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Protective effect of antioxidant medicinal herbs, Rosemary and Parsley, on subacute aflatoxicosis in *Oreochromis niloticus*

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Abstract: The object of this study was to conduct the ability of two medicinal herbs, namely rosemary and parsley, for amelioration of aflatoxicosis in *Oreochromis niloticus*. Two herbs' extracts at three concentrations of either (0, 2 and 4 g kg^{-1} B.W. divided into 2 doses at the start and the 6^{th} day of the experiment) and three concentrations of aflatoxin B_1, (AFB_1 0, 9 and 18 mg kg^{-1} B.W. as a single intraperitoneal administration) were tested either individually or in combination. The herbs and AFB_1 were dissolved in Dimethylsulphoxide (DMSO 25%) and injected to fish groups. Sixteen groups of fish were investigated in this study, where A group (control) was injected with saline 0.89%, group B injected with DMSO (control solvent), groups F_1 and F_2 were injected with AFB_1 alone (9 and 18 mg kg^{-1} B.W. respectively), R_1 and R_2 groups were injected with rosemary alone (2 and 4 g kg^{-1} B.W., respectively), groups F_1R_1, F_1R_2, F_2R_1 and F_2R_2 were injected with AFB_1 + rosemary, while groups P_1 and P_2 were injected with parsley alone (2 and 4 g kg^{-1} B.W., respectively); however, F_1P_1, F_1P_2, F_2P_1 and F_2P_2 groups were injected with AFB_1 + parsley. At the 12^{th} day of the experiment, blood and liver samples were taken from each group. The results indicated that the AFB_1 injected groups revealed a significant increase in mortality rate (MR%) compared with AFB_1-not injected, group F_2 was the highest while F_1R_1 and F_1P_1 were the lowest in MR% among all AFB_1 injected fish groups. Also, AFB_1 led to reduction of haemoglobin (Hb), total protein (TP) and globulin (Gl) concentrations and increase in activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). These alterations were significantly ameliorated when fish were injected with herbs' extracts. AFB_1 residues showed that the herbs level of 2g kg^{-1} B.W. have higher potency of reducing the AFB_1 residues than the level of 4 g kg^{-1} B.W. in case of AFB_1 level 9 mg kg^{-1} B.W. While, in case of AFB_1 level 18 mg kg^{-1} B.W., the groups F_2 and F_2P_1 showed absence of AFB_1 residues. Microscopically, AFB_1 presented histopathological changes in hepatopancrease which increased in severity with increasing AFB_1 level. These lesions may become less severer in all fish groups injected with AFB_1 combined with herbs' extracts especially with the lowest levels of herbs' extracts and AFB_1. So, this study concluded that either of rosemary or parsley was found to be safe and successful in protection from aflatoxicosis, particulary at the low level.

Key words: Tilapia – Aflatoxicosis – Parsley – Rosemary- Residues.

INTRODUCTION

Aflatoxins are secondary metabolites produced by the ubiquitous fungi *Aspergillus flavus* and *A. parasiticus*. Aflatoxin B_1 (AFB_1) has the highest potency as a toxin and is classified as group 1 carcinogen by International Agency for Research on Cancer (IARC, 1993). Aflatoxin has to be activated in order to exert its carcinogenic effect. Also, the free radical and reactive oxygen species (ROS) may, in part, be responsible for the carcinogenic activity of AFB_1 (Shen *et al*., 1996). So, inhibition of cytochromes (CYP_{450}) and/or stimulation of the antioxidant defence system, α- tocopherol, ascorbate and reduced glutathione (GSH), may reduce the risk of AFB-mediated carcinogenesis.

Previous studies referred to many medicinal herbs that serve as sources of antioxidant which have antiaflatoxigenic effects such as *Thonningia sanguinea* (Gyamfi and Ariya, 1998 and Gyamfi *et al*., 1999), *Cymbopogon citratus* Staf and *Murdannia ioriformis* (Vinitketkumnuen *et al*., 1999), *Oldenlandia diffusa* and *Seutellaria babata* (Wong *et al*., 1993), as well as *Ocimum sanctum* (Rastogi *et al*., 2007).

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While, parsley (*Petroselinum crispum*) and rosemary (*Rosmarinus officinalis*) are medicinal herbs which are widely used around the world that have shown a good antioxidant activity (Lampe *et al*., 2000; Hinneburg *et al*., 2006; Sacan Ozsoy *et al*., 2006 and Caillet *et al*., 2007). As well as, Lampe *et al*. (2000) reported that parsley has been shown to inhibit CYP_{1A2} in human and it was suggested that the inhibition was possibly related to its phytochemical content. Natural polyphenols found in rosemary have not only potent antioxidant activities but also anticarcinogenic properties. Rosemary components inhibit both the initiation and tumour promotion stages of carcinogenesis in mouse and rat models (Tokuda *et al*., 1986; Singletary and Nelshoppen, 1991; Yasukawa *et al*., 1991 and Huang *et al*., 1994). Also, Offord *et al*. (1997) reported that rosemary extract strongly inhibits metabolic activation of two important human procarcinogens, AFB_1 and benzo (a) pyrene. Additionally, the medicinal properties of parsley are stimulant, diuretic, carminative, emmenagogue, antipyretic and anti-inflammatory, while the medicinal properties of rosemary are mild irritant, carminative, stimulant and diaphoretic (Peter, 2001). The last author reported that the lethal dose (LD_{50}) of rosemary and parsley essential oils determined in rats are > 5 and 1-5g/kg B.W., respectively. The nutritive values of rosemary and parsley were reported by Farrel (1990), it was higher in parsley than in rosemary as shown from the following:

