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Impact of 90-Day Oral Dosing with Naturally Occurring Aflatoxin Mixture on Male Sprague-Dawley Rat Neurochemistry and Behavioral Pattern

[1]Sayed M. Rawi and [2]Abeer M. Waggas

[1]Department of Biology Faculty of Science and Arts, Khulais, King Abdulaziz University, KSA
[2]Department of Biology (Zoology), Science Faculty for Girls, King Abdulaziz University, Jeddah, KSA

Abstract: Our studies have identified 10µg AFB1 and 15.24µg AFG1/Kg in the collected food samples. Chronic daily exposure of rats to the recorded doses for 90 days is associated with significant elevation of brain and spinal cord glucose and total lipids and a significant decrease in the concentration of total protein content and AchE activity. The general pattern of most of the studied amino acids was an increase except for glutamine and glutamate. Moreover, the brain, renal and hepatic histopathological examination revealed inflammatory cells, necrobiotic, focal haemorrhagic areas and cellular degeneration.

Key words: Aflatoxins · Brain · Liver · Kidney · Histological · Biochemicals

INTRODUCTION

Mycotoxins are secondary fungal metabolites that can be produced in crops and other food commodities before and post harvest [1]. Environmental conditions especially high humidity and temperatures favor fungal proliferation resulting in contamination of food and feed. Aflatoxins (AFB1, AFB2, AFG1 and AFG2) are the famous group of mycotoxins [2] and aflatoxin B1 which is produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus* is the most famous and toxic form of all naturally occurring alfatoxin, as reported by several investigators, it is very reactive, unstable in tissues and metabolized into AF-M1, AF-P1, AF-Q, AF-B2 and aflatoxicol [3,4] by the cytochrome-P450 enzyme system found in the microsome, particularly in liver tissues and the degree of metabolism is differ according to sex, species and differences in the mitochondrial enzymatic reaction. According to Massey *et al*. [5] and Guengerich *et al.* [6]. AFB1-epoxide can bind to cellular macromolecules like DNA, RNA and protein causing chemical lesions which can lead to manifestation of biological effects and acute or chronic diseases in both animals and human [7]. The symptoms of mycotoxicosis depend on the concentration and the length of exposure as well as age, health and sex of exposed individual [8]. Many reports stated the harmful effects of aflatoxin on wide variety of animals and humans such as depression of growth and production, immune suppression, liver disorders, abnormalities of enzyme picture, mutagenicity and teratogenicity [3,4,9-13].

Till now Little is known about the toxicological effects of aflatoxin-mixture on the central nervous system of human and animals. Some studies indicated that aflatoxin B1 altered the levels of various biogenic amines and their precursors in rat and mouse brains [14,15], peripheral and central nervous system neuronal ATPases [16], reduces whole brain tryptophan hydroxylase [17] and alters brain AchE activity [18]. Also, according to Rawi and Shebl [19]aflatoxin administration increased brain transaminases activity, total lipid and glucose content and decreased brain total protein and albumin contents. In addition, Fahim *et al*. [20] found that chronic administration of 2 different doses of a mixture of aflatoxins B1 and G1 caused significant alterations in the total content of catecholamines and GABA of different brain areas of albino rats. It is clear that data recorded for aflatoxin mixtureon central nervous system, especially on the amino acids neurotransmitter are rare which gives a good indication for planning of the present study.

MATERIALS AND METHODS

Animals: Male rats (*Rattus rattus*)weighing 90-110 g were obtained from the breeding unit of king Fahad Research Center (Jeddah). The animals were housed in plastic cages with metal cover. Each cage contained six rats. Animals were maintained at atmospheric temperature (25± 3°C) as

Corresponding Author: Sayed M Rawi, Department of Biology, Faculty of Science and Arts, Khulais, King Abdulaziz University, KSA.

well as normal day light and dark. All animals were fed on standard diet and water *ad libitum*. The rats were kept under observation for 2 weeks to exclude any inter current infection prior to the initiation of the experiment. All animal's procedures are in accordance with the general guidelines of animal care and recommendations of the Canadian Committee for Care and use of animals [21].

