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PROTECTIVE EFFECTS OF ETHANOLIC *MORINGA OLEIFERA* LEAVES EXTRACT AGAINST AFLATOXIN B_1 AND CCL_4 INDUCED HEPATOTOXICITY IN RATS

Eman E. Yassien; El-Morsi A. El-Morsi; Mohammed A. El-Shafie; Adel M. Zaki and Magda E. Mahmoud

Department of Chemistry, Faculty of Agriculture, Minia University, Minia, Egypt

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ABSTRACT:

The present study is designed to screen the protective effect of 80% ethanolic extract of *Moringa oleifera* (*M. olieifera*) leaves at two dose (MO1 (250) and MO2 (500) mg / kg B.W) on Aflatoxin B1 (AFB1) (0.4 µg / kg B.W i.p.) once and CCl_4(1ml / kg B.W i.p.) twice a week for 9 weeks induced liver injury in male albino rats model, various biochemical parameters like Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and total protein (TP) were determined. Results showed that treatment rats with ethanolic *M. oleifera* leaves extract improved organs weight, hematological parameters, reduced the levels of serum biomarkers of liver, kidney function that were enhanced by AFB1 and CCl_4. *M. oleifera* leaves extract inhibited the elevation of reduced glutathione (GSH) by 37% for low dose, 43% for high dose and restored catalase (CAT) by 50, 87 % respectively and malondialdehyde (MDA) to its normal levels by 23, 29 % respectively. Histopathological results revealed that, the damaging effect of AFB1+ CCL_4 on organs tissue was clearly reduced by using *M. oleifera* leaves extract treatment. In conclusions, *M. oleifera* leaves extract afforded significant protection against AFB1 and CCl_4 induced liver and other organs injury and therefore may have applications in the field of drug development.

Keywords: *M. oleifera*, albino rats, AFB1, CCl_4, liver fibrosis, oxidative stress.

INTRODUCTION

Since ancient times, medicinal plants have been used by all civilizations as a source of medicines. There has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, due to their natural origin, cost effectiveness and lesser side effects (Naik *et al.,* 2003). Among those herbs: *M. oleifera* is one of the 14 species tropical perennial trees belonging to the family *Moringaceae* (Iqbal and Bhanger, 2006), and is considered one of the world's most useful trees. The plant, referred to number of names such as horse radish tree, drumstick tree (Shindano and Chitundu, 2009), and is the most widely distributed throughout the world in many tropic and sub-tropic regions, especially in Asian countries (Shindano and Chitundu, 2009 and Khalafalla *et al*., 2010). Also, *M. oleifera* was utilized by the ancient Romans, Greeks and Egyptians (Fuglie, 1999).

Every part of the tree can be used for food and it has some other beneficial properties. The leaves, especially young shoots, can be eaten fresh as greens in salads, in vegetable curries, and as pickles. Also, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value (Arabshahi *et al.,* 2007 and Fahey, 2005). *M. oleifera* leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Anwar *et al.*, 2005 and Makkar and Becker, 1996) also, a rich source of kaemferol (Siddhuraju and Becker, 2003).

Aflatoxins, produced by *Aspergillus fungi* are common contaminants of livestock feeds. The most common aflatoxins in feed are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2). Consumption of such contaminated feed affects liver and kidney and leads to damage (Umaya and Parvatham, 2009). *Aspergillus flavus* and *A. parasiticus*, are widespread fungi and members of the *Aspergillus* group, are aflatoxin producing strains (Strelic *et al.,* 2009). AFB1 is a major contaminant of foods in the humid tropical regions of Africa and Asia and has been linked, with hepatitis B and C infections, to the high incidence of liver cancer in these regions (Montalto *et al.,* 2002 and Sarin *et al.,* 2001). The mycotoxin requires bioactivation to exert its carcinogenic effects.

CCl_4 has been widely used to elicit experimental liver damage. CCl_4 induced liver damage has been thought to depend on the formation of reactive intermediates such as trichloromethyl free radical produced by cytochrome P450 mixed function oxidase system, and further converted to a peroxy radical. These free radicals react with polyunsaturated fatty acids to propagate a chain reaction leading to lipid peroxidation or bind covalently to lipids and proteins, resulting in destruction of membranes (Weber *et al.*, 2003 and Fang *et al.*, 2008).

The present study aims to investigate the role of ethanolic *M. oleifera* leaves extract against AFB1 and CCl_4-induced hepatic injury and oxidative stress in rats.

MATERIALS AND METHODS

Preparation of *M. oleifera* extract (MOE):

Leaves of *M. oleifera* were obtained from the Experimental Farm, Faculty of Agriculture, Minia University. *M. oleifera* leaves extract was prepared as described by El-Shemy *et al.,* (2007) and Khalafalla *et al.,* (2009). The leaves were cleaned, dried, and ground to fine powder using an electric grinding machine (Model MX 491N National). Leaves powder (1:10w/v) was extracted with 80% (v/v) ethanol for 12 h with constant stirring at room temperature (25°C). Suspensions were filtered through Whatman No. 1 filter paper to retain the clear solution and the residue was extracted again. The pooled moringa extract was vacuum evaporated at temperature below 50°C in a rotary evaporator and stored at 4°C for further process.

Preparation of Aflatoxin B_1 (AFB_1).

The pure crystalline AFB_1 toxin (purchased from Sigma Co, U.S.A, Lot. No. LB97509) 3µg/ml was dissolved in benzene: acetonitrile (98:2) then completed to the required volume by dimethylsulfoxide (DMSO) to perform the final concentration 0.4 µg / kg B.w. (Parsai, *et al.,* 2014).

Preparation of carbon tetrachloride (CCl_4) solution.

CCl_4 was added to paraffin oil (1:1 v/v) with shaking to obtain a solution of CCl_4 which were used for treating rats (1 ml/kg b.wt) as recommended by (Madkour *et. al.*, 2012).

Experimental animals:

36 male wistar rats (*Rattus norvegicus*) weighting 180-240 g were obtained from the animal house of Faculty of Agriculture, Minia University, Minia, Egypt. Animals were used after 10 days of acclimatization in plastic cages at room temperature (25±2◦C) and photoperiod 12 cycles. Animals were fed a commercial balanced diet and tap water *ad libitum* till the end of the experiment (9 weeks). The experiments were conducted according to ethical guidelines of Minia University, Egypt.

Experimental design:

The rats were randomly divided into six experimental groups of six animals each. Group I served as the control group. Group II and III rats were administrated orally daily with *M. oleifera* leaves ethanolic extract (250 and 500 mg/kg B.w respectively Babu *et al.,* 2011) for 9 weeks. Group IV rats were injected with AFB_1 (0.4 µg / kg b. wt once i.p.), then after 30 min injected with CCl_4 (1 ml/kg b.wt, i.p), twice weekly for 6 weeks to induce liver fibrosis. Group V and VI rats were administrated orally daily with *M. oleifera* leaves ethanolic extract (250 and 500 mg/kg b. wt respectively) for 3 weeks before, injected with AFB_1 (0.4 µg / kg b. wt once i.p.) and 6 weeks concurrently with CCl_4. Body weight was recorded weekly during the experimental period and daily food intake was determined. Feed efficiency was calculated daily during the adaptation and the experimental periods.

Sampling

Blood samples

At the end of 9 weeks, rats were fasted overnight and anesthetized to collect the blood samples from the retro-orbital plexus (Schermer, 1967). Suitable volumes of fresh blood were immediately taken in heparinized tube for

hematological examinations. The other parts of blood samples were allowed to coagulate at room temperature, and then centrifuged at 3000 r.p.m for 15 min at 4^0C, the clear non-haemolysed sera were separated and stored at -20^{o}C until required for assay of biochemical parameters.

Tissues samples

Rats were sacrificed and organs such as the brain, heart, lung, liver, spleen, kidney and testis were measured relative weight and excised, wiped with filter paper and weight. Small parts of these organs were fixed in 10% formalin solution and stored in 70 % (v/v) ethanol for histopathological examinations. Other parts of liver tissue were homogenized and used for measuring of CAT, GSH and MDA concentration.