Nutritive value of rosemary and parsley (approximate composition/ 100g of edible portion)

Composition	Rosemary	Parsley
Energy (Kcal)	331	276
Protein (g)	4.9	22.4
Fat (g)	15.2	4.4
Total carbohydrates (g)	64.1	51.7
Fibre (g)	17.7	10.3
Ash (g)	6.5	12.5
Ca (mg)	1280	1468
Fe (mg)	29	98
Mg (mg)	220	249
P (mg)	70	351
K (mg)	955	3805
Na (mg)	50	452
Zn (mg)	3	5
Vitamin C (mg)	61	122
Riboflavin (mg)	-	1
Niacin (mg)	1	8
Vitamin A (IU)	3128	23340

The aim of this study is to investigate the effects of rosemary and parsley at two levels (2 and 4g kg^{-1} B.W.) on aflatoxicosis B_1 by fish, *Oreochromis niloticus* at 0.25 and 0.5 of the LD_{50} of AFB_1.

MATERIALS AND METHODS

Preparation of Aflatoxin B_1

Aflatoxin B_1 was produced on liquid medium (Potato dextrose) by *Aspergillus parasiticus* (NRRL. 2999) according to Ready *et al*. (1971). Aflatoxin B_1 was dissolved in chloroform and quantitatively estimated by thin layer chromatography, TLC (AOAC, 2000). So, chloroform was evaporated to dryness on a rotary vacuum evaporator at 40ºC and redissolved in Dimethylsulfoxide (DMSO) 25% (1:3 water) to the requirement of each aflatoxin concentration. AFB_1 was freshly dissolved in DMSO before injection.

Herbal Materials and Preparation of Their Extracts

Fresh rosemary and parsley leaves were obtained from a local farm and carefully washed with tap water then left to dry in the dark at room temperature. Twenty gram of the ground leaves were extracted for 24 h by soaked in 500 ml of methanol (70%). The extract was then filtered and the filtrate was divided into two amounts (one part and its double) before evaporating them till dryness in a rotary evaporator (45ºC). The residues of the two amounts were dissolved in constant volume of 25% DMSO to obtain the two concentrations of herbs extract. The dose levels of 0, 2 and 4 g kg^{-1} B.W. were divided into double dose, the first was injected at the start of the experimental period and the second dose was injected one week later.

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Fish and Experimental Design

Two hundred and eighty eight fingerlings of *O. niloticus* were obtained from El-Serw fish farm, where this study was carried out in summer season 2007. The fish were acclimated to aquaria conditions for two weeks before the experiment was initiated. Six fish (approximately the same size, 20g average) were stocked into each of the 48 aquaria which contained 50 l of water, three glass aquaria (70X40X30 cm) for each treatment, the aquaria were provided with continuous aeration and their water was changed partially daily and totally weekly. All fish were received diet twice daily at a daily feeding rate of 3% of the actual body weight, six days weekly for two weeks. Fish were divided into 16 groups and were administered the test compounds interperitoneally (I.P.). Their effects were studied at the end of the 2nd week. The experimental setup used is shown in Table (1). AFB_1 was tested at three levels (0, 9 and 18 mg kg^{-1} B.W.) being 0, 0.25 and 0.50 the LD_{50}, respectively according to El-Barbary (2008) in a single dose, while both of rosemary and parsley extracts were used at three levels (being 0, 2 and 4 g kg^{-1} B.W.) divided into 2 doses (pretreatment at the start of the experiment and one week later). AFB_1 and herbs' extracts were mixed together directly before administration.

Table (1): Explanation of the experimental groups

Groups	Pretreatment first week	Second week
A	Saline	Saline
B	DMSO 25%	DMSO 25%
F_1	DMSO 25%	AFB_1 9 mg/kg B.W.
F_2	DMSO 25%	AFB_1 18mg/kg B.W.
R_1	Rosemary 1g/kg B.W.	Rosemary 1g/kg B.W.
F_1R_1	Rosemary 1g/kg B.W.	Rosemary 1g/kg B.W.+ AFB_1 9 mg/kg B.W.
F_2R_1	Rosemary 1g/kg B.W.	Rosemary 1g/kg B.W.+ AFB_1 18mg/kg B.W.
R_2	Rosemary 2g/kg B.W.	Rosemary 2g/kg B.W.
F_1R_2	Rosemary 2g/kg B.W.	Rosemary 2g/kg B.W.+ AFB_1 9 mg/kg B.W.
F_2R_2	Rosemary 2g/kg B.W.	Rosemary 2g/kg B.W.+ AFB_1 18mg/kg B.W.
P_1	Parsley 1g/kg B.W.	Parsley 1g/kg B.W.
F_1P_1	Parsley 1g/kg B.W.	Parsley 1g/kg B.W. + AFB_1 9 mg/kg B.W.
F_2P_1	Parsley 1g/kg B.W.	Parsley 1g/kg B.W. + AFB_1 18mg/kg B.W.
P_2	Parsley 2g/kg B.W.	Parsley 2g/kg B.W.
F_1P_2	Parsley 2g/kg B.W.	Parsley 2g/kg B.W. + AFB_1 9 mg/kg B.W.
F_2P_2	Parsley 2g/kg B.W.	Parsley 2g/kg B.W. + AFB_1 18mg/kg B.W.