Aflatoxin Production and Assays: *Aspergillus flavus* NRRL (3145) was obtained from the National Research Center (Dokki, Giza). Standards of Aflatoxins (B1, B2, G1 and G2) were purchased from Sigma Chemical Company, USA. The aflatoxin was produced from *Aspergillus flavus* using the method proposed by Koehler *et al*. [22]. The fungal Culture was grown on potato-dextrose agar medium (E-Merck, Germany) and were incubated at 28 °C for 18 days. Spores of *Aspergillus flavus* culture were scrapped loose with a loop after adding sterile distilled water and then used to inoculate 50 ml of yeast extract-sucrose medium (YES medium, 2% yeast extract and 20% sucrose)(E-Merck, Germany).The culture was incubated at 30 °C for 8 days. Afterwards, the culture was filtered through Whatman no.1 filter paper. Aflatoxins were extracted from culture filtrate by adding 50 ml of chloroform and shaking for 15 minutes in gyro rotatory shaker. The chloroformic extract was then concentrated by rotatory evaporator (Eyela N-1000, Japan) near to dryness. The residue of the sample was resolved in 10 ml of 40% methanol (HPLC grade) and diluted in 60ml of phosphate-buffered saline (pH 7.8). Qualitative and quantitative assay for aflatoxins has been carried out using HPLC (Agilent Technologies, Waldbronn, Germany) as recommended by AOAC [23]. After the determination of appropriate concentration of AFB1 and AFG1 in the YES medium, the medium was then taken as stock solution. Saline solution was used to dilute known amount of YES medium to obtain the selective dose in suitable volume (10µg AFB1 + 15.24µg AFG1 / Kg body weight).

Experimental Design: After 2 weeks of acclimatization to the laboratory environment, the selected animals were divided into 2 groups as follow: CONTROL GROUP, animals were daily injected intraperitoneally (ip) with pure non-toxic YES medium diluted with saline for 10 weeks. AFLATOXIN TREATED GROUP, animals were daily injected i.p. with the tested dose of aflatoxin (10µg AFB1 + 15.24 µg AFG1 / Kg body weight) for 10 weeks. Body weight of all animals was recorded initially and subsequently weekly during treatment. Also, all animals were observed during experiment to record any observations.

Blood and Tissue Sampling: Animals were killed by decapitation after being fasted for 6 hours. Six rats from each group were killed at time intervals 1, 5 and 10 weeks. Blood samples were collected in heparinized centrifuge tubes, chilled and centrifuged at 3000 r.p.m for 20 minutes. Plasma was then collected and kept frozen at-8°C until analysis. Brain and spinal cord were rapidly removed and samples of detectable and recorded weight from each tissue were taken and homogenized in suitable amount of cold sucrose solution for determination of glucose, total lipids, total protein, transaminases (AST and ALT) and AchE activities. Another brain samples were homogenized in 75% HPLC methanol and served for determination of amino acids. Selected samples of brain tissue were taken and fixed in buffer formalin for histological examination.

Biochemical Analysis: Biochemical analysis was carried out using reagent kits purchased from Bio Merieux-France. Glucose was determined in plasma, brain and spinal cord homogenates according to the method of Trinder [24]. Total lipid was determined as described by Zollnerand Kirsch [25]. Total protein determination was carried out according to Gornall *et al*. [26]. AST and ALT activities were determined according to the method of Reitman and Frankel [27]. AchE activity was determined by a modified method of Ellman *et al.* [28] as described by Gorun *et al*. [29]. The concentration of amino acids in the brain was determined by HPLC method according to Marquez *et al*. [30].