Hematological evaluation

Blood taken with EDTA was used for determination of total red blood cells (RBCs), white blood cells (WBCs) and hematocrit value (HCT %) or paked cell volume (PCV), was determined by centrifuging blood in heparinized microhematocrit tube (capillary tubes of 1mm internal diameter and 7.5 cm length) for 5 minutes at 15,000 r.p.m as described by Dacie and Lewis (1991), while haemoglobin concentration (Hb) was measured according to Drabkin and Austin (1932). Mean cell volume (MCV), mean capuscular hemoglobin (MCH), mean capuscular hemoglobin concentration (MCHC) were calculated from the following formula:

MCV (Fl) = Hct (ml/dl)/ RBCs (million/ml).

MCH (pg) = Hb (g/dl) / RBCs (million/ml)

MCHC (g/dl) = Hb (g/dl)/ Hct (ml/dl).

Fl= (femtoliter) = 10^{-15} liter

Pg=(Picogram) = 10^{-12} gram.

Biochemical assays

Serum enzyme activity of aspartate aminotransferase (AST; E.C.2.6.1.1.) and alanine aminotransferase (ALT; E.C.2.6.1.2.) activities were measured according to the method described by Reitman and Frankel, (1957). Total protein, albumin, urea and creatinine level were determined according to the methods described previously (Gornal *et al*., 1949; Doumas *et al.,* 1971; Fawcett and Scott, 1960; Murray, 1984). Globulin was determined by difference between total protein and albumin. Alkaline phosphatase (ALP), total and direct bilirubin were determined calorimetrically (Belfield and Goldberg, 1971; Walters and Gerarde, 1970). Triglycerides, Cholesterol, HDL-Cholesterol and LDL-Cholesterol were determined according to the methods described previously (Fossati and Prencipe 1982; Allain, *et al.,* 1974; Burstein, *et al*., 1970 and Wieland and Seidel, 1983) using enzymatic colorimetric procedures Kits from Bio-Diagnostic Co., Egypt.

Measuring of the biomarkers of liver oxidative stress:

Half gram of liver tissue from each animal was homogenized in 5 ml of 100 mM phosphate buffer, pH 7.4 using Universal laboratory Aid homogenizer, homogenates were then centrifuged at 3000 rpm for 15 min at 4^{o}C. The resulting supernatants were collected and preserved at -20 ^{o}C until measuring of catalase activity (CAT), reduced glutathione (GSH) and Malondialdehyde (MDA) concentration

according to methods described by Aebi, (1984), Beutler *et al.* (1963) and Ohkawa *et al.,* (1979) respectively.

Histopathological examination

Liver, kidney, heart, lung, spleen, testis and brain specimens were fixed in 10% formalin and processed for paraffin sections of 5µm thickness. Sections were stained with hematoxylin and eosin for routine histopathological examination and Masson's trichrome for demonstration of collagen fibers in liver (Banchroft *et al*., 1996).

Statistical analysis:

The results obtained in the present study were evaluated by One Way ANOVA test followed by Tukey Dunken SPSS. The results were expressed as mean ± standard error and values of P<0.05 were considered statistically significant (Snedecor and Cochran, 1986).

RESULTS AND DISCUSSION

Changes in body weight of rats:

The body weight gain was significantly ($P < 0.05$) decreased in the AFB1 + CCl_4 intoxicated group about 45% compared to the control group (Table1). Meanwhile, pretreated groups with ethanolic *M. oleifera* extracts lowered significantly ($P < 0.05$) the reduction of body weight gain about 7% in the low dose and 38% in the high dose compared to intoxicated group (AFB1+CCl_4). However, ethanolic *M. oleifera* extracts alone at two doses significantly increased the body weight gain compared to control group (Table 1).

Feed efficiency Ratio was increased in rats administrated with ethanolic *M. oleifera* extracts alone but not significant whereas it was decreased in rats injected with AFB1 + CCl_4 (i.p.). On the other hand, pretreated group with high dose ethanolic *M. oleifera* extracts improved the feed efficiency Ratio about 17% compared with intoxicated group (AFB1+CCl_4) while low dose had no effect on the feed efficiency Ratio compared to intoxicated group (AFB1+CCl_4).

Table (1). Effect of *M. oleifera* extracts and AFB_1 + CCl_4 on body weight, daily weight gain, daily feed intake and Feed efficiency ratio of rats in different groups for 9 weeks.

Groups	F.W (g)	B.W.G (g)	D.B.W.G (g) Mean	D. F.I. (g) Mean	Feed Efficiency Ratio (%) Mean
Cont.	289 ±4	127 ±3.02	2.00 ±0.10	20	10
MO1	315 ±4	151 [a] ±2.4	2.4 ±0.33	20	12
MO2	328 [a] ±3	151 [a] ±2.4	2.3 ±0.24	20	11.5
AFB_1+CCl_4	296 ±4	70 [a] ±3.5	1.1 [a] ±0.34	14 [a].3	7.7
MO1+AFB_1+CCl_4	269 ±3	75 [ab] ±3.3	1.2 [a] ±0.28	15 [a].0	8.0
MO2+ AFB_1+CCl_4	283 ±3	97 [ab] ±2.5	1.5 [a] ±0.19	16.8	8.9

Data represent the mean ±S.E. of observations from six rats. [a]Significantly different from control group at $P < 0.05$. bSignificantly different from AFCB1+CCl4 group at $P < 0.05$.

These results are accordance with that obtained by El bakry, *et al*., (2016), who reported that CCl_4 induced a significant reduction in the body weights of the intoxicated rats. Such reduction may be due to the

hepatotoxic effects of CCl_4 administration. While, Ara *et al.*, (2008) revealed that *M. oleifera* leaves extracts caused hypolipidemic activity and lowering in blood glucose, body weight and heart weight in rats treated with Atenolol.

M. oleifera alone appeared a significant increase in rat's body weight and improved the weight was loss by alcohol administration (Saalu *et al*., 2012). This is due to this part of the plant (leaves) is considered as a high delivery source of protein, β-carotene, vitamins A, B, C, E, riboflavin, nicotinic acid, folic acid and pyridoxine, amino acids, minerals and various phenolic compounds (Fakurazi *et al.*, 2012).

Changes in organs weight index:

As shown in Table 2 the changes in organ weights / body weight x 100. The weight of the liver, kidney and spleen in the intoxicated group significantly ($p<0.05$) increased about 84, 28 and 51% respectively, compared to control group. While pretreated groups with *M. oleifera* extracts at two doses, the weight of the liver was significantly ($p<0.05$) decrease, whereas high dose more effective to returned the spleen weight to control. The lung, heart, testis and brain weights were not significantly different in comparison with control.

Table (2). Effect of *M. oleifera* and AFB_1 + CCl_4 on organs relative weight of rats in different groups for 9 weeks.

Groups	liver%	kidney%	Spleen%	Brain%	Testis%	Lung%	Heart%
Cont.	2.94±0.11	0.68 ± 0.02	0.43± 0.03	0.57±0.04	1.11±0.05	0.59 ± 0.03	0.40±0.06
MO1	2.95 ±0.08	0.62 ± 0.03	0.46± 0.01	0.55±0.04	0.96±0.08	0.64 ± 0.11	0.32±0.02
MO2	2.77 ±0.15	0.62 ± 0.03	0.40± 0.04	0.54±0.01	1.03±0.03	0.47 ± 0.03	0.31[a]±0.04
AFB_1+CCl_4	5.42[a]±0.01	0.86[a] ±0.05	0.65 [a] ±0.06	0.66±0.02	.96 ± 0.10	0.75 ± 0.02	0.32±0.02
MO1+AFB_1+CCl_4	4.06[ab]±0.15	0.65[b]± 0.01	0.63 [a] ±0.04	0.61±0.02	1.11± 0.07	0.71 ± 0.06	0.34±0.01
MO2+AFB_1+CCl_4	4.69[ab]±0.38	0.71 [b] ±0.09	0.43 [b] ±0.02	0.60±0.04	1.10±0.07	0.63 ± 0.04	0.37±0.02

Data represent the mean ±S.E. of observations from six rats. aSignificantly different from control group at $P < 0.05$. bSignificantly different from AFCB1+CCl4 group at $P < 0.05$.

Ethanolic *M. oleifera* leaves extract could improve the hepatomegaly, one of the most common complications of liver fibrosis induced by chronic CCl_4 administration in rats. Ethanolic *M. oleifera* extract can retard the progression of liver fibrosis (El-bakry, *et al.*, 2016).

*M. olei*fera addition to diet was able to attenuate the adverse effect on rat renal tissues (Adeyemi and Elebiyo, 2014). Also, it has been shown to possess diuretic effect (Mbikay, 2012 and Kumar *et al.*, 2010a).