Analytical Methods

At the end of the 2nd week of the experiment, blood samples were withdrawn from the fish heart of each group to determinate some blood parameters using commercial colorimetric kits (Diamond, Diagnostic, Egypt), and the obtained data were statistically analyzed by one way analysis of variance using a software (SAS, 1996). Three fish from each group were homogenized and preparated to determinate the residues of AFB_1 in fish by TLC (AOAC, 2000). The histological examination of the fish livers were performed after the preparation of livers which were dissected out from each group and fixed in 10% neutralized formalin solution until use according to the technique of Roberts (2001).

RESULTS AND DISCUSSION

Mortality Rate

The mortality rate caused by interperitonal injection with AFB_1 alone or in combination with rosemary or parsley extracts was the highest in AFB_1 injected fish as shown in Table (2). The MR% gradually increased by increasing the AFB_1 level in all AFB_1 groups, while this increase in MR% was significantly reduced by using the herbs' extracts against AFB_1. The positive effects of the herbs' extracts on MR% were observed. The level of both extracts 2g kg^{-1} B.W. reflected the most significant decrease in MR% with the two levels of AFB_1 comparing with the level 4g kg^{-1} B.W. The reduction in MR% ranged from 37.39, 33.30, 37.39 to 42.90% in groups F_1R_1, F_2R_1, F_1P_1 and F_2P_1, respectively as compared with the AFB_1 groups (F_1 and F_2). While, this reduction in MR% ranged

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from 12.60 to 16.60% in groups F_1R_2 and F_2R_1, respectively and 0 to 33.30% in F_1P_2 and F_2P_2 as compared to F_1 and F_2 groups, respectively.

Table (2): Mortality rate (MR%) of Nile tilapia I.P. injected with AFB_1 with and without herbal plants extract (means ± standard errors).

Groups	MR%*
A	5.5^{e} ± 5.5
B	5.5^{e} ± 5.5
F_1	44.4^{bc} ± 5.5
F_2	66.6^{a} ± 0.0
R_1	5.5^{e} ± 5.5
F_1R_1	27.8^{cd} ± 5.5
F_2R_1	44.4^{bc} ± 5.5
R_2	11.1^{ed} ± 5.5
F_1R_2	38.8^{bc} ± 5.5
F_2R_2	55.5^{ab} ± 11.0
P_1	11.1^{ed} ± 5.5
F_1P_1	27.7^{cd} ± 5.5
F_2P_1	38.0^{bc} ± 5.5
P_2	5.0^{e} ± 5.5
F_1P_2	44.0^{bc} ± 5.5
F_2P_2	44.0^{bc} ± 5.5

a - e: Means in the same column superscripted with different letters are significantly different at ($P \leq 0.001$).
*** MR% = the beginning number of fish – the end number of the live fish X 100/ the beginning number of fish**

These results agree with the results of AFB_1-residues which confirmed that the level of both herbs' extracts 2g kg^{-1} B.W. was more effective in reducing the AFB_1-residues than the level 4g kg^{-1} B.W. especially with the AFB_1 level 9 mg kg^{-1} B.W. The significant effect of AFB_1 on MR% was confirmed in previous studies with Nile tilapia (Marzouk *et al*., 1994; Hussein *et al*., 2000; Abdelhamid *et al*., 2002 a; Tuan *et al*., 2002 and El-Barbary and El-Shaieb, 2006). The positive effect of parsley could be attributed to its medicinal property as anti-inflammation (Peter, 2001). Also, both of rosemary and parsley have antioxidant properties due to polyphenolic compounds including ferulic acid and syringic acid that are the major phenolic compounds in parsley (El-Barbary, 2008). Ferulic acid exhibit a wide range of pharmacological effects including antiageing, anti-inflammatory, anticancer, antiapoptotic, antidiabetic and neuroprotective (Srinivasan *et al*., 2006).

Quantitative Estimation of AFB_1 Residues

The residual analysis of AFB_1 in the whole body of fish which were injected with 9 mg AFB_1 kg^{-1} B.W. with or without herbs' extracts (F_1, F_1P_2, F_1R_2, F_1P_1 and F_1R_1) (Fig. 1) showed that all these groups revealed the presence of AFB_1 residues (1.6 to 7.5 ppb) which consider less than the permissible limit (20 ppb) that was recommended by WHO (Diener *et al*., 1985). In fish groups injected with AFB_1 with herbs' extracts, traces of AFB_1 were detected in their body at concentration ranged from 1.6 to 3.1 ppb.

Fig. (2) illustrates AFB_1 residues of the fish groups injected with the level of AFB_1 18 mg kg^{-1} B.W. with or without herbs' extracts (F_2, F_2P_1, F_2R_1, F_2P_2 and F_2R_2) which, showed contrast results to those in Fig. (1), since there were no-residues of AFB_1 in either F_2 (18 mg AFB_1 kg^{-1} B.W.) or F_2P_1 (18 mg AFB_1 + 2g parsley kg^{-1} B.W.). Whereas AFB_1-residues ranged from 2.1, 9.6 to 23.0 ppb in F_2R_1, F_2P_2 and F_2R_2, respectively.

These results indicate that the herbs' extracts have potency for reducing the AFB_1 residues, particularly in cases of the low levels of AFB_1 and the herbs' extracts. Also, parsley could have higher potency than rosemary.

The absence of AFB1 residues in F2 group (18 mg AFB1 kg-1 B.W.) could be attributed to the high level of AFB1 which acts as an acute dose, so its metabolism may be rapid and severely converted into other metabolites which could have more toxicity than AFB1.