Histological Examination: Selected pieces of brain cerebellum were obtained and fixed in 10% neutral buffered formalin solution, then washed in tap water and dehydrated by different grades of alcohol and cleared by xylene then embedded in paraffin and sectioned. The sections were stained with hematoxylin and eosin [31].

Statistical Analysis: The data were presented as mean ± standard deviation (SD). Significance of results was evaluated by using Student's "*t*" test between control and treated group [32]. *P* value of less than 0.05 was considered statistically significant.

RESULTS

General Toxic Observation: Animals treated with the tested dose of AF displayed noticeable behavioural changes during duration of the experiment. General weakness, loss of appetite, diarrhea, sometimes sedation, sometimes excitation, erection of body hair and presence of water under abdominal skin were observed.

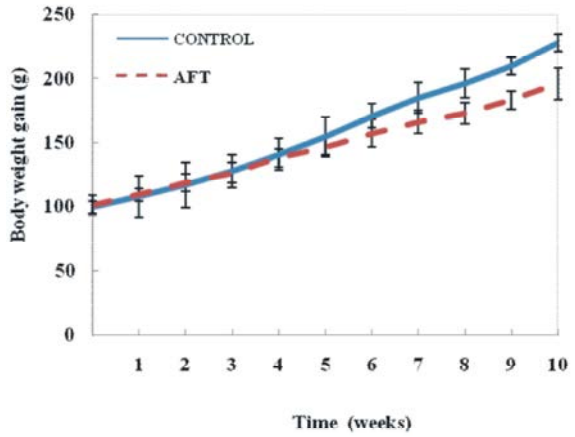

Fig. 1: Effect of intraperitonial injection of aflatoxin tested dose (10 µg AFB1+ 15.24 µg AFG1/Kg body weight) on body weight gain of male albino rat (*Rattus rattus*).

Effect on Mortality Rate: The administration of the tested dose level of AF led to death of some treated rats. It was observed that, the mortality rate was increased as the duration time was increased. At the end of the tested period the tested dose level has led 36.66% mortality rate.

Effect on Body Weight Gain: Data represented in Figure (1) showed that aflatoxin administration at the dose ((10µg AFB1 + 15.24 µg AFG1/ Kg body weight) induced reduction in the body weight gain of treated animals which were statistically significant at the latest weeks of experiment as compared to control group. In addition, some animals displayed noticeable behavioral changes during duration of experiment such as general weakness, loss of appetite, diarrhea, sometimes excitation and sedation, erection of body hair and presence of water under abdominal skin.

Histopathological Observations: Microscopic examination of brain sections of AF-treated animals showed more or less normal cerebellum with mild to moderate changes in the cerebral cortex represented as small areas of focal gliosis, some congested cerebral blood vessels, few neuronal degeneration and neurophagia. The degenerated neurons appeared as small dark basophilic or without pyknotic nuclei. As the experimental period was extended to 90 days, there was an increased neuronal degeneration, multiple areas of focal gliosis and neurophagia. In some cases areas of haemorrhage were observed in the cerebral cortex and the cerebral meninges seemed to be thicker