Hematological Determinations:

The hematological studies of the animals administrated with the ethanolic *M. oleifera* leaves extracts at two doses for 9 weeks showed no significant differences in the Hb, PCV, WBCs and RBCs levels when compared to the control. Intoxicated AFB1 + CCl_4 rats, Hb, PCV and RBCs were significantly ($p < 0.05$) decreased compared with control group (Table 3) about 29, 12 and 26% respectively, while WBCs was increased about 48.6 % relative to normal control.

On the other hand, pretreated groups with *M. oleifera* extract caused significant ($p < 0.05$) increase in blood Hb, PCV and RBCs level. Meanwhile WBCs was decreased significantly comparing with the mean value of intoxicated group. *M. oleifera* extract improved these parameters as protective agent (Eshak, *et al.*, 2015).

Data represent the mean ±S.E. of observations from six rats. [a]Significantly different from control group at $P < 0.05$. [b]Significantly different from $AFCB_1$+CCl_4 group at $P < 0.05$.

Also, aqueous *M. oleifera* leaves extract has showed marked effect on the haemopoietic system manifested by a positive increase in the levels of haemoglobin, packed cell volume, red blood cell and delayed the proliferation of the parasites in Trypanosoma brucei-infected rats (Edoga *et al.*, 2013).

Toxicity of CCl_4 lead to transient decrease in the Hb concentration and reticulocyte count as well as PCV and RBCs counts by extension (Saba *et al.*, 2010).

Liver biomarkers of rats

ALT, AST and ALP levels were used as biochemical markers for evaluation of hepatic injury. A significant ($P < 0.05$) elevation in serum AST, ALT and ALP levels about 1.7, 2.1 and 1.6 fold respectively was observed in AFB1 + CCl_4 -treated rats compared to control group. However, the serum levels of these biomarkers revealed significant ($P < 0.05$) decrease in pretreated rats with ethanolic *M. oleifera* extracts (250mg/kg and 500mg/kg b. wt) compared to AFB1+ CCl_4 -treated group. Serum ALT levels were reduced about 27 and 29% respectively while AST levels were diminished about 35 and 37% respectively and ALP levels 71 and 64 % respectively compared to AFB1+ CCl_4 group (Table 4). Nevertheless, treatment with ethanolic *M. oleifera* alone did not show any significant changes in all markers when compared to control group.

Table (3). Effect of *M. oleifera* extract on protective agent on hematological parameters in AFB_1 + CCl_4 intoxicated rats

Groups	HB (mg/dl)	PCV (mg/dl)	RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	MCV x 10^{-15}	MCH	MCHC
Cont.	13.33± 0.29	45.9 ± 0.69	6.10 ± 0.27	3.7 ± 0.22	75.2	2.2	0.29
MO1	12.54± 0.74	45.7 ± 0.23	5.34 ± 0.20	3.4 ± 0.11	85.6	2.3	0.27
MO2	11.91 ± 0.64	44.0 ± 0.54	5.54 ± 0.20	3.2 ± 0.15	79.4	2.1	0.27
AFB_1+CCl_4	9.5 ^{a} ±0.21	40.4 ^{a} ± 0.42	4.50 ^{a} ± 0.11	5.5 ^{a} ± 0.24	89.7	2.1	0.23
MO1+AFB_1+CCl_4	10.4 ^{a} ± 0.38	42.7 $^{a\,b}$ ± 0.33	4.82 ^{a} ± 0.23	4.1 ^{b} ± 0.10	88.6	2.1	0.24
MO2+AFB_1+CCl_4	11.5 $^{a\,b}$ ± 0.42	43.0 $^{a\,b}$ ± 0.91	5.1 $^{a\,b}$ ± 0.20	4.4 $^{a\,b}$ ±0.62	84.3	2.2	0.26

Table (4). Effect of *M. oleifera* extract and AFB_1 + CCl_4 on ALT, AST, ALP, Total bilirubin and Direct bilirubin levels in serum of rats in different groups for 9 weeks..

Groups	ALT U/ml	AST U/ml	ALP IU/L	Total bilirubin mg/dl	Direct bilirubin mg/dl
Cont.	26.5 ± 2.2	25.7 ± 1.7	123.36 ± 3.1	0.44 ±0.02	0.27 ± 0.12
MO1	27.00 ± 1.2	26.7 ± 1.4	122.5 ±2.3	0.40 ±0.02	0.30 ^{a} ± 0.03
MO2	27.7 ± 1.6	30.2 ± 0.75	130.96 ± 2.9	0.43 ±0.02	0.28 ± 0.04
$AFCB_1$+CCl_4	46.7 ^{a} ± 0.94	54.00 ^{a} ± 0.57	178.9 ^{a} ± 1.1	0.90 ^{a} ±0.03	0.58 ^{a} ± 0.02
MO1+ $AFCB_1$+CCl_4	34.00 ^{ab} ± 1.8	35.00 ^{ab} ± 1.9	135.33 ^{b} ± 1.3	0.73 ^{ab} ±0.02	0.50 ^{a} ± 0.03
MO2+ $AFCB_1$+CCl_4	33.00 ^{b} ± 1.1	34.00 ^{ab} ± 1.1	129.76 ^{b} ± 2.1	0.56 ^{ab} ±0.01	0.45 ^{ab} ± 0.02

doses ethanolic *M. oleifera* extract before administration of AFB_1+ CCl_4 about 19 and 38% respectively for total bilirubin and 14, 22 % respectively for direct bilirubin compared with intoxicated rats.

Methanolic *M. oleifera* extracts of both leaves and roots at a dose 500mg/Kg b. wt showed comparable results with Silymarin in rats treated CCl_4 and methanolic *M. oleifera* leaves extract showed better protection than methanolic *M. oleifera* extract (Kumar, *et al.,* 2010b). The antioxidant and hepatoprotective potential of *M. oleifera* leaves extract may be attributed to the presence of total phenolics and flavonoids in the extract and/or isolated active constituents β-sitosterol, quercetin and kaempferol which have hydroxyl group(s) (Singh, *et al.,* 2014).

Total Protein, Albumin:

Treatment with ethanolic *M. oleifera* extracts alone revealed no significant ($p>0.05$) changes in total protein, albumin levels compared to control group (Table 5). While, intoxicated rats with AFB1+ CCl_4 induced significant ($p< 0.05$) decreased in total protein, albumin and globulin levels about 30, 33 and 28 % respectively, relative to normal control.

Data represent the mean ±S.E. of observations from six rats. [a]Significantly different from control group at $P < 0.05$. [b]Significantly different from $AFCB_1$+CCl_4 group at $P < 0.05$.

These results agreement with Ezeonwu and Ugonna (2012) and El-bakry *et al.,* (2016) who reported that the bi-ethanolic *M. oleifera* extract (500mg/kg and 300mg/kg b. wt) showed a remarkable potentiality in restoring serum transaminases levels to normal in rats treated with CCl_4 induced liver damage. These diminish attributed to the stabilizing ability of the cell membrane preventing enzymes leakages (Saalu, *et al.,* 2012). Also, the data of serum total and direct bilirubin levels were summarized in the same table (4) revealed no significant ($p>0.05$) changes in groups treated with ethanolic *M. oleifera* extract alone compared to control group. While a significant ($P < 0.05$) increase in total and direct bilirubin levels in rats injected with AFB_1 + CCl_4 about 100 and 115 % respectively when compared with normal control group. While, significantly ($p< 0.05$) decreased were recorded for groups treated with two

Table (5). Effect of *M. oleifera* extract and AFB_1 + CCl_4 on Total protein, Albumin (A), Globulin (G) and A/G levels in different groups for 9 weeks.

Groups	Total Protein gm/dl	Albumin gm/dl	Globulin gm/dl	Albumin/ Globulin
Cont.	8.03 ± 0.10	4.02 ±0.10	4.01	1.00
MO1	8.04 ± 0.16	3.7 ±0.22	4.34	0.85
MO2	8.3 ± 0.58	3.7 ±0.13	4.6	0.80
AFB_1+CCl_4	5.6 [a] ± 0.18	2.7 [a] ±0.10	2.9	0.93
MO1+ AFB_1+CCl_4	6.7 [ab] ± 0.11	3.5 [ab] ±0.11	3.2	1.09
MO2+ AFB_1+CCl_4	7.06 [ab] ± 0.10	3.8 [b] ±0.37	3.2	1.18

Data represent the mean ±S.E. of observations from six rats.
[a]Significantly different from control group at P < 0.05. [b]Significantly different from $AFCB_1+CCl_4$ group at P < 0.05.