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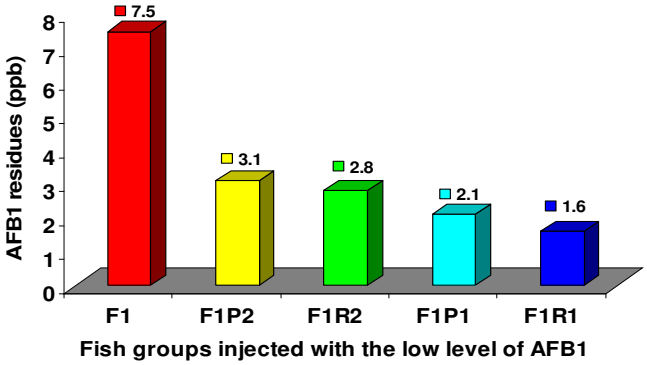

Fig. (1): AFB_1 residues in the experimented fish injected with the low dose of AFB_1.

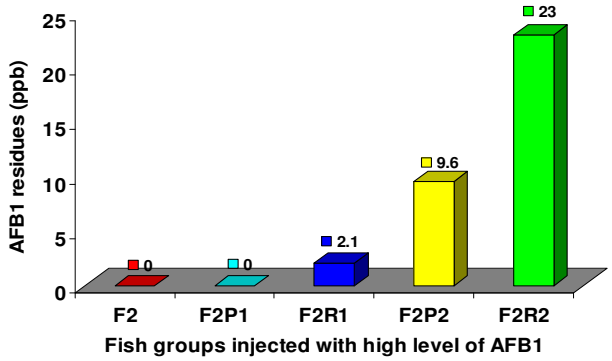

Fig. (2): AFB_1 residues in the experimented fish injected with the high dose of AFB_1.

The negative results of both MR% and histopathological examination in F_2 group may confirm that. On the other hand, the low level of AFB_1 acts as subacute dose; so, its metabolism may be gradual and slight so its MR% and histopathological manifestation had better results comparing with F_2 group. The mechanism of parsley and rosemary extract may be due to inhibition of the metabolic activation through inhibition of cytochrome P_{450} enzymes, and also through inducation of the detoxifying enzyme glutathione S-transferase. In this respect, similar results were recorded by Soliman *et al.* (1998 and 2000). Yet, Abdelhamid *et al.* (2004 b and 2007) recorded high level of AFB_1 in whole body of Nile tilapia fish. On the other hand, Abdelhamid *et al.* (2002 a, 2002 b and 2004 a) reported that there were no AFB_1 residues in *O. niloticus* body. These variable results may be due to AFB_1 level and exposure time as well as to sensitivity variation among fish species to AFB_1. Recently, Oliveira and Furlong (2008) reported that phenolic extracts of different edible plants have antifungal and antimycotoxigenic activity.

Blood Parameters

There were significant reduction in Hb, TP and Gl levels and increase in the albumin (Al) concentration and activity of hepatic transaminase enzymes (AST and ALT) at all AFB_1 groups, whether injected with AFB_1 alone or AFB_1 with herbs' extracts comparing to the control group (A). Table (3) indicates that the control solvent (B) showed approximately similar values of most blood parameters when compared to the control (A).That indicates the absence of toxicity due to DMSO administration. This alteration in haematology and biochemistry of blood was gradually increased by increasing the level of AFB_1. On contrary, these negative alterations due to AFB_1 were significantly improved by using rosemary and parsley extracts, particulary at the low level of AFB_1.

However, these improved values remained lower than the control values. Also, the study indicates that R_1 and P_1 showed significant alterations in some of the studied blood parameters when compared to the control.

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Table (3): The influence of AFB_1 with or without either of rosemary or parsley extract on some blood parameters of *O. niloticus* ($\bar{X} \pm SE$)