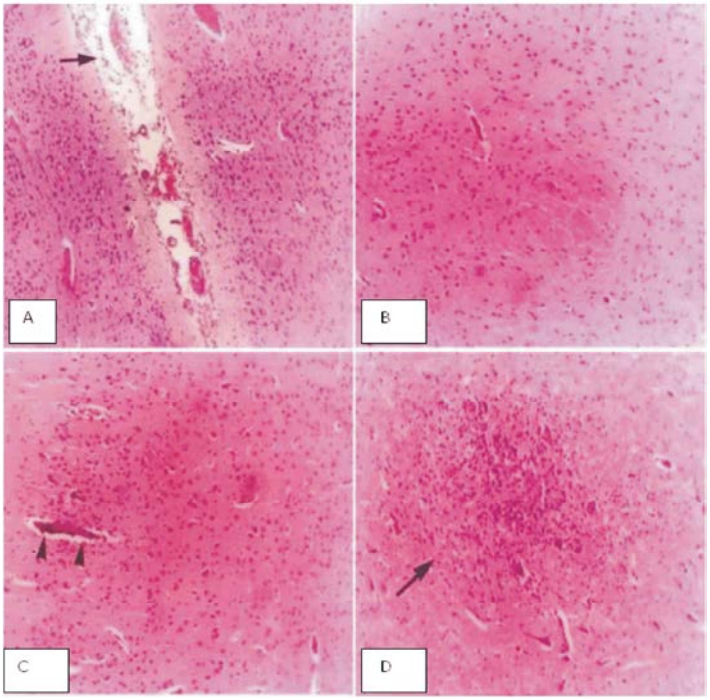

Plate 1: Brain section of control male albino rat (A) showing normal histological structure of cerebral cortex (H and E X100), (B) one week aflatoxin tested doseshowing ventricle with congested blood vessels and extravasated blood vessels (arrow), 5 weeks treated rats (C) showing perivascular cuffing of the cerebral blood vessels (arrow heads) and 10 weeks treated rats (D) showing large focus of glia cells surrounding by degenerated neurons (arrow) (H and E X 100).

than normal due to the presence of congested blood vessels with few numbers of inflammatory cells and extravasated RBCs. the ventricles of cerebrum also showed vascular congestion with local haemorrhagic areas. The examined sections also revealed marked neuronal degeneration especially of pyramid cells and large focus of glia cells was noticed. This focus mostly considered of reacted glia cells and surrounded by degenerated neurons (Plate 1).

Biochemical Parameters: Table (1) represents the effect of the tested dose of AF (10µg AFB1 + 15.24 µg AFG1 / Kg body weight) on some biochemical parameters in brain, spinal cord and plasma of male albino rats.

Glucose: AS comparing with the control value, It is evident from the results that AFB1 administration induced a remarkable significant elevation in brain and spinal cord after 10^{th} weeks of treatment.

Total Lipid: As regards to total lipid, the data recorded showed significant increase in spinal cord and plasma total lipid contents. Brain total lipid content was affected vigorously by aflatoxin tested dose after 5^{th} weeks. Significant elevation in its total lipid content was recorded throughout the study.

Total Protein: Results in Table (1) showed significant decrease in total protein content of brain and spinal cord after 5^{th} and 10^{th} week of treatment. In plasma, significant decrease was observed at the end of experiment.

Enzymes Activities: Data recorded in Table (2) showed the effect of the tested dose of aflatoxin (10µg AFB1 + 15.24 µg AFG1 / Kg body weight) on AST, ALT and AchE activities in brain, spinal cord and plasma of male albino rats.

Transaminases (AST and ALT): Marked elevation in AST and ALT activities in the tested tissues was observed throughout all the tested periods. This elevation was significant at the end of experiment.

Acetyl Cholinesterase (AchE): Comparing to control group, significant decrease in AchE activity was recorded in brain and spinal cord of treated rats post 5^{th} and 10^{th} week of treatment, while insignificant change was observed in plasma throughout the experiment.

Free Amino Acids: Data recorded in Table (3) revealed marked elevation in most of the studied brain amino acids levels. This elevation was significant post 5^{th} and 10^{th} week of treatment for aspartate, glycine and alanine and post 10 weeks for taurine, GABA, tryptophane and tyrosine. On other hand, brain glutamate and glutamine concentrations decreased significantly after 5^{th} and 10^{th} week of experiment.