While, pre-treated groups with both doses of ethanolic *M. oleifera* extract caused significantly (p< 0.05) elevated in total protein (19, 25%), albumin (29, 41%) and globulin (10%) compared to AFB1 + CCl_4 group.

These results agreement with many authors who found a significant reduction in total protein and albumin levels in serum rats exposure to CCl_4 which causes considerable liver damage through induction of peroxidation of lipids (Ezeonwu and Ugonna, 2012; Singh, *et al.*, 2014 and El-bakry *et al.*, 2016) and inhibit protein synthesis which attributed to trichloromethyl free radical covalent bindings (Lee, *et al*., 2004). The treatment with *M. oleifera* extract stabilized the serum total protein and albumin levels which attributed to enhanced protein synthesis in the hepatic cells (Mandal, *et al.*, 1993).

Changes in kidney functions:
Urea and Creatinine:

Serum urea and creatinine levels were assessed as markers of renal functions. AFB_1 + CCl_4 groups showed significant increase (p < 0.05) in serum creatinine and urea levels about 1.4 fold compared to control group (table 6). Ethanolic *M. oleifera* extract treatments at both doses significantly (p>0.05) decreased these markers. ethanolic *M. oleifera* pretreatment, low dose reduced the elevated serum urea nearly 18 % while high dose diminish about 26% compared to intoxicated group (AFB1+CCl_4).

Meanwhile, pretreated groups with ethanolic *M. oleifera* extracts reduced the elevation of serum creatinine about 35% in the low dose and 33% in the high dose compared to intoxicated group (AFB1+CCl_4).

Table (6). Effect of *M. oleifera* extract and AFB_1 + CCl_4 on Urea and Creatinine levels in serum of rats in different groups for 9 weeks**.**

Groups	Urea gm/dl	Creatinie gm/dl
Cont.	42.8 ± 2.8	0.67 ± 0.15
MO1	47.2 ± 0.87	0.71 ± 0.11
MO2	46.8 ± 1.1	0.74 ± 0.02
AFB_1+CCl_4	62.2 [a] ± 1.6	0.95 [a] ± 0.02
MO1+ AFB_1+CCl_4	51.00 [ab] ± 2.4	0.62 [b] ± 0.05
MO2+ AFB_1+CCl_4	46.1 [b] ± 0.79	0.64 [b] ± 0.02

Data represent the mean ±S.E. of observations from six rats.
[a]Significantly different from control group at P < 0.05. [b]Significantly different from $AFCB_1+CCl_4$ group at P < 0.05.

Whereas both doses of ethanolic *M. oleifera* extracts alone have no effect on renal function markers

compared to control. While, groups treated with both doses of ethanolic *M. oleifera* extract, the levels of blood urea and creatinine were significantly ($p < 0.05$) decreased nearly about 18 and 12% respectively in serum urea and about 35 and 33% respectively in creatinine levels compared to the AFB_1+ CCl_4 group. The feeding of diets containing different concentrations of *M. oleifera* (5, 10, 15 %) to rats were prevented the elevation of the indices for renal dysfunction (Adeyemi and Elebiyo, 2014). These findings are in support of previous studies which showed that *M. oleifera* offered nephro- and hepatoprotection (Sharma and Paliwal, 2012 and Awodele, *et al*., 2012).

The harmful effects of AFB_1 have been attributed to the metabolism of this mycotoxin and reactive metabolites can bind to cellular macromolecules. (Iyer *et. al.,* 1994)

Estimation of lipid profile:

Treated rats with ethanolic M. oleifera extract alone appeared no significant ($p<0.05$) changes in TC, TG, HDL, LDL and VLDL levels relative to normal control group (Table 7). At the same time intoxicated rats were treated with AFB1+CCl_4, the levels of TC, TG, LDL and VLDL were significantly ($p<0.05$) increased about 35, 42, 222 and 42% respectively, while, a significant ($p<0.05$) decreased in HDL level about 26% in animals treated with AFB1+CCl_4 compared to control group.

Table (7). Effect of *M. oleifera* extract and AFB_1 + CCl_4 on T.C, T.G, HDL, LDL and VLDL levels of rats in different groups for 9 weeks.

Groups	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Cont.	75.8 ± 1..6	115±3	40.5 ± 1.3	12.3±0.9	23±1.3
MO1	72.5 ± 1.89	118±3	39.5 ± 0.99	9.4±1	23.6±1.2
MO2	73.2 ± 1.3	112±2	38.5 ± 0.79	12.3±1.5	22.4±1.3
AFB_1+CCl_4	102.3 [a] ± 1.2	163 [a] ±2	30 [a] ± 1.6	39.7 [a] ±1.7	32.6 [a] ±1.1
MO1+ AFB_1+CCl_4	91.2 [ab] ± 1.1	150 [ab] ±2	34.02 [ab] ± 0.88	27.18 [ab] ±1.2	30 [a] ±1.1
MO2+ AFB_1+CCl_4	85.01 [ab] ± 0.98	132 [ab] ±3	35.5 [ab] ± 1.5	23.11 [ab] ±1.3	26.4 [b] ±1.1

Data represent the mean ±S.E. of observations from six rats. [a]Significantly different from control group at $P < 0.05$. [b]Significantly different from $AFCB_1$+CCl_4 group at $P < 0.05$.

The pretreated groups with 250 and 500 (mg/Kg b.wt) appeared a significant ($p<0.05$) decrease in TC (11, 17 %), TG (8, 19 %), LDL (31, 42 %) and VLDL (8, 19 %). While, HDL was a significantly ($p<0.05$) increased about 13, 18 % compared to AFB1+CCl4-intoxicated group. These results agreement with El Serwy, (2011); and Al-Seeni, *et al*., (2016) who investigated that the CCl4 showed a significant increase in TC, TG, LDL and VLDL. In contrast, HDL was

decreased compared with the negative control.

CCl_4 is assumed to initiate the biochemical processes leading to oxidative stress, which is the direct cause of many pathological changes in liver, kidney, testes, lungs, nervous system and blood tissues by producing free radicals (Abraham, *et al*., 1999). These free radicals frequently damage different cell (Darlington and Stone, 2001) and *M. oleifera* leaves extract had lipid lowering effect particularly of TG and VLDL, is an encouraging one (Ara *et al.,* 2008)**.**

Oxidative stress parameters:

AFB1+CCl_4 -induced oxidative stress in rat liver was evaluated by assessing lipid peroxides, GSH and CAT levels. As shown in Table 8, AFB1+CCl_4 significantly increased MDA level about 44% and significantly ($P < 0.05$) decreased CAT, GSH levels about 49 and 39 % respectively, in comparison with control group.

Table (8). Effect of *M. oleifera* extract and AFB_1 + CCl_4 on CAT, GSH, and MDA level in different groups for 9 weeks.

Groups	CAT U/L	GSH Mg/g	MDA nmole/g
Cont.	3.1 ±0.06	10.1 ±0.16	4.8 ± 0.27
MO1	3.1 ± 0.03	9.5 ±0.17	4.6 ±0.11
MO2	3.2 ±0.03	9.9 ±0.27	4.3 ±0.35
AFB_1+CCl_4	1.6 [a] ± 0.1	6.2 [a] ±0.07	6.9 [a] ±0.33
MO1+ AFB_1+CCl_4	2.4 [b] ±0.06	8.5 [ab] ±0.2	5.3 [b] ±0.36
MO2+ AFB_1+CCl_4	3.00 [b] ±0.2	8.9 [ab] ±0.2	4.9 [b] ±0.16

Data represent the mean ±S.E. of observations from six rats. [a]Significantly different from control group at $P < 0.05$. [b]Significantly different from $AFCB_1$+CCl_4 group at $P < 0.05$.

On the other hand, pretreatment of rats with either low dose of ethanol *M. oleifera* extract or high dose afforded significant protection against AFB1 + CCl_4 -intoxication and maintained level of lipid peroxides at near basal level with significant increase in GSH level compared to AFB1 + CCl_4 treated group. Furthermore, animal's treated with ethanol *M. oleifera* extract alone did not show any significant alterations in oxidative stress markers as compared to control group.

