Assiut University Journal of Chemistry (AUJC) 48(2019) 26-41

Journal homepage: www.aujc.org

(ISSN 1678-4919) (Print) (ISSN 2357-0415) (Online)

Vol(49)	No(2)	2020

Full Paper

Ameliorative effects of bradykinin potentiating factor, butylated hydroxy toluene and oltipraz on biochemical and histological structure of lymphoid organs in aflatoxicated female rats

***Safaa A. Mahgoub*[1], *Ahmed Y. Nassar*[2**], *Hossam-El-Din M. Omar*[3], *Amany A. Osman*[1*]**

[1] *Chemistry department, Faculty of Science, Assiut University, Egypt.*

[2] *Biochemistry department, Faculty of Medicine, Assiut University, Egypt.*

[3] *Zoology department, Faculty of Science, Assiut University, Egypt.*

Email address of corresponding author:

***AYNassar41@yahoo.com*,**

****Amanyabdelrahman6@gmail.com***

Article history: Received: 12/1/2020; Revised: 20/9/2020; Accepted: 6/10/2020; Available Online: 1/12/2020;

Abstract:

Aflatoxins (AFs) are chemically secondary metabolites produced by *Aspergillus, Penicillium* and *Fusarium* genera. It has adverse effects on humans and animals health due to inhibition of macromolecule synthesis. The current research investigate the pathological and biochemical changes in lymph follicles of female rats exposed to aflatoxin B_1 (AFB_1) for one month and the efficacy of isolated bradykinin potentiating factor (BPF) from cobra snake venom, butylated hydroxy toluene (BHT) and oltipraz (OPZ) to ameliorate those changes. Aflatoxicosis cause significant increase of lipid peroxidation (LPO) and nitric oxide (NO) in addition to significant decrease in the level of total thiols, glutathione (GSH) and the activities of glutathione peroxidase (GPx) and glutathione S-transferase (GST). Moreover, AFB_1 caused

histopathological changes in lymph follicles represented by depletion of the lymphoid cells. Treatment of aflatoxicosed rats with BPF, BHT or OPZ resulting in amelioration of the oxidative stress markers and improvement in the histological structure of lymph follicles represented by increase of lymphoid cell population with presence of mast cells and collagen bundle. In conclusion BPF, BHT or OPZ ameliorate the aflatoxicosis with priority for the BPF.

Key Words: lymph follicles, aflatoxin B_1, oxidative stress, butylated hydroxy toluene, bradykinin potentiating factor, oltipraz.

1- Introduction:

Aflatoxins (AFs) are chemically secondary metabolites produced by *Aspergillus*, *Penicillium* and *Fusarium* genera which causes immunosuppression, genotoxicity, carcinogenic and teratogenic effects on humans and animals health due to inhibition of macromolecule synthesis [1,2]. Also, AFs deplete the cell populations of the lymph organs and decreases the concentrations of immunoglobulins and phagocytic activity [3,4]. Several kinds of animal venom from spiders, snakes, cone snails, scorpions contain short polypeptide such as bradykinin-potentiating peptides and natriuretic peptides [5,6]. The Egyptian cobra *(Naja haje haje)* contains a number of low molecular weight basic polypeptides has low toxicity and distinct immunochemical properties [7]. Moreover, BPF extracted from *scorpion* venom *Buthus occitanus* have antioxidants, anti-inflammatory, immunomodulatory effects that improve cellular growth of the uterus and ovarian follicle in mice and healing of guinea skin burned [8-11]. Also, BPF generates thymus and spleen cellularity promoted vascular permeability and mitogenesis in irradiated guinea pigs [12-14].

Oltipraz (OPZ) [4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione] as a cancer chemopreventive drug due to enhancement of the antioxidant response element binding activity of NFE_2 related factor-2 and the induction of the glutathione S-transferase (GST) A_2 gene in cirrhotic liver [15,16]. High doses OPZ may increase the production of its oxidized metabolites via extensive metabolism and made changes in target gene transactivation [17].

Butylated hydroxy toluene (BHT) is an antioxidant phenolic acid which used as additive in polymers such as foamed plastic, food grade plastics, natural and synthetic rubbers [18]. BHT protect against oxidative stress, cancer, HIV and cardiovascular diseases [19,20].

From the for mentioned the current research was planned to investigate the pathological and biochemical changes in lymph follicles of female rats exposed to AFB_1 for one month and the efficacy of isolated BPF from the cobra snake venom, the BHT and OPZ to ameliorate those changes.

2- Materials and methods:

2.1- Chemicals used:

Butylated hydroxy toluene (W218405), oltipraz (O9389), thiobarbutric acid (T5500), naphthylethylene diamine dihydrochloride (N9125), 5,5- dithiobis-2-nitrobenzoicacid) (D8130), sulphanilamide (S9251) and coomassie brilliant, blue R stain (B0149) were purchased from Sigma-Aldrich Company (St. Louis, MO), USA.

2.2- Extraction, purification and identification of AFB_1:

AFB_1 was extracted from harvested media of growing *Aspergillus flavus* according to the method of Booth [21], purified by TLC method of El- Kady and Moubasher [22].

2.3- Extraction, purification and identification of BPF:

The collected amount of the Egyptian cobra snake crude venom was dialyzed according to the method of Abdel-Raheim *et al*. [11]. Isolation and purification of BPF from the dialyzed performed by the chemical method of Ferreira [23]. Isolated BPF was identified by its hypotensive effect on arterial blood pressure of rabbit by oscillograph in the Central Lab of Zoology Department, Faculty of Science, Assiut University. The amino acid content of the isolated BPF was analyzed by amino acid analyzer SW (Hydrolisate Separation Method) at Desert Research Center, Ministry of Agriculture and Land Reclamation, Cairo, Egypt.

