Effect of aflatoxin on malondialdehyde, glutathione levels, and stress index in *Toxoplasma gondii* infected mice

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Abstract

This study was conducted for the determination of the combined effect of aflatoxin and *Toxoplasma gondii* on malondialdehyde, glutathione levels, and stress index in sixty young inbred Swiss female albino mice BALB/C, which were randomly divided into six equal groups; Group 1(untreated control) animals were maintained without any treatment; group 2 were injected intraperitonealy with *T. gondii* tissue cysts; groups 3 and 4 were fed diets contaminated with 0.5 and 1 ppm aflatoxin respectively; group 5 and 6 were fed 0.5 and 1 ppm aflatoxin and injected with *T. gondii* tissue cysts. All animals were maintained for 40 days. One ml, containing 100 *Toxoplasma gondii* tissue cysts was obtained from brain tissue of naturally infected local breed rabbit was injected intraperitonealy. Aflatoxins (AFs) were prepared through inoculation of rice with *Aspergillus parasiticus* NRRL 2999 and were incorporated into the diet to provide the described level of 0.5 and 1 ppm. At the end of the experiment, blood samples were taken to determine Heterophils/lymphopcytes ratio (H/L), while brain was taken to determine glutathione and malondialdehyde concentration. Results showed that mice injected with *T. gondii* tissue cysts alone and those groups fed aflatoxin at both rates of 0.5 and 1 ppm were exhibited a significant (P<0.05) increase in H/L ratio, and malondialdehyde, while there is a significant (P<0.05) reduction on the level of glutathione. The results revealed that aflatoxin could exacerbate *T. gondii* infection and induce stress through suppression of glutathione and elevation of malondialdehyde concentration and H/L ratio.

تاثير سموم الافلا على مستويات المالونداايالديهايد والكلوتاثايون ومؤشر الكرب في الفئران المخمجة بطفيلي المقوسة الكوندية

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الخلاصة

صممت هذه التجربة لمعرفة التاثير التراكمي لسموم الافلا ومقوسات كوندي على مستوى المالونداايالديهايد والكلوتاثايون ونسبة H/L باستخدام ستون من اناث الفئران البيضاء السويسرية BALB/C بعمر 4- 6 أسابيع والتي وزعت عشوائيا الى ستة مجاميع متساوية. المجموعة الاولى (سيطرة غير مُعالجة)؛ المجموعة الثانية حُقِنت بأكياس نسجية لطفيلي *T.gondii*؛ المجموعات الثالثة والرابعة أعطيت عليقه ملوثة بـ 0.5 و 1 جزء بالمليون سموم الافلا على التوالي؛ المجموعة الخامسة والسادسة أعطيت عليقة ملوثة بـ 0.5 و 1 جزء بالمليون سموم الافلا وحَقِنتْ بأكياس البيض لطفيلي *T. gondii* ولمدة 40 يوما. تم حقن واحد مليلتر يَحتوي ١٠٠ كيس نسجي لطفيلي *T.gondii* عن طريق البريتون (تم الحصول عليها من أنسجة الدماغ لأرانب محلية مصابه بطفيلي المقوسة الكوندية) لوحده او مع سموم الافلا بتركيز (0.5-1) جزء بالمليون مع العليقة، والتي تم الحصول عليها بتلقيح الرز بالعفن (*A. parasiticus*, NRRL 2999). عند انتهاء التجربة أخذت عينات الدم لتحديد نسبة الخلايا المتغايرة إلى الخلايا اللمفية كما واخذ نسيج الدماغ لتحديد مستويات الكلوتاثايون والمالونداايالديهايد. لقد

أظهرت الفئران المحقونة بالاكياس النسجية للطفيلي لوحدها ومع المجاميع التي استهلكت سموم الافلا في كلا التركيزين 0.5 و 1 جزء بالمليون زيادة معنوية (P<0.05) في نسبة الخلايا المتغايرة إلى الخلايا اللمفية وحدث انخفاض معنوي (P<0.05) في مستوى الكلوتاثايون رافقه ارتفاع معنوي (P<0.05) في مستوى المالوندايالديهايد في نسيج الدماغ وكان التأثير المعنوي أكثر وضوحا في المجاميع المعاملة بسموم الافلا والطفيلي معا. يستنتج من الدراسة إن سموم الافلا تزيد من التأثير التفاقمي لطفيلي المقوسة الكوندية ولها القابلية على احداث الاجهاد وزيادة نسبة الخلايا المتغايرة / اللمفية وارتفاع المالوندايالديهايد وانخفاض الكلوتاثايون في الانسجة.