Groups	Hb (g dl^{-1})	TP (g dl^{-1})	AL (g dl^{-1})	GL (g dl^{-1})	AST U/I	ALT U/I
A	$6.74^{a} \pm 0.04$	$4.31^{b} \pm 0.06$	$1.35^{e} \pm 0.01$	$2.96^{a} \pm 0.05$	$56.37^{ef} \pm 0.33$	$22.47^{ef} \pm 0.23$
B	$6.48^{ab} \pm 0.06$	$4.38^{a} \pm 0.05$	$1.40^{d} \pm 0.01$	$2.98^{a} \pm 0.06$	$56.40^{ef} \pm 0.35$	$21.53^{gh} \pm 0.40$
F_1	$5.36^{f} \pm 0.03$	$3.71^{g} \pm 0.05$	$1.67^{b} \pm 0.02$	$2.05^{g} \pm 0.07$	$69.37^{a} \pm 1.30$	$24.01^{d} \pm 0.13$
F_2	$4.60^{g} \pm 0.20$	$3.10^{i} \pm 0.01$	$1.51^{c} \pm 0.04$	$1.59^{h} \pm 0.11$	$70.23^{a} \pm 0.55$	$28.80^{a} \pm 0.06$
R_1	$6.32^{bc} \pm 0.11$	$4.04^{d} \pm 0.08$	$1.37^{e} \pm 0.02$	$2.68^{c} \pm 0.11$	$55.20^{f} \pm 0.60$	$22.07^{fg} \pm 0.09$
F_1R_1	$5.25^{f} \pm 0.04$	$3.75^{g} \pm 0.02$	$1.53^{c} \pm 0.07$	$2.22^{f} \pm 0.08$	$63.40^{cd} \pm 0.90$	$24.03^{d} \pm 0.12$
F_2R_1	$4.89^{g} \pm 0.01$	$3.97^{d} \pm 0.03$	$1.42^{d} \pm 0.01$	$2.55^{d} \pm 0.01$	$61.47^{d} \pm 0.86$	$29.13^{a} \pm 0.03$
R_2	$6.12^{cd} \pm 0.39$	$4.42^{a} \pm 0.02$	$1.64^{b} \pm 0.03$	$2.77^{b} \pm 0.02$	$56.13^{ef} \pm 1.01$	$20.67^{i} \pm 0.12$
F_1R_2	$5.95^{d} \pm 0.05$	$4.22^{c} \pm 0.01$	$1.49^{c} \pm 0.03$	$2.73^{b} \pm 0.03$	$58.47^{e} \pm 0.37$	$24.00^{d} \pm 0.31$
F_2R_2	$4.77^{g} \pm 0.10$	$3.56^{h} \pm 0.03$	$1.45^{cd} \pm 0.03$	$2.10^{g} \pm 0.03$	$58.13^{e} \pm 0.70$	$23.00^{e} \pm 0.17$
P_1	$6.32^{bc} \pm 0.49$	$4.05^{d} \pm 0.02$	$1.36^{de} \pm 0.01$	$2.69^{c} \pm 0.03$	$58.33^{e} \pm 1.22$	$21.37^{h} \pm 0.01$
F_1P_1	$5.90^{e} \pm 0.05$	$3.72^{g} \pm 0.07$	$1.51^{c} \pm 0.03$	$2.21^{ef} \pm 0.07$	$66.27^{b} \pm 0.50$	$25.84^{c} \pm 0.04$
F_2P_1	$4.68^{g} \pm 0.06$	$3.82^{fg} \pm 0.10$	$1.53^{c} \pm 0.01$	$2.28^{e} \pm 0.02$	$61.20^{d} \pm 0.73$	$26.55^{b} \pm 0.33$
P_2	$6.28^{bcd} \pm 0.04$	$4.04^{d} \pm 0.03$	$1.33^{e} \pm 0.03$	$2.71^{b} \pm 0.06$	$57.90^{e} \pm 0.87$	$22.05^{fg} \pm 0.05$
F_1P_2	$5.41^{f} \pm 0.05$	$3.89^{e} \pm 0.02$	$1.61^{b} \pm 0.01$	$2.28^{e} \pm 0.03$	$66.77^{b} \pm 0.99$	$23.01^{e} \pm 0.15$
F_2P_2	$4.56^{g} \pm 0.14$	$3.90^{de} \pm 0.02$	$1.92^{a} \pm 0.02$	$1.98^{g} \pm 0.04$	$64.77^{bc} \pm 1.10$	$24.06^{d} \pm 0.09$

a - i: Means in the same column superscripted with different letters are significantly different at ($P \leq 0.001$).

So, the positive effects of the two levels of these extracts were clearly observed with the low level than the high level of AFB_1.While, the high level of rosemary and parsley was better with the high level of AFB_1.These alterations in blood parameters among fish groups may be due to the alterations in histological structure of livers of AFB_1-injected fish leading to inhibition of blood synthesis, where liver plays an important role in this process. Similarly, Abdelhamid *et al*. (2002 a) and El-Barbary and El-Shaieb (2006) reported that AFB_1 reduced TP, Al and Gl of *O. niloticus*. In the same trend, Abdelhamid *et al*. (2007) found that AFB_1 caused significant decrease in TP, Al and Gl of aflatoxicated *O. niloticus* fish.

This reduction in TP levels may be due to the hepatotoxic effect of AFB_1. Whereas the reduced Gl levels in AFB_1 injected fish may have been the result of lymphocytolysis (Sahoo *et al*., 1998). While Youssef and Ashry (1999) attributed the increase in activity of AST and ALT enzymes to the hepatotoxic effect of AFB_1 and consequently hepatic cell damage and liver dysfunction.

Clinical and Histopathological Findings

The present study revealed that the toxicity sings began to exist together with mortalities. The most common clinical sings observed were lethargy, loss of appetite, sluggish movement, dark discoloration of the skin and respiratory manifestations. Macroscopically, the common lesions in all necropsied aflatoxicated fish were accumulation of fluids in abdomen, congested gills and dark liver. These symptoms were varied according to the treatment. The photomicrograph of liver showing microscopically the parenchemal architecture of hepatocytes in the control group (A) with central nuclei (Fig. 3A). Fish of the solvent control group (B) presented normal structure of the liver (Fig. 3B). Also, no clear histological changes were observed in any of fish groups which injected with herbs' extracts alone at the different levels (R_1,R_2,P_1,P_2). In contrast, fish injected with AFB_1 presented pathological changes in hepatopancreas which increased in severity with increasing the level (groups F_1, F_2 at 9 and 18 mg kg^{-1} B.W respectively). These changes in F_1 group were severe hemolysis in the portal blood vessels (PBV) and presence melanomacrophages (MMC) (Fig. 3C). In addition, lysis of hepatocyte membranes (necrotic cells) besides dilation and congestion in blood sinusoid were noticed (Fig. 3D); while the lesions among F_2 were severe hemolysis, dilation in the portal blood vessels and large area of degenerated hepatocytes (Fig. 3E). Also, liver shows diffuse vaculation and necrosis of hepatocytes and nuclei displaced to the cell periphery; in addition to, congestion and dilation in blood sinusoid (Fig. 3F), thrombosis formation in blood vessels (Fig. 4G) and accumulation of MMC and hemosiderin besides coagulative necrosis in hepatocytes were recorded too (Fig. 4H). These severe pathological alterations in hepatopancrease caused by AFB_1 alone became less severer when these fish were injected with AFB_1 plus either rosemary or parsley at the different levels. The effects of the low level of rosemary on the two levels of AFB_1 were observed in Figs. 4 I and J; whereas, group F_1R_1 showed slight

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degeneration of hepatocytes (Fig. 4I), while the lesions in group F_2R_1 were necrosis in hepatocytes besides congestion of some pancreatic acini (Fig. 4J).