2.4- Experimental animals:

In this study, 120 female Wistar albino rats were used and obtained from the Animal House of the Faculty of Medicine, Assiut University, Assiut, Egypt with mean body weight of 130±10 gm. Rats were housed in cages, kept at room temperature with normal 12h light/12h dark cycle and supplemented with standard commercial pellets for feeding, water, *ad libtium.* All of the animal procedures were performed in accordance with the guidelines for the care and use of experimental animals established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the protocol of the National Institutes of Health (NIH) [24].

2.4.1- Design of the experiment:

Rats were divided randomly into 6 groups of 20 rats each as follows:

G I: Reference normal group.

G II_0: Intoxicated group treated with AFB_1 for one month.

G II_1: Self- recovery for the same period after intoxication.

G II_2: Treated with BPF for the same period after intoxication.

G II_3: Treated with BHT for the same period after intoxication.

G II_4: Treated with OPZ for the same period after intoxication.

2.4.2- Administration protocol of intoxicating solutions and the other treating agents:

The animals of GII_0 were individually intoxicated by oral administration three times weekly (500µg, AFB_1 suspended in milk/kg b.wt) for one month according to Raisuddin *et al*. [25]. GII_1 animals after intoxication were left without treatment for self-recovery. GII_2 intoxicated animals were treated by intrapetionally three doses weekly for one month (1µg BPF in PBS, pH 7.4/ kg b.wt) according to Omar and Meki [26]. GII_3 intoxicated animals were orally treated by 0.5mg BHT in PBS, pH 7.4/ kg b.wt three times weekly for one month according to Hocman [27]. Similarly to GII_3, the animals of GII_4 were orally administrated by OPZ (2mg in PBS, pH 7.4)/ kg b.wt) according to Dimitrov *et al*. [28].

2.4.3- Collection and preparation of the samples for biochemical determination and histopathological examinations:

At the end time of the experiment, blood samples were collected from the heart under an aesthesia by ether and sacrificed by cervical dislocation. At time of scarifying, the blood was collected in tubes and centrifuged after blood clotting at 4,000 rpm for 10 min to separate serum. Spleen, thymus and axillary lymph node (ALN) was quickly removed, washed with saline solution and small slices were fixed in glutraldehyde for electron microscopic examinations and the remnant was imbedded in liquid nitrogen and kept frozen at -80 for biochemical studies. 10% w/v homogenate of ALN in 0.1M phosphate buffer (pH 7.4) was prepared then the homogenate was preserved at -20^0C for the subsequent biochemical indices.

2.5- Biochemical determinations:

Total protein content in the supernatant of ALN tissue homogenates was performed according to the method of Lowry *et al*. [29]. Alanine aminotransferase (ALT) activity in serum was determined by ALT-Liquizyme (4+1) E.C.2.6.1.2 kit which purchased from Egyptian Company for

Biotechnology. Nitric oxide (NO) was determined by Gries reagent according to Ding [30]. Lipid peroxidation as MDA was evaluated according to the method of Wills [31]. Total thiols content was assayed according to the method of Ellman [32]. Glutathione (GSH) was estimated according to the method of Beutler *et al*. [33]. The activity of GSH-peroxidase (GPx) and transeferase (GST) were assayed according to Habig *et al*. [34].

2.7- Histopathogical features:

Semi-thin section and transmission electron microscope observations were done in Electron Microscope Unit (E.M.U) of Assiut University according to Bozzola and Russell [35]**.**

2.8- Statistical analysis:

Statistical differences of parameters are presented as Mean ± SEM, statistical significance was determined using the one way ANOVA test in Graph Pad Prism 5.03 at $P<0.05$ where ^{a} is a significant of aflatoxicosed GII_0 with respect to reference normal GI, ^{b} is a significant of treated groups $GII_{1, 2, 3, 4}$ with respect to reference normal GI and ^{c} is a significant of treated groups $GII_{1, 2, 3, 4}$ with respect to aflatoxicosed GII_0.

3-Results

3.1- Identification and characterization of AFB_1 and isolated BPF from Cobra snake venom:

The results were identified and data not shown in the current paper (submitted and under revision in the journal of applied animal research (JAAR-2020-0109)) [36].

3.2- Biochemical indices:

3.2.1- ALT activity (U/L) in serum of experimental groups

ALT in serum was significantly increased in the intoxicated rats (GII_0) in comparison to the normal (GI) and still elevated in the self-recovery group (GII_1). However, in treated rats with BPF (GII_2), BHT (GII_3) and OPZ (GII_4), the levels were decreased but still higher than normal (GI) as shown in Fig (1).

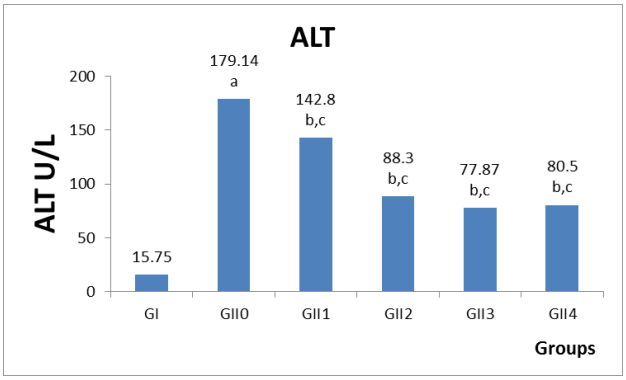

Fig (1): ALT activity in serum of experimental groups.