The oxidative stress induced by CCl_4 results an increased utilization of GSH and subsequently the levels of GSH are decreased in plasma and tissues (Kamalakkannan, *et* al., 2005 and Kazeem, *et al.*, 2011). And a significant decrease in hepatic CAT activities in rats treated with AFB_1 initiated hepatotoxicity. AFB_1 is a potent carcinogen for liver as indicated by the elevation of the specific tumor markers. Moreover, AFB_1 could inhibit the synthesis of hepatic nucleic acids (Abdel-Wahhab, *et al.*, 2006).

Methanolic *M. oleifera* leave extract seemed to offer protection and maintain the structural integrity of the hepatocellular membrane (Ahmed and Khater 2001). While, administration of

ethanol *M.* oleifera leave extract to CCl_4 treated rats enhanced the SOD and CAT profiles, dose dependently, by acting as a strong free radical quencher and protecting the hepatic cells. The decline of GSH level in the CCl_4 treated group might be due to its utilization by the excessively generated quantity of free radicals in the hepatocytes leading to hepatic injury (Singh, *et al.,* 2014; Abd El-Rahman, *et al.,* 2015 and El-bakry, *et al.,* 2016).

Treatment with ethanolic *M. oleifera* flowers and leaves extracts indicated its ability to break the chain reaction of lipid peroxidation in liver homogenate of acetaminophen intoxicated rats. The therapeutic potential of *M. oleifera* extracts is dependent on an antioxidant mechanism (Fakurazi, *et al.,* 2012).

Histological examination

Histological changes were screened to support the tested biochemical markers of organs injury. The histopathological results showed that *M. oleifera* alone was found to be safe and did not induce any histopathological changes in all organs (Fig. C and D).While, pretreated groups with *M. oleifera* leave extract was partially prevented and markedly reduced as a protective or therapeutic agent respectively which might be due to its antioxidant components (Singh *et al.,* 2014 and Eshak *et al.,* 2015).

The following photomicrograph showed the effect of *M. oleifera* leave extract on:

1-Liver

It is noticed that the sections taken from liver of rats intoxicated with AFB1 and CCl4 showed marked hepatocellular damage in the form of sever fatty change of hepatocytes, fibroblasts proliferation around the hepatocytes and apoptosis of hepatocytes. Nevertheless, these finding were ameliorated by treatment with *M. oleifera* which showed a mild in fatty change of hepatocytes, fibroblasts proliferation around the hepatocytes and there is no apoptosis of hepatocytes (Fig. 1). There was marked hepatocellular damage in CCl_4-intoxicated group (Heeba and Mahmoud, 2014). CCl_4 caused massive fibrosis of liver tissue (Morsy *et al.,* 2011).

Kidney:

Sections taken from kidney intoxicated rats with AFB_1 and CCl_4 showed marked damage in the form of sever vacuolation of tubular epithelium, moderate in vacuolation of glomerular tufts, proteinaceous material in renal tubules and a mild focal tubular necrosis. Pretreatment with *M. oleifera* effectively reversed the histopathological alteration induced by AFB_1 and CCl_4 (Fig. 2). These confirm that medicinal protection afforded by *M. oleifera* against drug-induced tissue damage (Adeyemi and Elebiyo, 2014 and Sharma and Paliwal, 2012).

Heart:

Sections taken from heart intoxicated rats with AFB_1 and CCl_4 showed a marked damage in the form of moderate inflammatory cells infiltration, intermuscular oedema and a mild myolysis of sporadic myocytes. Meanwhile, pretreatment with *M. oleifera* return histological changes to normal (Fig. 3)

Lung:

Sections taken from lung intoxicated rats with AFB_1 and CCl_4 appeared a marked damage in the form of sever focal interstitial pneumonia

and moderate perivascular inflammatory cells infiltration. While, photomicrographs from pretreatment with *M. oleifera* revealed mostly focal interstitial pneumonia, perivascular inflammatory cells infiltration and with absence focal pulmonary haemorrhage (Fig. 4)

Spleen

Sections taken from spleen intoxicated rats with AFB_1 and CCl_4 showed marked damage in the form of moderate thickening of the capsule with connective tissue and inflammatory cells infiltration in the capsule. Meanwhile, pretreatment with *M. oleifera* return histological changes to normal (Fig. 5). Spleen tissues assumed improved histomorphology due to the effects of moringa extract treatment (Owolabi and Ogunnaike, 2014).

Testis

Sections taken from testis intoxicated rats with AFB_1 and CCl_4 indicated marked damage in the form of sever inflammation of capsule and a mild congestion and degeneration of spermatogoneal cells. While, pretreatment with *M. oleifera* return histological changes to normal (Fig. 6). Owolabi and Ogunnaike, (2014) investigated that *M. oleifera* leaf extract produced extensive histological disruptions of the reproductive organs-testis and epididymis, thus acting as an anti-fertility agent.

Brain

Sections taken from brain intoxicated rats with AFB_1 and CCl_4 showed marked damage in the form of sever neuronophagia, a moderate in necrosis of neurons and a mild focal gliosis and cellular oedema. While, pretreatment with *M. oleifera* reduced neuronophagia, necrosis of neurons and focal gliosis to a mild (Fig. 7). In the same line Owolabi and Ogunnaike, (2014) found that *M. oleifera* leaf extract did not produce histologically observable deleterious effects on the brain cerebrum, cerebellum and hippocampus

CONCLUSION

The present study concluded that treatment rats with *M. oleifera* leaves alone did not show any significant changes in all parameters when compared with control group. While, pretreated rats with ethanolic *M. oleifera* extracts able to significantly attenuates the oxidative stress induced by AFB1 and CCL_4 in rats curing some health problems associated with toxication status, this was established by its positive effect on some of hematological, biochemical and histopathological examination of the experimental animals.

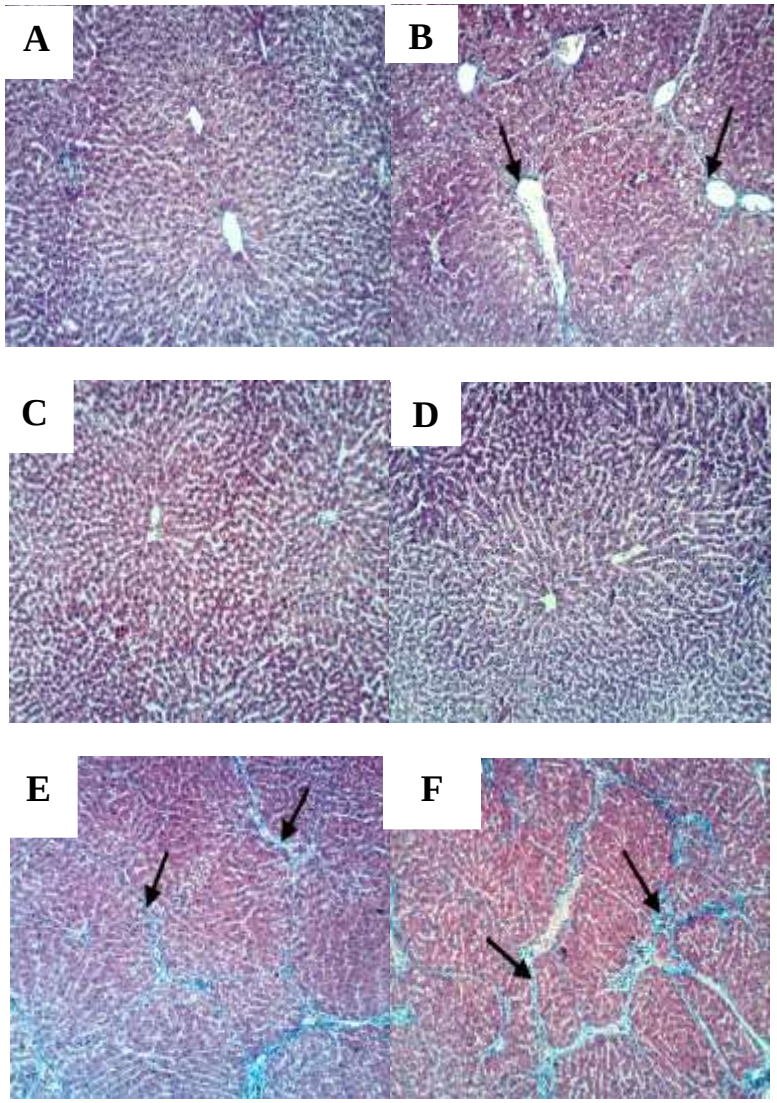

Figure (1 a): Photomicrograph of the cross section in the liver cortex of (A): control, (B): AFB_1- CCl_4, (C): *M. oleifera* (250mg/kg), (D):*M. oleifera* (500mg/kg), (E): *M. oleifera*(250mg/kg) + AFB_1-CCl_4, (F): *M. oleifera* (500mg/kg) + AFB_1-CCl_4 (Masson's Trichrome stain x 100)