Introduction

Numerous toxigenic fungi and their metabolites have been identified from a variety of substrates. Most of these are produced by genera of *Aspergillus, Peniàllium* and *Fusarium* and are most often isolated from cereal grains or corn. These fungi are among the most common food contaminants in animal feed, causing great economic loss in animal mass production and aquaculture (1). Aflatoxins are secondary toxic fungal metabolites produced by *Aspergillus flavus* and *A. parasiticus* (2). Problems associated with mycotoxins tend to be worse in the tropics where high humidity and temperature create optimal conditions for fungal growth and contamination. Recently, Luyendy *et al.* (3) reported that aflatoxin B1 (AFB1) is a food contaminant fungal toxin that has been implicated as a causative agent in human acute hepatotoxicity, hepatic and extrahepatic carcinogenesis. Souza *et al,* (4) have reported significant rise in lipid peroxidation in the liver of rats 72 hours after a single intraperitoneal dose of AFB1. The role of reactive oxygen species ROS has been postulated in the development of aging, chronic degenerative diseases, inflammatory diseases and cancers (5). Oxidative damage is one type of damage caused by AFB1 in human lymphocytes (6). An elevated level of ROS was induced by AFB1 in rat hepatocytes (7), lipid peroxidation (8) and 8-hydroxydeoxyguanosine (8-OHdG) (9), formation was also observed in rat liver after AFB1 administration, (10). However, evidence in humans is rare and complicated by the coexistence of hepatitis virus, chemical and physical carcinogens and many other physiologic/pathologic conditions contributing to the generation of ROS (11). *Toxoplasma gondii* is a highly frequent obligate intracellular protozoan parasite. It is reported that about one-third of the world population is infected with *T. gondii*, and the disease has asymptomatic progress in 90% of the patients with sound immune systems (12). Toxoplasmosis can cause serious pathological changes including hepatitis, pneumonia, blindness, and severe neurological disorders. These types of diseases are seen particularly in people with weak immune systems (13). Yet, the pathogenic mechanisms in healthy people could not be explained completely. It is assumed that the malondialdehyde MDA arising from the lipid peroxidation is an indicator of the oxidative stress in tissue and cells. Glutathione GSH, an endogen-originated peptide which can be synthesized in the liver without need for genetic data, is made up of glutamic acid, cysteine and glycine amino acids, and is an important antioxidant. It defends the cells against oxidative damage by undergoing reaction with free radicals and peroxidase (14). Most of the basic antioxidants have been examined in protozoans and helminthes (15). The aim of the present study was to determine that could the ingestion of low and high levels of aflatoxin with or without *T. gondii* infected mice induce stress through the measurement of MDA and GSH brain tissue levels.

Materials and Methods

Animals and treatments

Sixty young inbreed Swiss female albino mice BALB/C weighting 20-25 g were obtained from animal house at the College of Veterinary Medicine, University of Mosul. All were negative for anti-toxoplasm antibodies in the latex agglutination test (Biokit. S. A; Spain). Animals were provided with feed and water of *ad-libitum* and maintained under laboratory conditions. Animal feed was contaminated with aflatoxin. These mice were randomly divided into six groups (10 mice per group) and caged separately. Group 1 (untreated control); Group 2 were injected with *T. gondii* tissue cysts; groups 3 and 4 were fed diet contaminated with 0.5 and 1 ppm aflatoxin respectively; groups 5 and 6 were fed diets contaminated with 0.5 and 1 ppm aflatoxin and injected with *T. gondii* tissue cysts respectively. The experiment extended for 40 days.

Toxoplasma gondii tissue cysts

T. gondii tissue cysts were obtained from brain tissue of naturally infected local breed rabbit. Brain tissue was ground, suspended in phosphate buffer saline (PBS), at pH 7.2, and then filtered through gauze. One ml (containing 100 tissue cysts) was injected in Swiss mice intraperitoneally as described by (16). Mice were daily inspected for any febrile sign.