Data presented as M ± SEM, ^{a} is a significant of aflatoxicosed (GII0, GII00) with respect to reference normal GI, ^{b} is a significant of treated groups GII1, 2, 3, 4 with respect to reference normal GI and ^{c} is a significant of treated groups GII1, 2, 3, 4 with respect to aflatoxicosed GII0.

3.2.2- Oxidative stress markers in ALN tissue

LPO as MDA, NO in ALN tissue were significantly increased in rats that treated with AFB_1 for one month (GII_0) in comparison to the control group (GI) and still elevated in the recovery group (GII_1), however, in the groups co-treated with BPF (GII_2), BHT (GII_3) and OPZ (GII_4) was similar to the control one. Total thiols and GSH content were significantly reduced in the ALN tissue of rats that treated with AFB_1 for one month (GII_0) and the recovery (GII_1) group in comparison with control one (GI), however, in groups of rats that co-treated with BPF (GII_2), BHT (GII_3) and OPZ (GII_4) were increased but still lower than control group. The enzymatic activities of GPx and GST were significantly depleted in the ALN tissue of rats that treated with AFB_1 for one month (GII_0) and the recovery (GII_1) group in comparison with control one (GI), however, in groups of rats that co-treated with BPF (GII_2), BHT (GII_3) and OPZ (GII_4) were increased but still lower than control group as shown in Table (1).

Table (1): Oxidative stress markers in ALN tissue of the tested groups (Mean± SEM)

Groups / Parameters	GI	GII_0	GII_1	GII_2	GII_3	GII_4
MDA (nmol/ mg protein)	3.41 ± 0.03	4.68 ± 0.04[a]	4.67 ± 0.06[b,c]	3.84 ± 0.06[b,c]	3.75 ± 0.09[b,c]	3.85 ± 0.07[b,c]
NO (nmol/ mg protein)	31.6 ± 0.65	47.8 ± 0.53[a]	44.7 ± 1.08[b,c]	36.4 ± 1.52[b,c]	34.9 ± 1.06[b,c]	35.8 ± 1.05[b,c]
Total thiols (nmol/mg protein)	30.8 ± 0.03	23.4 ± 0.04[a]	24.8 ± 0.06[b,c]	26.3 ± 0.54[b,c]	27.2 ± 0.61[b,c]	26.9 ± 0.52[b,c]
GSH (nmol/ mg protein)	14.2 ± 0.50	12.7 ± 0.29[a]	12.9 ± 0.38[b,c]	13.1 ± 0.35[b,c]	13.7 ± 0.41[b,c]	13.5 ± 0.23[b,c]
GPx (nmol/min/ mg protein)	22.2 ± 1.22	12.8 ± 1.07[a]	14.6 ± 2.20[b,c]	16.4 ± 1.35[b,c]	17.4 ± 2.79[b,c]	19.8 ± 1.81[b,c]
GST (nmol/ mg protein)	1.35 ± 0.05	0.59 ± 0.16[a]	0.65 ± 0.05[b,c]	0.89 ± 0.05[b,c]	0.86 ± 0.07[b,c]	1.06 ± 0.09[b,c]

Lipid peroxidation as malondialdehyde (MDA), Nitric oxide (NO) and Glutathione (GSH) and the activities of glutathione peroxidase (GPx) and transeferase (GST).[a] is a significant difference between aflatoxicosed and control group, [b] is a significant difference between treated groups and control group and [c] is a significant difference between treated groups and aflatoxicosed group.

3.3- Histopathological examinations:

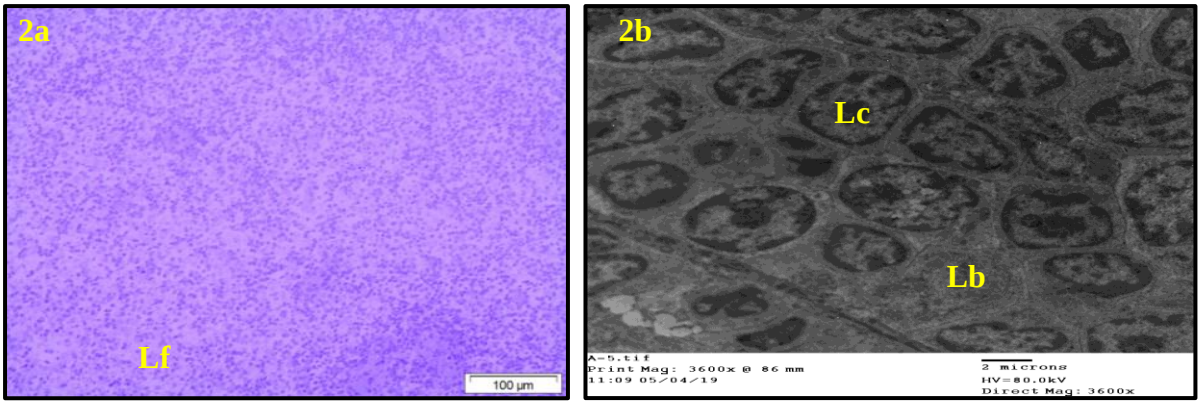

Fig 2a: Light micrograph of semi thin section of lymph follicles of normal rats (GI) showing normal population of lymphoid cells of lymph follicles (Lf). Toluidine blue T.B. stain.

Fig 2b: T.E. micrograph of the lymphoid follicle of normal rats (GI) showing lymphoid tissue formed by lymphoblasts (Lb) and lymphocytes (Lc).