Table 1: Effect of aflatoxin mixture (10µg AFBI and 15.24µ AFG1/ Kg body weight)on some biochemical in brain, spinal cord and plasma of male albino rat (*Rattusrattus*)

			AFT (n=6)		
			Time of decapitation (weeks)		
Tissue	Biochemical Parameters	Control (n=18)	1	5	10
Brain	Glucose(mg/g tissue)	12.94±6.76	13.55±4.26	16.09±2.97	48.51±22.16*
	Total Lipid (mg/g tissue)	63.64±18.98	107.21±34.15*	97.77±22.32*	80.08±12.13
	Total Protein (mg/g tissue)	309.5±71.9	301.4±27.2	228.0±44.1*	233.2±27.5*
Spinal Cord	Glucose (mg/g tissue)	41.29±16.88	43.34±14.51	45.20±18.89	166.75±38.27*
	Total Lipid (mg/g tissue)	53.41±18.77	72.17±8.68*	102.96±28.44*	137.11±23.18*
	Total Protein (mg/g tissue)	642.5±92.5	631.0±89.7	511.3±81.3*	502.4±81.01*
Plasma	Glucose (mg/ml)	2.42±0.80	2.55±0.16	2.29±0.19	2.63±0.48
	Total Lipid (mg/ml)	2.80±0.81	0.99±0.11*	1.82±0.11*	0.75±0.11*
	Total Protein (mg/ml)	69.3±12.5	65.8±11.7	57.7±17.3	55.7±11.6*

Number of animals (n) presented between parentheses.

Data are expressed as mean ± SD.

* Significant difference comparing to Control group at $P< 0.05$.

Table 2: Effect of aflatoxin mixture (10µg AFBI and 15.24µ AFG1/ Kg body weight)on the activity of some enzymes in brain, spinal cord and plasma of male albino rat (*Rattus rattus*)

			AFT (n=6)		
			Time of decapitation (weeks)		
Tissue	Enzyme	Control (n=18)	1	5	10
Brain	AST (mMole/g tissue)	0.155±0.033	0.168±0.013	0.174±0.021	0.207±0.053*
	ALT (mMole/g tissue)	0.042±0.009	0.045±0.002	0.052±0.008	0.056±0.003*
	AchE (µMSH/min/g tissue)	9.132±0.202	9.048±0.148	8.031±0.194*	7.657±0.248*
Spinal cord	AST (mMole/g tissue)	0.339±0.091	0.321±0.072	0.442±0.081*	0.442±0.041*
	ALT (mMole/g tissue)	0.109±0.081	0.121±0.004	0.133±0.019	0.138±0.015*
	AchE (µMSH/min/g tissue)	12.159±0.358	12.001±0.204	10.333±0.219*	10.136±0.116*
Plasma	AST (mMole/ml)	1.563±0.411	1.738±0.452	1.805±0.305	2.035±0.421*
	ALT (mMole/ml)	0.572±0.090	0.596±0.063	0.615±0.062	0.953±0.071*
	AchE (µMSH/min/ml)	0.638±0.036	0.734±0.037	0.615±0.012	0.601±0.071

Number of animals (n) presented between parentheses. Data are expressed as mean ± SD.

* Significant difference comparing to Control group at $P< 0.05$.

Table 3: Effect of aflatoxin mixture (10µg AFBI and 15.24µ AFG1/ Kg bodyweight) on some free brain amino acids (µ Mole/g tissue) of male albino rats (*Rattus rattus*)

		AFT (n=6)		
		Time of decapitation (weeks)		
Amino acid	Control (n=18)	1	5	10
Aspartate	1.83±0.45	2.41±0.044	3.97±0.81*	
3.12±0.90*Glutamate	11.38±0.48	13.88±0.81	8.74±0.93*	
6.92±0.50*Glutamine	3.95±0.47	4.30±0.87	2.65±0.70*	2.45±0.42*Taurine
	7.17±0.58	0.102±0.016	5.74±0.84	11.51±0.85*GABA
	2.37±0.37	2.91±0.43	3.37±0.76	4.75±0.83*Glycine
	0.87±0.12	1.10±0.26	1.54±0.26*	1.43±0.13*
Alanine	1.07±0.02	1.18±0.15	1.49±0.22*	1.43±0.22*
Tryptophan	0.035±0.008	0.025±0.004	0.036± 0.004	0.053± 0.009*
Tyrosine	0.092 ± 0.013	0.114± 0.013	0.112 ± 0.022	0.138± 0.015*

Number of animals (n) presented between parentheses.