Aflatoxins

Aflatoxins (AFs) were prepared through inoculation of rice with *A. parasiticus* NRRL 2999 (obtained from the College of Agriculture and Foresty, Mosul University, Mosul, Iraq) as described by (17) and modified by (18). Fermented rice was then autoclaved and ground. The aflatoxins content were measured by spectrophotometer

analysis (19; 20). Of the total AFs content in the rice powder, 81 percent was AFB1, 14 percent was AFG1, 4 percent was AFB2, and 1 percent was AFG2. The rice powder was incorporated into the diet to provide the described level of 0.5 and 1 ppm.

Heterophils/lymphopcytes ratio

Blood samples from tail, cardiac and saphenous were obtained. Approximately 0.7 to 1.0 ml of blood were used for heterophils and lymphopcytes count. Blood smears were performed and stained with gaimsa-stain (21).

Determination of Glutathion and Malondialdehyde

Determination of tissue glutathion was done as described by Moron method (22). Determination of tissue malondialdehyde was performed as described by Gilbert method (23)

Statistics

All data were expressed as means ± standard error of the mean. Analysis was done by using a two-ways analysis of variance (ANOVA), $P \leq 0.05$ was considered significant (24).

Results

Stress index: Heterophil/Lymphocyte ratio (H/L ratio)

From Table 1, it is obvious that group of mice injected with *T. gondii* tissue cysts (group 2), and those groups fed aflatoxins at both rates of 0.5 and 1 ppm (3, 4 groups), were exhibited a stress condition manifested by a significant (P<0.05) elevation in H/L ratio after 40 days of treatments compared with control group (group1). An additive dose dependant negative stress effects were clearly found when *T. gondii* injected groups fed diets contaminated with both levels of AF in groups 5 and 6, being significantly (P<0.05) different not only from those injected with *T. gondii* in group 2, but with those fed both doses of AF in groups 3and 4, and with control group (group1).

Glutathione level

T. gondii tissue cysts and aflatoxins had significant (P<0.05) reduction effects (when each was given alone) to groups (2, 3 and 4), on the antioxidant glutathione level at 40 days, compared with control group (group 1) (Table 2). Further significant (P<0.05) reduction in glutathione antioxidant parameter were also evident in groups 5 and 6, of mice fed two levels of aflatoxins and injected with *T. gondii.*

Malondialdehyde level

The injection of mice with *T. gondii* tissue cysts and feeding them aflatoxins at a rate of 1 ppm in groups groups 2 and 4, were responsible for significant (P<0.05) increase

Table 1: Effect of aflatoxins and *Toxoplasma gondii* tissue cysts on Heterophils / Lymphocytes ratio in mice after 40 days of treatments.

Treatments	Heterophils / Lymphocytes ratio
Control	0.216 ± 0.070 d
Injected tissue cysts (100 cysts/ml)	0.336 ± 0.058 bc
Aflatoxin 0.5 ppm	0.328 ± 0.097 bc
Aflatoxin 1 Ppm	0.347±0.072 bc
Injected tissue cysts (100 cysts/ml) + Aflatoxin 0.5 pm	0.465±0.065 b
Injected tissue cysts (100 cysts/ml) + Aflatoxin 1 ppm	0.522±0.031 a

Different letters in column differ significantly at level of P<0.05, Mean±SE for 10 mice.

Table 2: Effect of aflatoxins and *Toxoplasma gondii* tissue cysts on tissue glutathione concentration in mice after 40 days of treatment.

Treatments	Tissue glutathione concentration (micromole/L)
Control	4.63± 0.686 a
Injected tissue cysts (100 cysts/ml)	3.53± 0.542 b
Aflatoxin 0.5 ppm	3.37± 0.730 b
Aflatoxin 1 Ppm	3.30± 0.851 b
Injected tissue cysts (100 cysts/ml) + Aflatoxin 0.5 pm	2.46± 0.496 c
Injected tissue cysts (100 cysts/ml) + Aflatoxin 1 ppm	1.96± 0.812 d

Different letters in column differ significantly at level of P<0.05, Mean±SE for 10 mice.

Table 3: Effect of aflatoxins and *T. gondii* tissue cysts on malondialdehyde in mice after 40 days of treatments.

