64[b] ±3.07	13.920[*]
75	230.36[a] ±5.01	89.86[c] ±4.05	129.87[b] ±3.45	17.320[*]
Improvement % of body gain	100	39.01	56.38	----

• ([*]): Significance at P≤0.05. •n=30 •Data were analysed by One Way ANOVA.
•LSD: Least significance difference among means at P≤0.05
•Means with different alphabetical superscripts in the same row are significantly different.

Table (4): Some hematological parameters after treatment in chickens fed ration contaminated with aflatoxin-B1 and Treated with Artemisia herba alba aqueous extract.

• (*): Significance at P≤0.05. •n=30 •Data were analysed by One Way ANOVA.

Parameters	Groups			LSD at P≤ 0.05
	Group (1) Chickens fed standard ration without aflatoxin-B1 (-Ve Control)	Chickens fed ration contaminated with aflatoxin-B1 (500µg /kg ration)		
		Group (2) Not treated (+Ve Control)	Group (3) Treated with Artemisia herba alba extract	
Erythrocytes RBCs)) cell x10^6/ml))	3.01^a ±0.043	2.03^c ±0.063	2.82^b ±0.066	0.2100*
Total Leukocytes WBCs)) (cellx10^3/ml)	18.25^a ±0.354	14.18^c ±0.271	16.42^b ±0.461	1.0400*
Hemoglobin (Hb) (gm%)	9.55^a ±0.270	8.18^c ±0.213	9.41^b ±0.179	0.7400*

•LSD: Least significance difference among means at P≤0.05

•Means with different alphabetical superscripts in the same row are significantly different.

Table (5): Some serum biochemical parameters after treatment in chickens fed ration contaminated with aflatoxin-B1 and Treated with Artemisia herba alba aqueous extract.

Parameters	Groups			LSD at P≤ 0.05
	Group (1) Chickens fed standard ration without aflatoxin-B1 (-Ve Control)	Chickens fed ration contaminated with aflatoxin-B1 (500µg /kg ration)		
		Group (2) Not treated (+Ve Control)	Group (3) Treated with Artemisia herba alba extract	
Serum Total protein (g/L)	4.152^a ±0.060	3.262^c ±0.078	3.783^b ±0.069	0. 2100*
Serum albumin (g/L)	1.926^a ±0.051	1.287^b ±0.047	1.622^b ±0.049	0.1540*
Serum globulin (g/L)	2.155^a ±0.091	1.886^b ±0.126	2.174^a ±0.068	0.6950*
Serum AST (U/L)	28.565^c ±0.428	35.010^a ±0.387	30.745^b ±0.389	1.9700*
Serum ALT (U/L)	19.782^c ±0.415	26.134^a ±0.411	23.142^b ±0.540	1.460^{0*}
Total bilirubin (µmol/L)	3.352^c ±0.174	6.431^a ±0.155	4.150^b ±0.075	0. 5940*
Serum Urea (mg/dl)	10.06^b ±0.458	11.74^a ±0.654	9.88^b ±0.611	1.2990*
Creatinine (mg/dl)	1.28^c ±0.125	1.88^a ±0.134	1.40^b ±0.076	0.0210*

• (*): Significance at P≤0.05. •n=30

•LSD: Least significance difference among means at P≤0.05

•Means with different alphabetical superscripts in the same row are significantly different at P≤0.05.

•Data were analysed by One Way ANOVA.

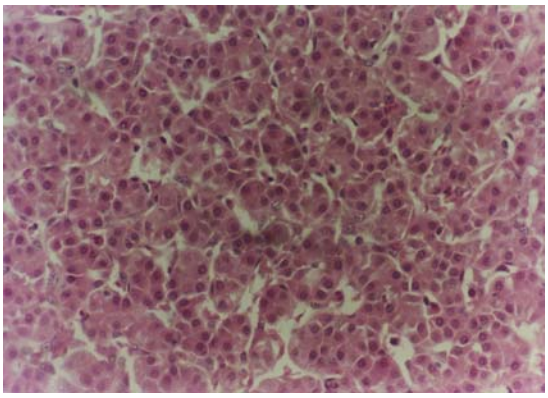

Fig. (1) : Group (1), liver showing normal histological structures of hepatocytes. H & E., (X 400).