Data are expressed as mean ± SD.

* Significant difference comparing to Control group at $P< 0.05$.

DISCUSSION

The ability of aflatoxin producing fungi (*ASperigillus flavus* and *Asperigillus parasiticus*) to grow on wide range of food and feed stuffs under certain conditions constitutes a threat to both animals and human. In developing countries, where food availability has often to be considered before food safety, there is a lack of legislation of acceptable limits for aflatoxins and populations are undoubtedly exposed to high amounts of aflatoxin [33]. The present study investigated the effect of a mixture of aflatoxin (10µgAFB1 + 15.24µg AFG1/Kg body weight) on nervous system of male albino rat.

Our results indicated that, the tested dose of aflatoxin caused reduction in body weight gain of treated rats mainly at the end of experiment. Similar results were reported in rats treated with aflatoxins [13,34]. Basmacioglue *et al.* referred the depression in body weight gain through aflatoxicosis to the disruption of protein synthesis which was also recorded in our study [35]. Additionally, Abdel-Fattah *et al.* [13] suggested that the decrease in body weight gain to reduction in food and water intake of aflatoxin treated rats which were also observed in our study. On the other hand, the decrease in body weight gain in animals treated with aflatoxins may be due to the effects of these aflatoxins on the balance

between orexigenic and anorexigenic circuits that regulate the homeostatic loop of body weight regulation leading to cachexia [36]. In this regards, Abdel-Wahhab *et al*. reported that rats treated with AFB1 showed significant decrease in leptin [37]. Low leptin concentration is usually associated with the high levels of cortisol and IL-6 which together act to influence the feeding response, causing weight loss in patients with pancreatic cancer [38]. This correlation may explain the recorded decrease in body weight in animals treated with aflatoxins. Since leptin and its receptors are the key players in the regulation of energy balance and body weight control [37].

The present study was also extended to include the effect of aflatoxin mixture on the histological characteristics of brain tissue cells of male albino rat. The examined sections showed congestion in cerebral cortex blood vessels and presence of inflammatory cells with few numbers of extravasated RBCs. Also, focal hemorrhagic areas as well as neuronal degeneration and gliosis especially in the cells of cerebral cortex were recognized. Perivascular cuffing of cerebral blood vessels was also noticed. The histopathological findings which recorded herein as a result of aflatoxicosis are similar to those recorded by many authors [20,39]. All of the recorded histopathological changes arised mainly from direct or indirect interaction of AFB1 or its metabolites with some component of the cells such as nucleic acid, protein and key enzymes involved in regulatory pathways of cell cycle [40,41]. Also, aflatoxin induced lipid peroxidation along with reduction in enzymatic and non-enzymatic antioxidant in the cells [42,43]. All of these interactions disrupt cell vital activities and lead to water and ionic shifts which finally caused cell damage and death.

The continuous administration of aflatoxin tested dose induced marked elevation in glucose level of brain and spinal cord tissues of treated rats, mainly at the end of experiment, while plasma glucose level showed insignificant increase throughout all the tested periods. Many authors recorded alterations in glucose level as a result of aflatoxicosis [44,45]. As reported by Abd El-Galiland Rawi and Shebl, the recorded change can be explained on the effect of the tested dose of aflatoxin via glucose transport, energy production, glycogenesis and glycolgenolysis which depend on the activity of some enzymes such as NA^{+}, K^{+}, Mg^{+2}-ATP ase, cyclic AMP, glucokinase as well as the activity of epinephrine [19, 46]. All the previous parameters reported to be altered by aflatoxin treatment [20,47]. IKegwuonu postulated that, nerve tissue requirement to glucose molecules were reduced during aflatoxicosis [16]. So, reduction in glucose metabolism in addition to the acceleration of active transport of glucose may explain glucose hyper content of neuronal tissues. This disturbance in glucose metabolism can explain the fluctuation in plasma glucose level which recorded in the present study.