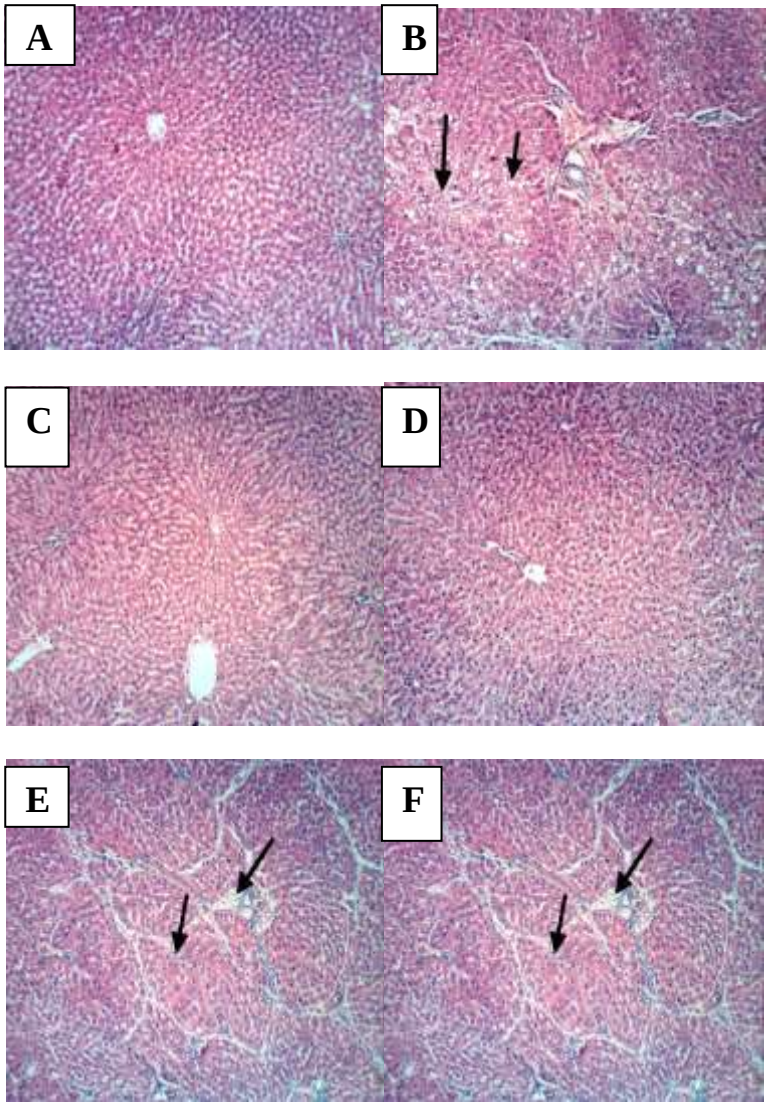

Figure (1 b): Photomicrograph of the cross section in the liver cortex of (A): control, (B): AFB_1- CCl_4, (C): *M. oleifera* (250mg/kg), (D):*M. oleifera* (500mg/kg), (E): *M. oleifera*(250mg/kg) + AFB_1-CCl_4, (F): *M. oleifera* (500mg/kg) + AFB_1-CCl_4 (H & E ×100).

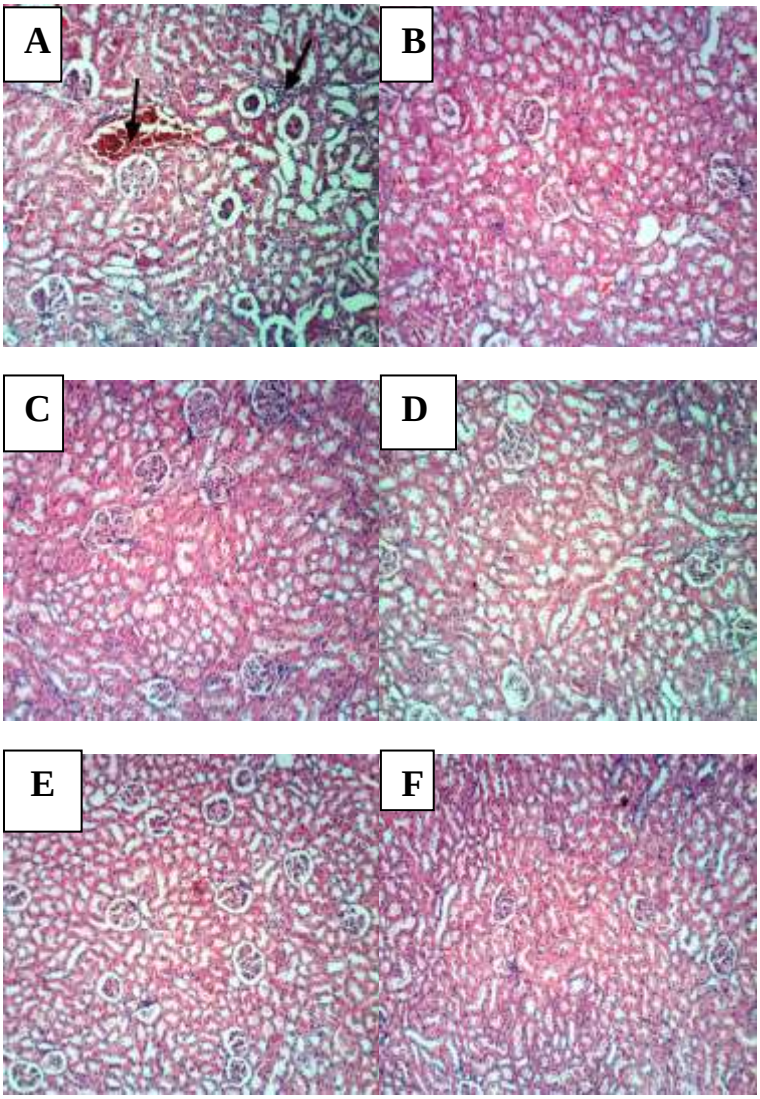

Figure (2):): Photomicrograph of the cross section in the kidney cortex of (A): control, (B): AFB_1- CCl_4, (C): *M. oleifera* (250mg/kg), (D):*M. oleifera* (500mg/kg), (E): *M. oleifera*(250mg/kg) + AFB_1-CCl_4, (F): *M. oleifera* (500mg/kg) + AFB_1-CCl_4 (H & E ×100).