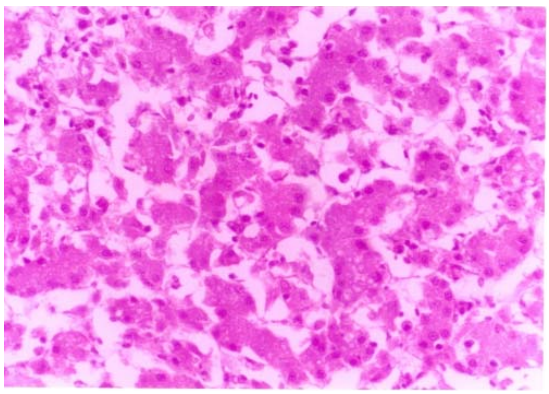

Fig. (2) : Group (2), liver showing focal necrosis, fatty changes and vacuolar degeneration in the hepatocytes. H&E., (X 400).

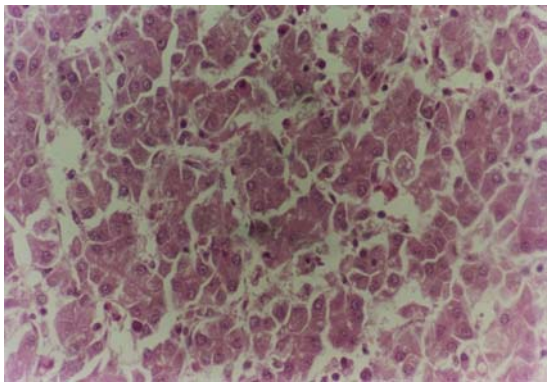

Fig. (3) : Fig., (3): Group (3), liver showing mild degenerative changes in the hepatocytes. H&E., (X 400).

DISCUSSION

In our study, the body weight and body weight-gain of Group (3) were improved. The body weight in Group (3) was higher than that in Group (2). This may be due to hepatoprotective activities of Artemisia crude aqueous extract **(Yashphe et al, 1979 and Saleh et al, 2006)**, besides its antimicrobial and antifungal activities **(Israpil et al, 2002 and Kim et al, 2003)**. Moreover, the body weight-gain in Group (3) was higher than that in Group (2). This may be due to the bactericidal, fungicidal and hepatoprotective activities of Artemisia herba alba **(Yashphe et al, 1979 and Saleh et al, 2006)**.

The body weight and body weight-gain of chickens fed on rations contaminated with aflatoxin-B1 and treated with crude aqueous extract of Artemisia herba alba were improved at the age of 75 days. The body weight improvement (24.64%) was higher in Group (3) than that of Group (2), whereas the body weight-gain improvement (17.37%) was higher in Group (3) than that of Group (2). This may be due to the antioxidant effect of the essential oil of Artemisia herba alba that has antioxidant activity equivalent to 18% of the reference compound (alpha-tochopherol). The essential oil may protect the liver cells from damage and fatty changes (hepatoprotective) as reported by **Juteau et al (2002); Israpil et al (2002) and Kim et al (2003)**. Moreover, it enhanced the bilirubin clearance **(Huang et al, 2004)**. Such oil is effective against some Gram-positive and Gram-negative bacteria **(Yashphe et al, 1979)** besides its antifungal activity associated with two major volatile compounds (carvone and piperitone) according to **Saleh et al (2006)**.

The Artemisia plant has been used for the remedy of liver diseases **(Huang et al, 2004)** such as hepatitis, jaundice and fatty liver in the traditional oriental medicine. The liver and kidney functions were enhanced at the age of 75 days. The values of the total protein, albumin and globulin were elevated while the AST, ALT, total bilirubin, urea and creatinine were lowered in Group (3) were improved when compared with Group (2). This result coincide with the fact of **Gilani et al (2005)** who used different doses of Artemisia aqueous-methanol extract to reduce the toxin-induced rise in the plasma ALT and AST. Similar findings were reported by **Tedesco et al (2004) and Gilani et al (2005)** but contradicted with the findings of **Del Bianchi et al (2005)** who reported that the prolonged oral administration of aflatoxin-B1 was not induced a change in the hematological and serological parameters of broiler chickens, but may caused relevant lesions in the liver and kidneys.

Microscopically, the liver of chickens of Group (3) showed a marked improvement in the hepatic fatty change and regeneration inspite of the presence of mild hydropic degeneration. This protection may be due to the presence of essential oil of Artemisia herba alba that has antioxidant activity equivalent to 18% of the reference compound (alpha-tochopherol) as reported by **Juteau et al (2002)**.

It could be concluded that the Artemisia heba alba alleviated the toxicosis induced by aflatoxin-B1. This was deduced from the increased gain in the body weight and the elevated blood proteins.

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