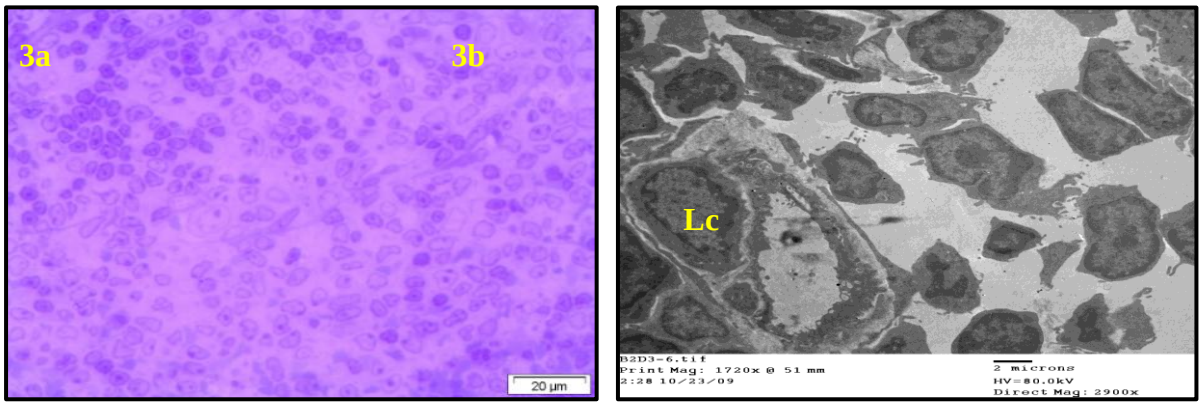

Fig 3a: Light micrograph of lymph follicles of rats treated with AFB_1 for one month (GII_0) showing marked depletion of the lymphoid cells in the lymph follicles. T.B. Stain

Fig 3b: T.E. micrograph of lymph follicles of rats treated with AFB_1 for one month (GII_0) showing marked depletion with prominent decreases of the lymphoid cells (Lc).

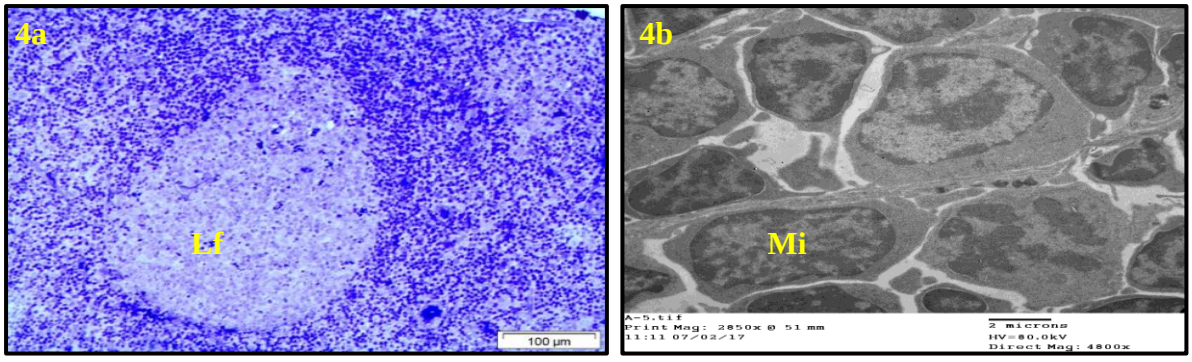

Fig 4a: Light micrograph of lymph follicles of rats left for self-recovery for one month (GII_1) showing proliferation of lymphoid cells in the lymph follicle (Lf).T.B. stain.

Fig 4b: T.E. micrograph of lymph follicle of rats left for self-recovery for one month (GII_1) showing lymphoblast in state of mitosis (Mi).