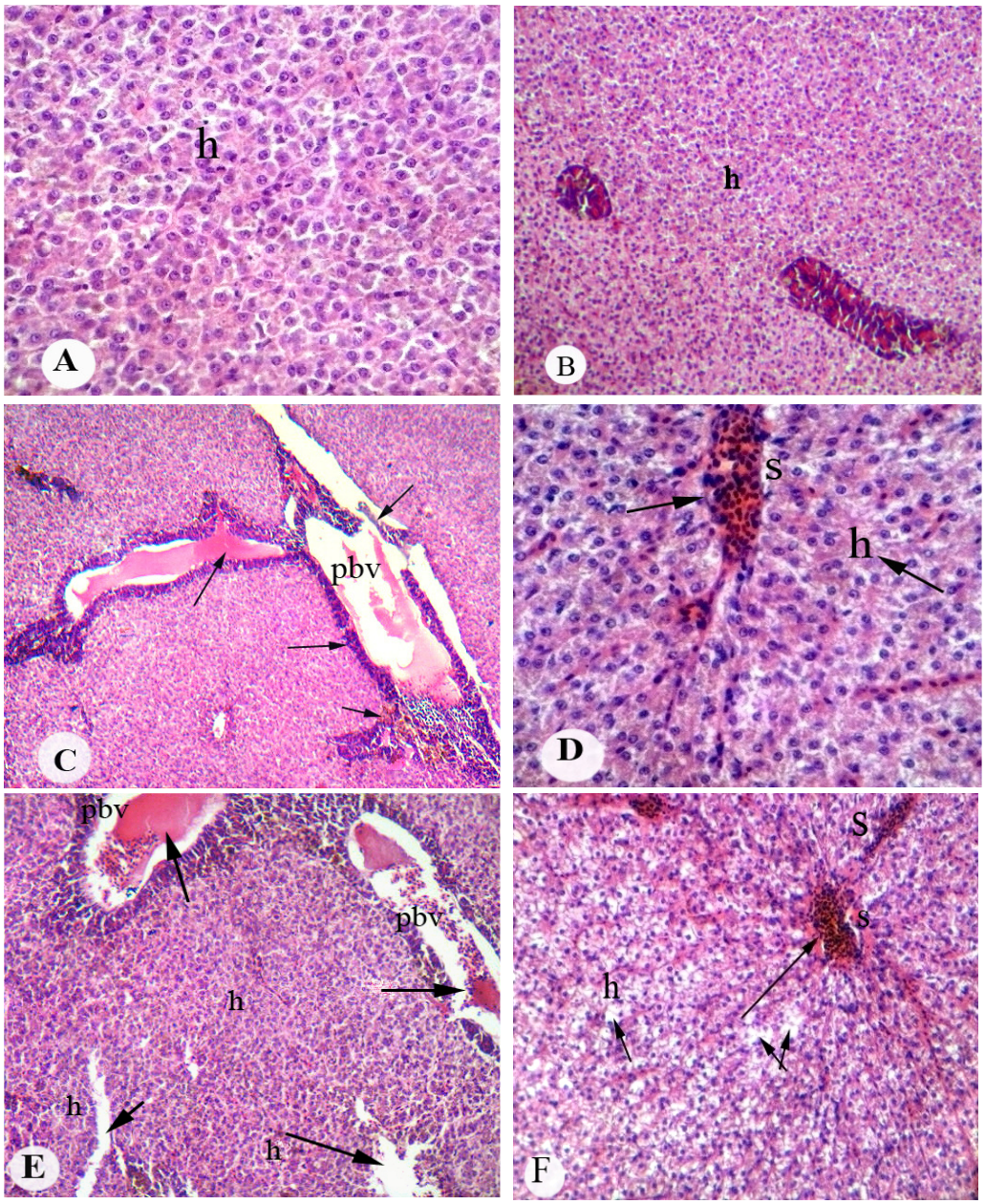

Fig. 3: Histopathological changes in liver of *O niloticus* injected with different levels of AFB_1 as compared to control (stained with H&E). (A&B): The control and control solvent fish groups showing normal structure of the liver (X600&250, respectively). (C&D); fish injected with AFB_1 (9 mg kg^{-1} B.W., F_1 group) showing hemolysis and dilation in pbv (C, X200) besides congestion and dilation in sinusoid with necrosis (D, X600). (E&F); fish injected with AFB_1 (18 mg kg^{-1} B.W., F_2 group) showing dilation and hemolysis in pbv, degeneration in hepatocytes (E, X300), vaculation and necrosis in hepatocytes and congestion with dilation in sinusoid (F, X450). h; hepatocyte. s; sinusoid. pbv; portal blood vessel.