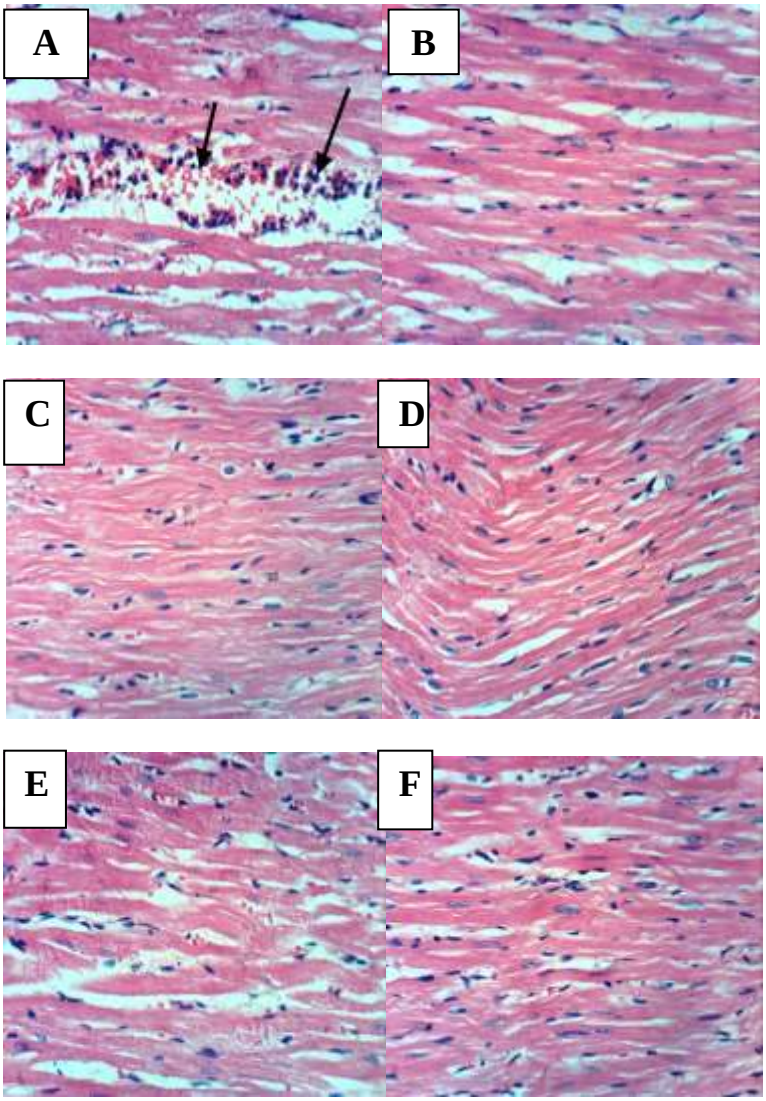

Figure (3):): Photomicrograph of the cross section in the heart cortex of (A): control, (B): AFB_1- CCl_4, (C): *M. oleifera* (250mg/kg), (D):*M. oleifera* (500mg/kg), (E): *M. oleifera*(250mg/kg) + AFB_1-CCl_4, (F): *M. oleifera* (500mg/kg) + AFB_1-CCl_4 (H & E ×400).

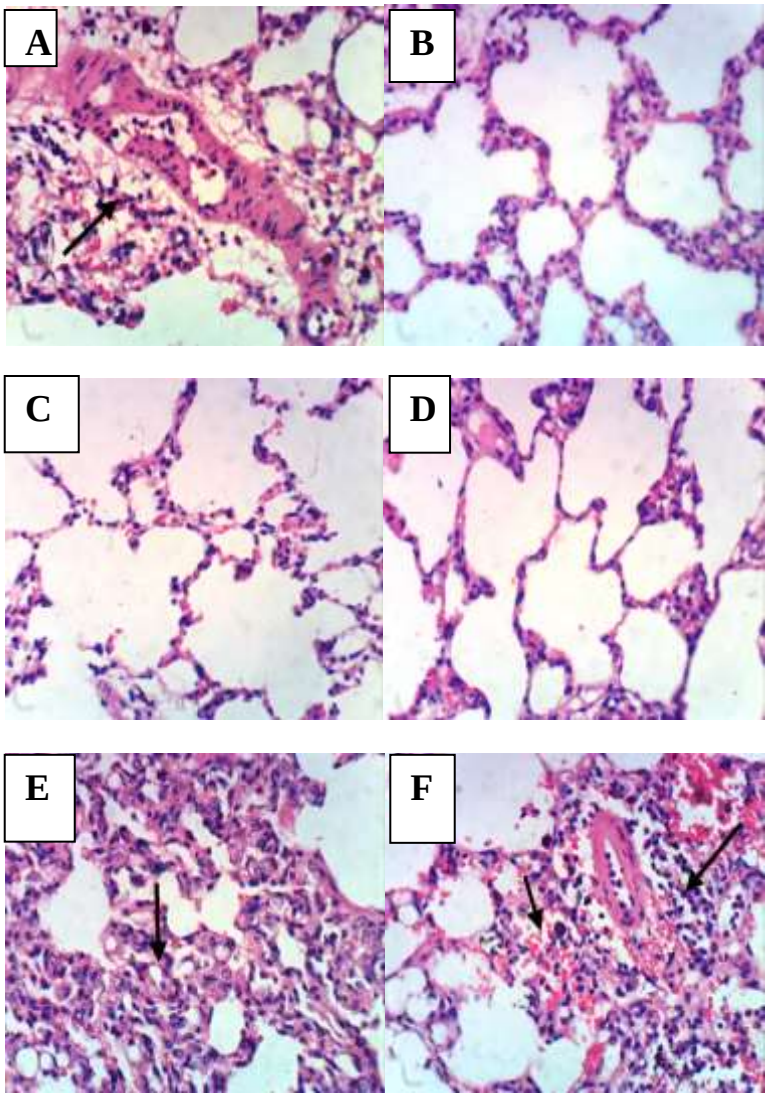

Figure (4):): Photomicrograph of the cross section in the lung cortex of (A): control, (B): AFB_1- CCl_4, (C): *M. oleifera* (250mg/kg), (D):*M. oleifera* (500mg/kg), (E): *M. oleifera*(250mg/kg) + AFB_1-CCl_4, (F): *M. oleifera* (500mg/kg) + AFB_1-CCl_4 (H & E ×100).

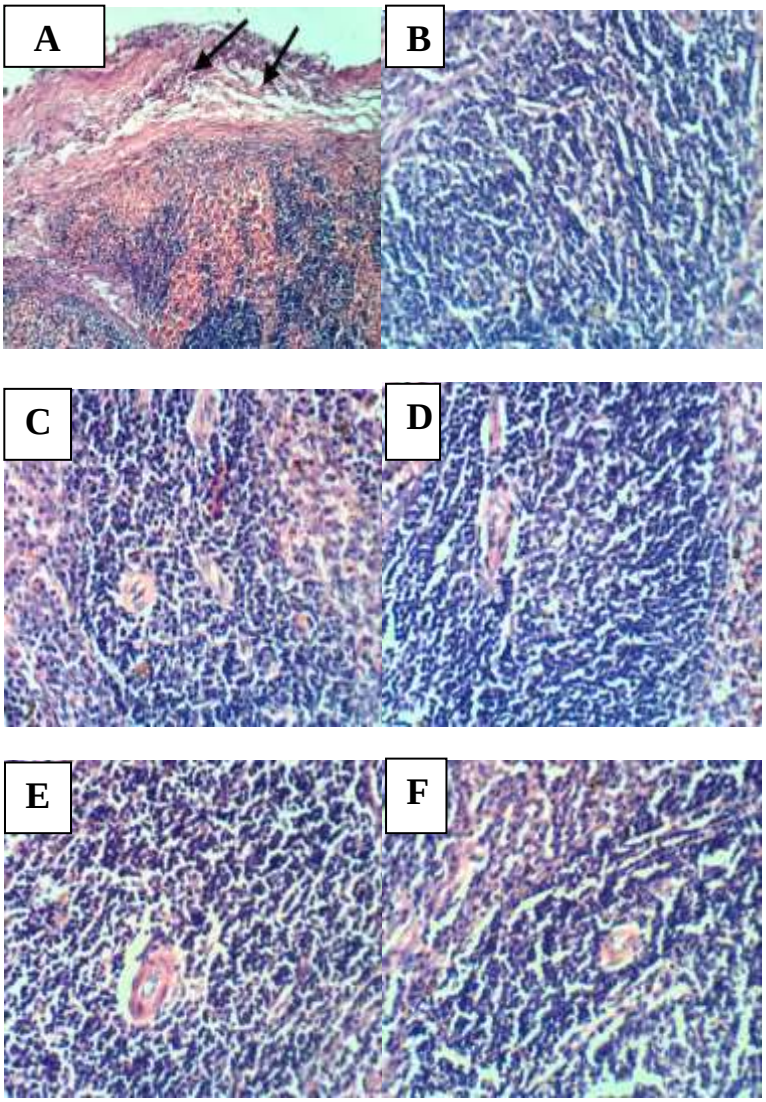

Figure (5):): Photomicrograph of the cross section in the spleen cortex of (A): control, (B): AFB_1- CCl_4, (C): *M. oleifera* (250mg/kg), (D):*M. oleifera* (500mg/kg), (E): *M. oleifera*(250mg/kg) + AFB_1-CCl_4, (F): *M. oleifera* (500mg/kg) + AFB_1-CCl_4 (H & E ×400).