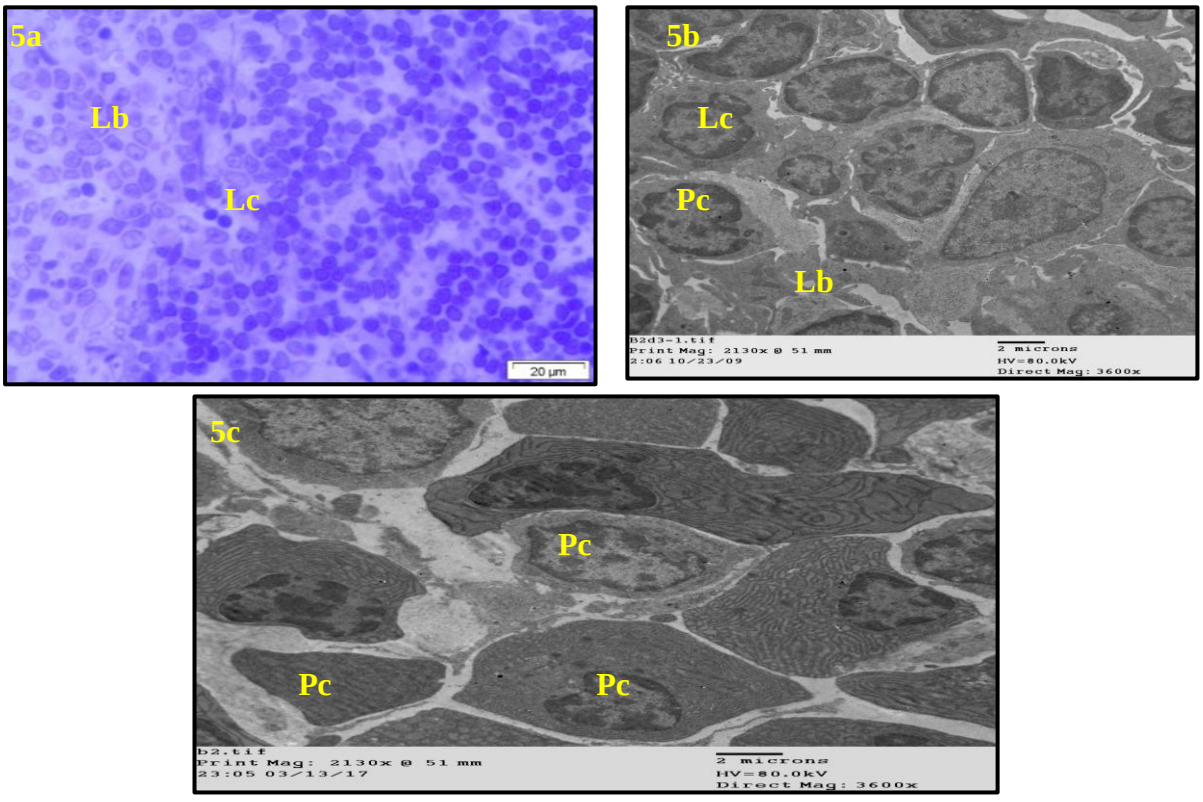

Fig 5a: Light micrograph of lymph follicle of rats treated with AFB_1 for one month then treated with BPF for one month (GII_2) showing presence of large number of lymphocyte (Lc) and lymphoblast's (Lb). T.B. stain.

Fig 5b: T.E. micrograph of lymphoid tissue of rats treated with AFB_1 for one month then treated with BPF for one month (GII_2) showing presence of large number of lymphocyte (Lc) and lymphoblast's (Lb) as well as plasma cells (Pc).

Fig 5c: T.E. micrograph of lymph follicles of rats treated with AFB_1 for one month then treated with BPF for one month (GII_2) showing presence of large number of plasma cells (Pc).

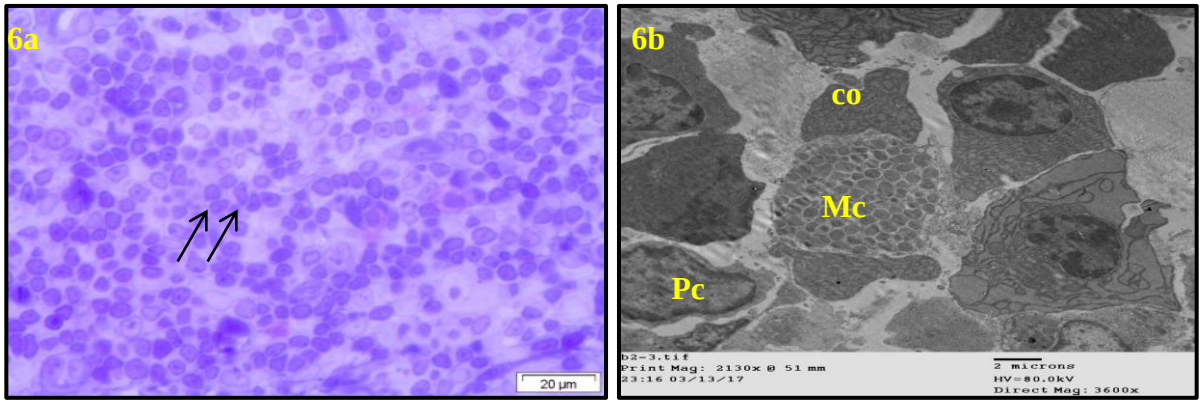

Fig 6a: Light micrograph of lymph follicles of rats treated with AFB_1 then treated with BHT (GII_3) for one month showing increase lymphoid cell population with presence of mast cells (arrow). T.B.stain.

Fig 6b: T.E. micrograph of lymphoid tissue of rats treated with AFB_1 then treated with BHT (GII_3) for one month showing presence of plasma cells (Pc), mast cell (Mc) and collagen bundle (co).

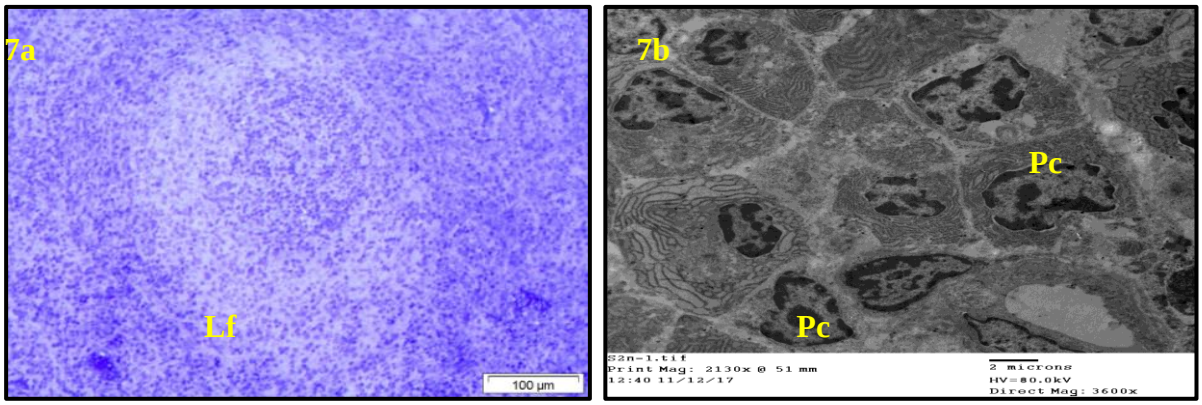

Fig 7a: Light micrograph of lymph follicles of rats treated with AFB_1 then treated with OPZ (GII_4) for one month showing lymph follicle (Lf) of normal morphological appearance. T.B.stain.