In view of total lipid content of treated animals, data recorded showed dramatic increase in spinal cord tissue of rats at almost all the tested periods. Brain total lipid content markedly elevated during the early periods of experiment. On the other hand, marked decrease in plasma total lipid level was observed. In this regard, data presented may emphasize a particular role of lipid anabolism in aflatoxicosis and concordant largely with the finding of several investigators [19,48]. These findings can be explained on the basis of aflatoxin effect on specific enzyme activity that affects alteration in total lipid and lipid fractionation [48]. Also, Horton *et al*. demonstrated loss of feed back control of cholesterol synthesis in aflatoxin treated animals [49]. In general, the marked elevation in tissue lipid depot which observed herein may be attributed to the direct effect of commutative doses of aflatoxin or aflatoxin metabolites on increasing the rate of lipid synthesis as well as to the inhibition of lipid transport from these tissues.

Concerning the effect on total protein content, data recorded revealed that the tested dose of aflatoxin induced depression in total protein content of the tested tissues. Our results in accordance with many investigators [50,51] who recorded deleterious effect of aflatoxin treatment in most animal species. The observed alterations might be due to changes in protein synthesis and / or metabolism and could be attributed to an under nutrition and due to decreased amino acids uptake and disturbances in amino acid levels which was observed in our study. In addition, the effect of aflatoxin and its metabolites are mediated through covalent binding to cellular protein and nucleic acids to form AFB-DNA adducts and thus cause disruption of synthesis of DNA and RNA and inhibiting protein synthesis [42,52].

In brain, spinal cord, liver as well as in other tissues, some enzymes have been reported to be associated with protein and amino acids metabolism. Transaminases are one of the most important of such enzymes. The recorded data for ALT and AST activity revealed marked elevation at most of the tested periods. The disturbance in the activities of both assayed transaminases during aflatoxicosis was proved by many authors [13,53]. Since the transamination in conjugation with the operation of tricarboxylic acid cycle represents the major mechanism for regulating the steady state concentration of aspartate

and glutamate [54] and the balance between these two systems and the extent of carbohydrate metabolism will be determining factors in the changes of glutamic acid concentration. So, the marked elevation in the neuronal transaminases (ALT and AST) activities which observed in the present study with the corresponding increase in brain alanine, aspartate and glucose which observed also herein, suppose an activation or at least disturbance in the transamination mechanism within the glutamate system. In addition, the elevation of transaminases in the plasma is mainly due to cell damage, especially hepatic cells and leakage of the enzymes into the blood [45].

In the present study, significant inhibition of AchE activity in both brain and spinal cord of treated rats was recorded. Our results are in accordance with [55] who reported that AFB1 non-competitively inhibited mouse brain AchE by blocking access of the substrate to the active site or by inducing a defective conformational change in the enzyme through non-covalent binding interacting with the AchE peripheral binding site or through both mechanisms. The prolonged release and accumulation of acetylcholine in response to the continued inhibition of AchE may result in nervous degeneration which observed in brain tissue.