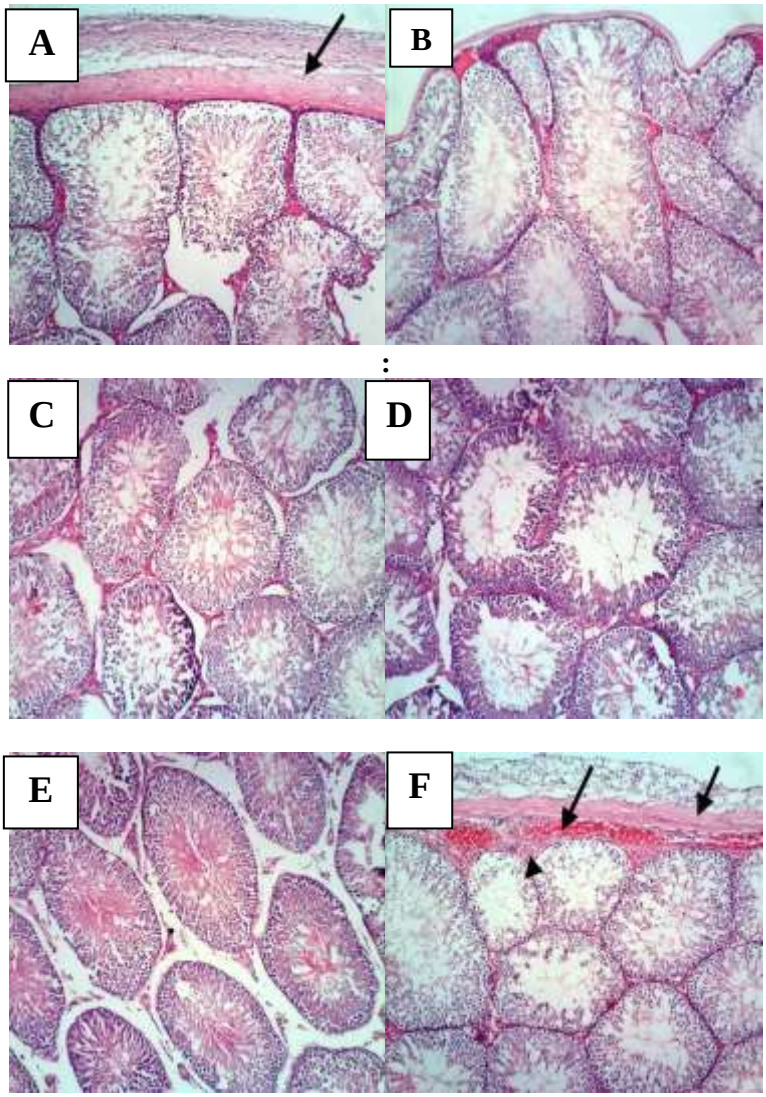

Figure (6):): Photomicrograph of the cross section in the testis cortex of (A): control, (B): AFB_1- CCl_4, (C): *M. oleifera* (250mg/kg), (D):*M. oleifera* (500mg/kg), (E): *M. oleifera*(250mg/kg) + AFB_1-CCl_4, (F): *M. oleifera* (500mg/kg) + AFB_1-CCl_4 (H & E ×100).

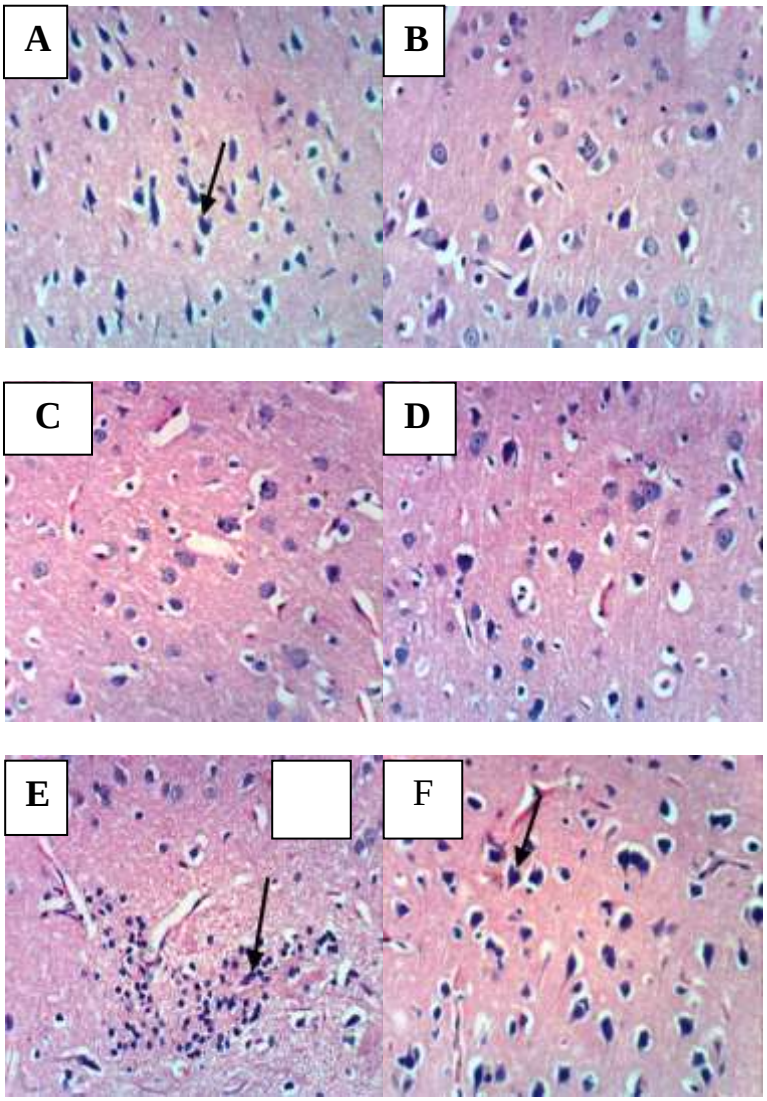

Figure (7):): Photomicrograph of the cross section in the brain cortex of (A): control, (B): AFB_1- CCl_4, (C): *M. oleifera* (250mg/kg), (D):*M. oleifera* (500mg/kg), (E): *M. oleifera*(250mg/kg) + AFB_1-CCl_4, (F): *M. oleifera* (500mg/kg) + AFB_1-CCl_4 (H & E ×400).

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الملخص العربي

التأثير الوقائي لمستخلص ايثانول أوراق المورينجا ضد الافلاتوكسين ب1 ورباعي كلوريد الكربون المسبب سمية لكبد فئران التجارب

إيمان الحسيني يس، المرسى أبو الفتوح المرسى، محمد عبدالعزيز الشافعي، عادل محمد زكى، ماجدة عويس محمود

تهدف هذه الدراسة إلى دراسه التأثير الوقائي لمستخلص ايثانول 80% أوراق نبات المورينجا بتركيزين هما (250 ، 500ملجم/كجم وزن جسم) والتي تم إعطائها يوميا عن طريق الفم لمدة 9 أسابيع على الفئران المعاملة بالافلاتوكسين ب1 بتركيز 0.4 ميكروجرام/ كجم وزن جسم مرة واحدة خلال التجربة ورباعى كلوريد الكربون بتركيز 1مل/كجم وزن جسم مرتين أسبوعيا لمدة 6 أسابيع لعمل ضرر في الكبد وبعض أعضاء الجسم الأخرى منها الكلى، الخصيتين، الطحال، القلب، المخ والرئة. بعد الانتهاء من التجربة تم سحب عينات دم من الفئران وتشريحها بعد ذلك لأخذ عينات نسيجية من الأعضاء السابق ذكرها لمعرفة تأثير المستخلص، وقد أظهرت النتائج أن معاملة الفئران بهذا المستخلص أدى إلى تحسن في وزن الأعضاء وخاصة الكبد والكلى والطحال والتقديرات التي أجريت على الدم كذلك حدث بها انخفاض في تقديرات وظائف الكبد والكلى التي زادت بسبب الافلاتوكسين ب1 ورباعى كلوريد الكربون، أيضا قام مستخلص أوراق نبات المورينجا بتثبيط الزيادة التي حدثت في الجلوتاثيون المختزل وتحسين الكاتاليز وعودة المالون داى الدهيد إلى المستوى الطبيعي. أما العينات النسيجية للأعضاء أظهرت أن الافلاتوكسين ب1 ورباعي كلوريد الكربون أحدثوا تحطم في جميع الأعضاء وان المعاملة الوقائية بالمستخلص أدت إلى تقليل التحطم الذي حدث في هذه الأنسجة.