Fig 7b: T.E. micrograph of lymphoid tissue of rats treated with AFB_1 then treated with OPZ (GII_4) for one month showing presence of numerous plasma cells (Pc).

4- Discussion

ALT is commonly measured liver function test and used as a way of screening for liver damage. ALT activity was significantly increased in AFB_1 intoxicated rats (GII_0) in comparison to control one (GI) and still elevated in the self-recovery (GII_1), however, in treated rats with BPF (GII_2), BHT (GII_3) and OPZ (GII_4), the levels were elevated to those of normal (GI) as in Fig (1). These results agreed with who reported that these changes represent the initial protective response of the mitochondria against the aflatoxin toxicity and the increased activity of these enzymes provided evidence for their

release from damaged hepatocytes [37]. In the present study, the reduction of serum ALT activity after treatment with BPF, BHT and OPZ suggested healing of hepatic parenchymal cells and regeneration of hepatocytes [38-40]. In the current study, the authors found a significant increase in the levels of LPO as MDA, NO in aflatoxicosed rats and a significant decrease in the levels of total thiols, GSH and the activities of GPx and GST. AFs transformed *in vivo* into active metabolites which inhibits RNA and protein synthesis leading to change in cell cycles, impaired proliferation, differentiation of immune cells and promotes inflammatory responses by regulating cytokines expression [41, 42]. AFs affected cellular and humoral immune reactions and caused immunosuppression [43]. As well as AFs depresses the T-cell dependent functions of splenic lymphocytes and affects the lymphoid follicles result in depleting the lymphocytes [44]. In the present study, lymph follicles of intoxicated rats with AFB_1 showed marked depletion with prominent decreases of the lymphoid cells, moreover, lymph follicles of self-recovery rats showed lymphoblast in state of mitosis. All the previous changes by AFB_1 may return to elevation of LPO which is considered a key event in cellular damage [45]. Treatment of aflatoxicosed rats with BPF improved the oxidative stress parameters and the histological changes such as presence of large number of lymphocyte and lymphoblast's as well as plasma cells.

In this aspect, BPF stimulated the release of prostaglandin and cytokines that important in reconstituting the hematopoietic organs and increases immunoglobulin production [26, 46]. Moreover, the authors found increase in hepatic IL-1β gene expression (data not shown) which is known enhance the synthesis of prostaglandin E_2 via the induction of phospholipase A_2 and cyclo-oxygenase activities by potentiation the response to BK [47]. In the present study, treatment of aflatoxicosed rats with BHT improved the oxidative stress parameters and the histological changes in lymph follicles such as increase lymphoid cell population with presence of mast cells, plasma cells and collagen bundle could be return to the increased of GST activity which is important for the detoxification process [48,49]. Moreover, BHT as antioxidants transfer immunoprotective effects during aflatoxicosis that increase immune sensitivity and/or the activation of NK cells which related to the alleviation of lymphedema [50,51]. In the current study, treatment of aflatoxicosed rats with OPZ enriched the oxidative stress parameters

and the histological changes such as presence of lymphoid cells with numerous plasma cells and showed normal morphological appearance. In this aspect, Velayutham *et al*. [52] suggested that OPZ enhanced phase 2 enzyme induction via Nrf2 activation and inhibit CYP_1A_2 and CYP_3A_4, the principal enzymes involved in AFB_1 detoxification, especially GSTs. OPZ have anti-inflammatory effects that provoke the induction of phase II detoxification enzymes and enhancer of wound healing and fibroblast proliferation [53].

From the previous discussion we conclude that AFB_1 induced biochemical and pathological changes in lymphoid tissue and any of the BPF, BHT and OPZ have the capacity to ameliorate those biochemical and pathological changes with the priority for the BPF.

No conflict between the authors; the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

5- Reference:

1- M. Maresca and J. Fantini, Some food-associated mycotoxins as potential risk factors in humans predisposed to chronic intestinal inflammatory diseases, Toxicon, 56 (2010) 282.

2- M. Tola and B. Kebede, Occurrence, importance and control of mycotoxins, Cogent Food and Agriculture, 2 (2016) 1191103.

3- R. R. Dugyala and R. P. Sharma, The effect of AFB_1 on cytokine mRNA and corresponding protein levels in peritoneal macrophages and splenic lymphocytes, Int J Immunopharmacol, 18(10) (1996) 599.

4- J. H. Jiang, Y. N. Yin, L. Y. Yan and Z. H. Ma, Biological control of aflatoxin contamination of crops, Journal of Zhejiang University Science B, 9 (2008) 787.

5- X. Xu, B. Li, S. Zhu and R. Rong, Hypotensive peptides from snake venoms: structure, function and mechanism, Current Topics in Medicinal Chemistry, 15 (7) (2015) 658-69.

6- D. C. Yang, J. R. Deuis, D. Dashevsky, J. Dobson, T. N. Jackson, A. Brust, B. Xie, I. Koludarov, J. Debono, I. Hendrikx, W. C. Hodgson, P. Josh, A. Nouwens, G. J. Baillie, T. J. Bruxner, P. F. Alewood, K. K. Lim, N. Frank, I. Vetter and B. G. Fry, The snake with the scorpion's sting: novel three-finger toxin sodium channel activators from the venom of the long glanded blue coral snake

(*Calliophis bivirgatus*), Toxins (Basel), 8(10) (2016) 303.