Regarding to brain amino acids, data recorded showed that the tested dose of aflatoxin altered the levels of all the studied brain amino acids of treated rats. The general behavioral pattern of most amino acids in the brain was an increase at most of the tested periods. On the other hand, glutamine and glutamate brain levels showed marked decrease during the experiment. Our results are in agreement with many investigators,who reported significant alterations in amino acids levels and their metabolites during aflatoxicosis [14,17,20,56]. The rate of protein breakdown in different organs should be related to the rate of protein turnover. Presumably those organs that have high rate of turnover would have higher free amino acids when protein synthesis was blocked than those tissues where the half-life of the proteins is larger [57]. The same authors in 1971 cited that if protein synthesis blocked, protein breakdown should occur, free amino acids throughout the body would rise [58]. This investigation, when connected with the drop in protein synthesis which recorded by the depletion in protein content in neuronal tissues within the present work explained the increase in most of the studied brain amino acids. On the other hand, it has been found that glucose is converted to amino acids faster in brain than in other organ [59]. A number of non-essential amino acids, but chiefly aspartate, alanine, glutamate and glycine can be synthesized in the brain through reactions interconnecting with the tricarboxylic acid cycle and glycolysis. The transaminases involved in these reactions are AST and ALT [60]. That means, those agents which influence the levels of glycolytic intermediates as aflatoxin can cause immediate and sometimes drastic changes in the concentration of brain amino acids. Data recorded in the present study revealed a parallel increase in brain glucose level and both ALT and AST activities concomitant with increase in brain aspartate and alanine. That mean large pool of this amino acids in brain tissue, may be due to glucose conversion. This, when one connected glucose conversion with protein breakdown, as a result of protein synthesis blocking, good explanation for large pool of brain amino acids during the present aflatoxicosis can investigated.

In the present work, the decrease in glutamate family seems to be a shift from glutamate to aspartate and GABA. Furthermore, Guidotti *et al*. reported that taurine regulates glutamate level in CNS apparently by stimulating a conversion of glutamate to glutamine [61]. The increase in taurine often compensates the decrease in the members of glutamate family. This suggestion is confirmed by the data recorded in the present study, where a marked decline in glutamate family was recorded concomitant with significant elevation in brain taurine which may suggest that aflatoxin may act to increase glutamate family level through taurine metabolism. Many authors concluded that the major role of taurine and also hypotaurine at least in some tissues is to afford protection against oxidant and free radicals that can cause severe cellular [62,63]. It may react with those toxic agent via its amino group to detoxify them and it also exert a direct protective effect in preventing the ionic and water shifts that result in cellular damage and death. This marked elevation in taurine level which recorded in the brain of treated animals strongly confirms all the previous finding and might be regarded to its role as a direct or indirect antitoxic agent during aflatoxicosis.

Data recorded in the present study also revealed marked elevation in brain tryptophan and tyrosine levels in aflatoxin treated rats. Several studies have shown that central and systemic tryptophan and tyrosine levels and metabolism were altered during aflatoxicosis [14,15,17]. Since tryptophan is an essential amino acid, its brain level can only originate from brain proteolysis or circulating amino acids derived from dietary or tissue pool. However, tryptophan was reported to be the least abundant amino acid in the cellular pool available for protein synthesis

[64]. On the other hand, brain tyrosine was reported to be derived from the circulation, brain proteolysis or hydroxylation of phenylalanine [65]. Insulin appears to regulate in part, the large neutral amino acids (tryptophan, tyrosine and phenylalanine) fluxes into tissues [14]. On the other hand, disturbance in 5-HT level disturbs the blood-brain barrier [66]. In the present study, AFT treatment has been shown to inhibit protein synthesis in brain, as assayed by the decline in total protein content, altered insulin level as assayed by drastic changes in glucose level and also disturbed 5-HT level as assayed by the disturbance in brain tryptophan level. Thus, the increase in tryptophan and tyrosine levels which observed in the present study during aflatoxicosis may be occurred as a result of direct alteration in their metabolic pathways, changes in tissue uptake process or as a result of modifications in tissue proteolysis. On the other hand, the disturbance in tryptophan metabolism and its major metabolite, serotonin, eventually disturb the blood-brain barrier which might gave the way for the entrance of aflatoxin B1 or its metabolites into the brain resulting in biochemical and histological changes [67], as recorded in the present study.

In conclusion, this work indicated that, the upper limits of aflatoxin which allowed in some countries all over the world, in both food and feed still dangerous. Since the closely related selected tested dose induced the observed biochemical and histological changes. So, the reduction of aflatoxin limits strongly suggested by the present study to avoid the causes of certain idiopathic and debilitating diseases in humans and animals.

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