The effects of the high level of rosemary with AFB_1 were observed, where the hepatocytes showed condensed cytoplasms and loss contact between hepatocytes (Figs.4 K & L), in addition to presence of few numbers of inflammatory cells (Fig. 4L), besides mild congestion and hemolysis in blood vessels and necrosis of hepatocytes (Fig. 4K).Concerning the effect of the low level of parsley with different levels of AFB_1 ,it may have a positive effect in amelioration the toxicity of AFB_1, since the liver of group (F_1P_1) showed normal structure of hepatocytes with slight hemolysis of hepatic acini and presence of few numbers of MMC (Fig. 5M).Similar

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findings were observed in group F_2P_1 but with diffuse of hemosiderin and MMC (Fig. 5N).The effect of the high level of parsley against AFB_1 was presented in groups F_1P_2 and F_2P_2, since the liver of group F_1P_2 showed normal hepatocytes (Fig. 5O).While group F_2P_2 showed slight hemolysis and presence of few inflammatory cells (Fig. 5P). Similar hepatic lesions were reported by El-Banna *et al*. (1992), Hussein *et al*. (2000) and Abdelhamid *et al*. (2002 a) who described congestion, vacuolar degeneration of hepatocytes and activation of melanomacrophages in aflatoxicated tilapia. Also, El-Barbary and El-Shaieb (2006) reported that the liver of aflatoxicated fish showed severe vacuolation of hepatocytes of mainly fatty changes besides focal coagulative necrosis, focal replacements of the hepatic parenchyma with extravagated blood, and severe congestion and hemorrhage. In the same trend, also Mehrim *et al*., (2006) emphasized similar clinical sings and histopathological lesions in the liver of aflatoxicated Nile tilapia.

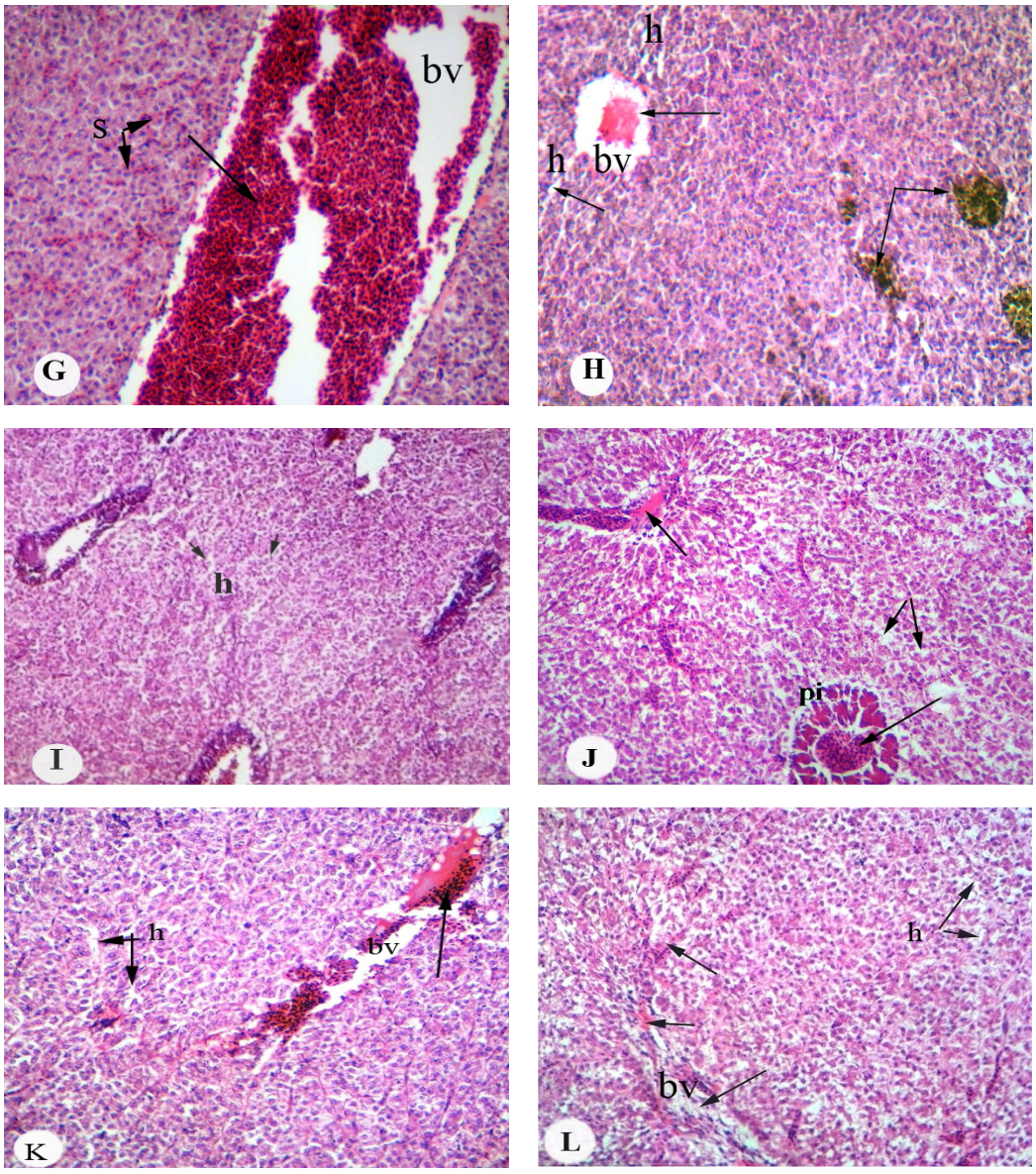

Fig. 4: Histopathological changes in liver of *O niloticus* injected with AFB_1 with or without rosemary at the different levels (stained with H&E). (G, H); fish injected with (18 mg kg^{-1} B.W., F_2 group) showing thrombosis formation in bv (G, X250), coagulative necrosis, besides diffusion of MMC and hemosiderin (H, X250). (I); fish injected with AFB_1 + rosemary (9 mg and 2g kg^{-1} B.W., F_1R_1 group) showing slight necrosis (X250). (J); fish injected with AFB_1 + rosemary (18 mg and 2g kg^{-1} B.W., F_2R_1 group) showing congestion and necrosis in pi (X400). (K); fish injected with AFB_1 + rosemary (9 mg and 4g kg^{-1} B.W., F_1R_2 group) showing necrosis and congestion (X400). (L); fish injected with AFB_1 + rosemary (18 mg and 4g kg^{-1} B.W., F_2R_2 group) showing necrosis and congestion (X400). bv; blood vessel. pi; pancreatic acini.