7- M. W. Caldwell, R. L. Nydam, A. Palci and S. Apesteguı´a, The oldest known snakes from the middle Jurassic-lower cretaceous provide insights on snake evolution, Nat Commun, *6* (2015) *5996.*

8- M. M. Salman, Effect of a single dose of a BPF isolated from scorpion venom (*Buthus occitanus*) on total protein and albumin in serum of irradiated growing male Guinea pigs, Acad. J. biolog. Sci., 1(1) (2009) 33.

9- M. M. Salman, A. M. Kotb, M. A. Haridy and S. Hammad, Hepato- and nephroprotective effects of BPF from scorpion (*Buthus occitanus*) venom on mercuric chloride treated rats, Excli J, 15 (2016) 807.

10- A. Y. Nassar, G. Abu-Sinna and S. Abd El-Rahim, Effect of a BPF from venom of the Egyptian scorpion *Buthus occitanus*, on the ovaries and endometrium of mice, Toxicon, 28(5) (1990) 525.

11- M. M. Abdel-Raheim, A. Y. Nassar and H. Rochat, A BPP (peptide K_{12}) from the venom of Egyptian scorpion Buthus occitanus, Peptides, (16) 8 (1995) 1359.

12- G. Abu-Sinna, Y. Kafafy, A.Y. Nassar and A. Salman, Synergistic effect of bone marrow transplantation and BPF isolated from venom on thymus and spleen of sublethally irradiated guinea pig, Egypt J Radiat Sci. Appl., 18 (2005) 249.

13- H. Sarin, Pressuromodulation at the cell membrane as the basis for small molecule hormone and peptide regulation of cellular and nuclear function, J Transl Med., 13(2015) 372.

14- M. M. Salman, Antioxidant effects of BPF isolated from scorpion venom in liver injury induced by carbon tetrachloride (CCl_4) in male albino rats, Journal of clinical toxicology, 8(6) (2018) 399.

15- M. Ramos, M. K. Kwak, P. M. Dolan, K. Itoh, M. Yamamoto, P. Talalay and T. W. Kensler, Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice, Proc Natl Acad Sci U.S.A, 98(6) (2001) 3410.

16- I. J. Cho, S. H. Kim and S. G. Kim, Inhibition of TGF beta1- mediated π-induction by OPZ through selective interruption of smad activation, Cytokine, 35 (2006) 284.

17- P. J. Dwyer, M. Clayton, T. Halbherr, C. B. Myers and K. S. *Yao,* Cellular kinetics of induction by OPZ and its keto derivative of detoxification enzymes in human colon

adenocarcinoma cells, Clin Cancer Res, 3 (1997) 783.

18- A. K. Lundebye, H. Hove, A. Måge, V. J. Bohne and K. Hamre, Levels of synthetic antioxidants (ethoxyquin, BHT and BHA) in fish feed and commercially farmed fish, J Food Additives and Contaminants: Part A, 27(12) (2010) 1652.

19- L. A. Cohen, M. Polansky, K. Furuya, M. Reddy, B. Berke and, J. H. Weisburger, Inhibition of chemically induced mammary carcinogenesis in rats by short-term exposure to BHT: interrelationships among BHT concentration, carcinogen dose and diet, J. Nati. Cancer Inst., 72 (1984) 165.

20- B. Jaouad and B. Torsten, Exogenous antioxidants Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses, Oxid Med Cell Longev., 3(4) (2010) 228–37.

21- Booth, Methods in Microbiology, vol(4), chapter I; Introduction to general methods (1971) 1-47 and chapter II ; Fungal culture media 49-94.

22- I. A. El-Kady and M. H. Moubasher, Toxigenicity and toxins of Stachybotrys isolates from wheat straw samples in Egypt, Experimental Mycology, 6(1) (1982) 25.

23- S. H. Ferreira, BPF present in the venom of *bothrops jararaca*, Br J Pharmacol Chemother, 24(1) (1965) 163.

24- M. M. S. Ragab, Kh. A. Sary, H. E. Tarek, B. Gamal, H. M. Mohamed and M. O. Hossam, Effect of a high fat, high sucrose diet on the promotion of non-alcoholic fatty liver disease in male rats: the ameliorative role of three natural compounds. Lipids in Health and Disease. 14 (2015) 83.

25- S. Raisuddin, K. P. Singh, S. I. Zaidi, B. N. Paul and P. K. Ray, Immunosuppressive effects of AFs in growing rats, Mycopathologia, 124(3) (1993) 189.

26- M. H. Omar and M. A. Meki, A bradykinin potentiating fraction isolated from the venom of Egyptian scorpion *Buthus occitanus* induced prostaglandin biosynthesis in Female guinea pigs, Comp. Biochem. Physiol, 3(1997) 183.

27- G. Hocman, Chemoprevention of cancer: phenolic antioxidants (BHT, BHA), Int J Biochem, 20(7) (1988) 639.

28- N. V. Dimitrov, J. L. Bennett, J. McMillan, M. Perloff, C. M. Leece and W. Malone, Clinical pharmacology studies of OPZ, A potential chemopreventive agent, Investigational New Drugs, 10(4) (1992) 289.

29- O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, Protein measurement with the folin phenol reagent, J.Biol.Chem, 193(1) (1951) 265.

30- A. H. Ding, C. F. Nathan and D. J. Stuehr, Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages: comparison of activating cytokines and evidence for independent production, J. Immunol, 141(1988) 2407.

31- E. D. Wills, Lipid peroxide formation in microsomes; general considerations, Biochem J., 113(2) (1969) 315.

32- G. L. Ellman, Tissue sulfhydryl groups, Arch. Biochem. Biophys, 82(1) (1959) 70.

33- E. Beutler, O. Duron and B. M. Kelly, Improvement method for the determination of blood glutathione, J. Lab. Clin. Med, 61 (1963) 882-888.

34- W. H. Habig, M. J. Pabst and W. B. *Jakoby*, GSTs, the first enzymatic step in mercapturic acid formation, J Biol Chem, 249(22) (1974) 7130.

35- J. J. Bozzola and L. D. Russell, Electron microscopy principles and techniques for biologists: Jones and Bartlitt publishers 20 park plasa Boston Ma o (1991) 2116.

36- A. Y. Nassar, S. A. Mahgoub, H. M. Omar, S. M. Bakkar and A. A. Osman, Comparative ameliorative actions of extracted bradykinin potentiating fraction from cobra snake venom and synthetic antioxidants on hepatic tissue of aflatoxicosed rats (submitted and under revision in the journal of applied animal research (JAAR-2020-0109)).

37- O. A. Rotimi, S. O. Rotimi, J. M. Goodrich, I. B. Adelani, E. Agbonihale and G. Talabi, Time-Course Effects of Acute AFB_1 Exposure on Hepatic Mitochondrial Lipids and Oxidative Stress in Rats, Front. Pharmacol. (2019) 467.

38- O. Ashry, M. Moustafa, A. A. Baset, G. E. Abu Sinna and H. Farouk, Outcome of venom bradykinin potentiating factor on rennin-angiotensin system in irradiated mice. *Int. J. Radiat. Biol. 88 (*2012) *840*.

39- B. Dassarma, D. K. Nandi, S. Gangopadhyay and S. Samanta Hepatoprotective effect of food preservatives (BHA, BHT) on carbon tetrachloride induced hepatotoxicity in rat. *Toxicol. Rep. 5* (2018) *31*.

40- A. Giudice, A. Barbieri, S. Bimonte, M. Cascella, A. Cuomo, A. Crispo, G. D'Arena, M. Galdiero, M. E. DellaPepa, G. Botti, M. Caraglia, M. Capunzo, C. Arra and M.

Montella, Dissecting the prevention of estrogen-dependent breast carcinogenesis through Nrf2-dependent and independent mechanisms. Onco Targets Ther. 12 (2019) 4937.

41- M. H. Dennis, J. M. Michael, A. R. Richard, F. C. Sabine, E. S. Rene, S. Joseph, W. Alan, and W. C. Ming, Immunotoxicity of AFB_1 in rats, effects on lymphocytes and the inflammatory response in a chronic intermittent dosing study, Toxicological Sciences, 73 (2003) 362.

42- L. Miao, Z. Yi, L. Peng, Y. Shu-Hua, Z. Wen-Kui, H. Jian-Xin, W. Yuan and H. Jian-Bin, Intervention of grape seed proanthocyanidin extract on the subchronic immune injury in mice induced by AFB_1, Int J Mol Sci., 17(4) (2016) 516.

43- R. A. Coulombe and J.r, Nonhepatic disposition and effects of AFB_1, The Toxicology of Aflatoxins, Human Health, Veterinary and Agricultural significance (1994) 89.

44- P. K. Sahoo, S. K. Chattopadhyay and A. Sikdar, Immunosuppressive effects of induced aflatoxicosis in rabbits, Journal of Applied Animal Research, 9 (1996) 17.

45- N. G. E. Neal, H. M. Godoy, D. J. Judah and W. H. Butler, Some effects of acute and chronic dosing with AFB_1 on rat liver, Cancer research 36 (1976) 1771.

46- Y. Őztürk, Kinin receptors and their antagonists as novel therapeutic agents, Curr. Pharm., 7(2) (2001) 135.

47- M. Murakami, H. Kuwata, Y. Amakasu, S. Shimbara, Y. Nakatani, G. Atsumi and I. Kudo, Prostaglandin E_2 amplifies cytosolic phospholipase A_2- and cyclooxygenase-2-dependent delayed prostaglandin E_2 generation in mouse osteoblastic cells. Enhancement by secretory phospholipase A_2, J Biol Chem, 272(32) (1997) 19891.

48- N. Y. Marcus, K. Blomenkamp, M. Ahmad and J. H. Teckman, Oxidative stress contributes to liver damage in a murine model of alpha-1-antitrypsin deficiency, Exp. Biol. Med., 237(10) (2012) 1163.

49- S. W. Sohn, J. W. Jung, S. Y. Lee, H. R. Kang, H. W. Park, K. U. Min and S. H. Cho, Expression pattern of GSTP1 and GSTA1 in the pathogenesis of asthma, Exp. Lung Res, 39 (4-5) (2013) 173.

50- D. Nisha and S. Peter, Targeting reactive oxygen species in development and progression of pancreatic cancer, Expert Rev Anticancer Ther, 17(1) (2017) 19.

51- D. Barsha, K. N. Dilip, G. Somnath, S. Saptadip, Hepatoprotective effect of food preservatives (butylated hydroxyanisole, butylated hydroxytoluene) on carbon tetrachloride-induced hepatotoxicity in rat, Toxicology Reports (2018) 9.

52- M. Velayutham, F. A. Villamena, J. C. Fishbein and J. L. Zweier, Cancer chemopreventive OPZ generates superoxide anion radical, Archives of Biochemistry and Biophysics 435(1) (2005) 83.

53- N. Ibrahim, S. K. Wong, I. N. Mohamed, N. Mohamed, K. Y. Chin, S. Ima and A. N. Shuid, Wound Healing Properties of Selected Natural Products, Int J Environ Res Public Health, 15(11) (2018) 2360.