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The positive effects of both of the parsley and rosemary on overcoming the toxic effects of AFB_1 could be attributed to the antioxidative and nutritive properties of these herbs. These results showed that the ability of parsley extract to counteract the toxic effects of AFB_1 on the fish could be better than rosemary extract. That may be attributed to the high nutritive value of parsley that included too high percent of vitamins (A, C, riboflavin and niacin) and minerals (Fe, Mg, P, K, Ca, Na and Zn) compared to rosemary. Vitamin C is related to the immunological system performance, and has antioxidant properties. This antioxidant activity of Vit. C makes it as a hunter of free radicals, thus preventing the autointoxication of immunological cells, such as macrophages which are the first processors of the information about the alien bodies and maximizing the defense of fish (Brake, 1997). Also, metal ions such as Se, Zn, Cu, Mn and Fe are essential for most organisms. Essential trace elements are important parts of antioxidant enzymes as superoxide dismutase and glutathione peroxidase and may affect the antioxidant defense system (Hung *et al*., 2007).

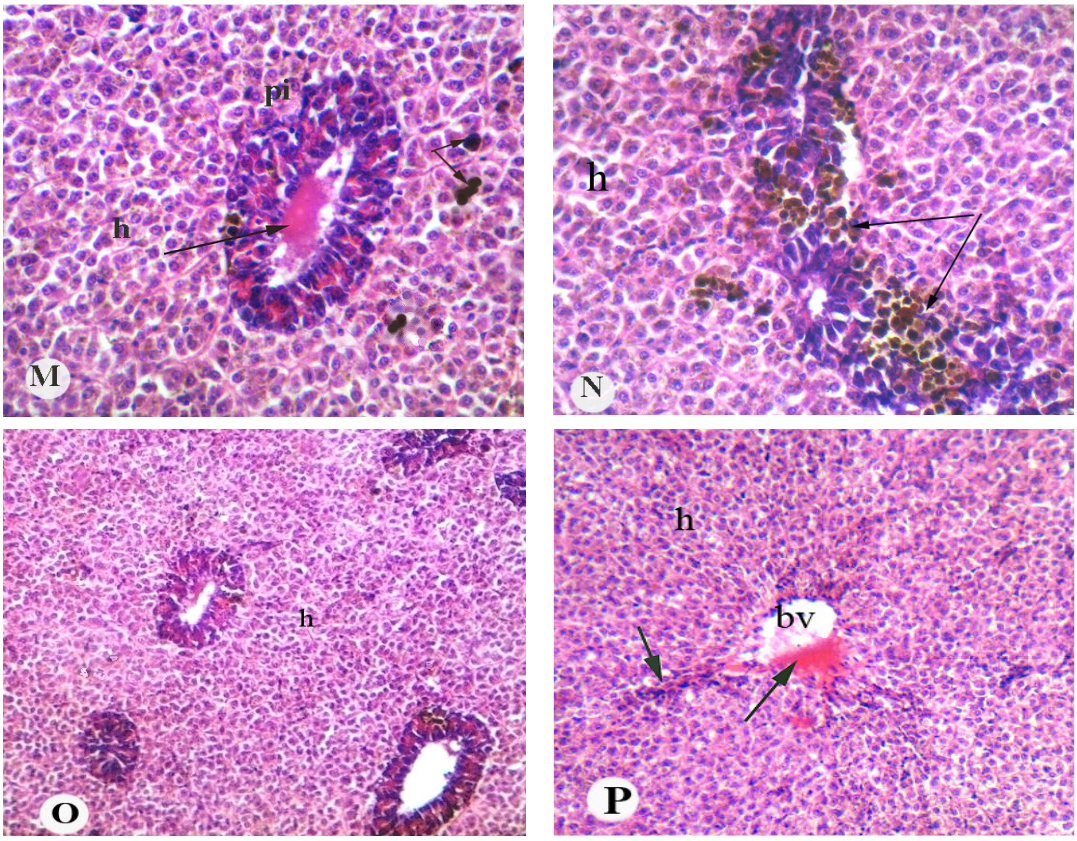

Fig. 5: Histopathological changes in liver of *O niloticus* injected with AFB_1 + parsley at the different levels (stained with H&E). (M); fish injected with AFB_1+ parsley (9 mg and 2g kg^{-1} B.W., F_1P_1 group) showing hemolysis in pi and presence of MMC (X600). (N); fish injected with AFB_1+ parsley (18 mg and 2g kg^{-1} B.W., F_2P_1 group) showing diffusion of MMC and hemosiderin (X600).(O); fish injected with AFB_1+ parsley (9 mg and 4g kg^{-1} B.W., F_1P_2 group) showing normal structure (X250). (P); fish injected with AFB_1+ parsley (18 mg and 4g kg^{-1} B.W., F_2P_2 group) showing slight hemolysis and infiltration (X400).

CONCLUSIONS

From previous results, it could be recommended the useful using of both of medicinal herbs, namely rosemary and parsley, at low level (2 g kg^{-1} B.W.) to eliminate the drastic effects of aflatoxin B_1 on *O. niloticus*. Yet, it must be exist more scientific efforts to use the medicinal herbs and other natural materials against the contamination with the mycotoxins. But, it will be still the prevention from toxic effects of aflatoxin B_1 and other toxins are more useful usually.

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