Treatments	Malondialdehyde (micromole/L)
Control	96± 2.828 a
Injected tissue cysts (100 cysts/ml)	112.9± 2.983 b
Aflatoxin 0.5 ppm	106.8± 2.149 b
Aflatoxin 1 Ppm	117.6± 2.836 b
Injected tissue cysts (100 cysts/ml) + Aflatoxin 0.5 pm	131.5± 1.702 c
Injected tissue cysts (100 cysts/ml) + Aflatoxin 1 ppm	178.2± 4.098 d

Different letters in column differ significantly at level of P<0.05, Mean±SE for 10 mice.

in the lipid peroxidation byproduct malondialdehyde level, compared with value in control group (group 1) (Table 3). Combined treatments of *T. gondii* tissue cysts injection and feeding aflatoxins at two levels were responsible for a significant ($P<0.05$) increase the lipid peroxidation by-product malondialdehyde in a dose dependant fasion compared with all remaining groups including control one.

Discussion

Farm animals cannot be completely excluded from aflatoxicosis due to contamination of food stuffs especially under conditions favored its production of warm, moist environments, like tropical and subtropical latitudes (25). Aflatoxin also is a common contaminant of foods, particularly in the staple diets of many developing countries and it is considered by the US Food and Drug Administration (FDA) to be an unavoidable contaminant of foods (26). *T. gondii* infection was reported here in Iraq in pregnant women (27) in ruminants and birds (28,29). Aflatoxin contamination of human foods (30), animal feeds (31) was also reported in Mosul province. So it is highly likely the concurrent occurrence of *T. gondii* infection and aflatoxicosis in humans and farm animals under practical conditions. In two experiments, conducted in mice to determine wether ingestion of low levels of aflatoxin, after *T. gongii* infection ((16), or before *T. gondii* infection (32). Activation of toxoplasmosis was reviled by the development of specific IgG to *T. gondii*, with severe encephalitis and a significant increase in the number of tissue cysts in brain tissue (32). To make a line connecting between the results of these two experiments and our findings, we should stress on the role of AF and *T. gongii* in inducing ROS, The ability of AF and its active metabolite AFB1-8, 9-epoxide on the formation of reactive oxygen species ROS was reported through the significant increase in lipid peroxide level of hepatic, renal and testis of treated mice (33) which are known to increase the permeability of the cell to calcium (34). In turn, calcium and prooxidant significantly reduced mitochondrial glutathione and NADPH, (substrate of the antioxidant enzymes glutathione peroxidase) and glutathione reductase, respectively, which favors the accumulation of (H_2O_2). The higher lipid peroxidation observed in the present investigation could be due to a lower antioxidant capacity of the cells, and oxidative stress occurs in a cell or tissue when the concentration of reactive oxygen species ROS generated exceeds the antioxidant capability of that cell (35). There is increase evidence concerning failure of placentation or placental dysfunction, a characteristic feature of toxoplasmosis, to the imbalance of free radicals, which will further affect placental development and function and may subsequently have an influence on both the fetus and its

dam or mother (36). The alteration of serum GSH in patients with toxoplasmosis could also caused by aflatoxicosis as was seen in mice brain specimens in our experiment. In response to acute or chronic toxoplasmosis, Glutathione peroxidase GP and superoxide dismutase SOD activity show an evident increase to counteract the increase of radical, peroxide, and so playing a major role in detoxifying lipid peroxide and hydrogen peroxide (H_2O_2) (37). Depression of these antioxidants by aflatoxin, the main determinants of the antioxidant defense mechanism of the cell, so it may exacerbate toxoplasmosis infection. The decreased level of non-enzymatic antioxidant, GSH, has been reported in ewes infected with toxoplasmosis (38). Malondialdehyde MDA is the most abundant lipid peroxides, as indicative of oxidative stress in cells and tissues (39). Therefore, measurements of MDA, is widely used as indicator of lipid peroxidation, and increased levels of lipid peroxidation products have been associated with a variety of chronic diseases in both human and model system (40). Aflatoxin here was olsa induce significant increase in MDA level and exacerbate the level induced by *Toxoplasma* tissue cysts treated mice. The combined effects of AF and *Toxoplasma* tissue cysts could increase the generation of free radicals which may exceeds the cellular defenses resulting in oxidative stress (41). It is important to note that the combined negative effect of AF and *T.gondii* on glutathione production may disrupt the damaging effect of glutathione on reactive oxygen species ROS during toxoplsmosis infection (42).The production of free radicals due to toxoplsamosis in this study is in agreement with (43). The other possible explanation in aflatoxin exacerbation toxoplasmosis could be attributed to the reduction of cytokines secretion from activated macrophage and monocytes, like TNF-γ, is responsible for the toxoplasmocidal effects of macrophages, and is necessary for the synthesis of NO, which can supress the proliferation of *Toxoplasma* (27). Unfortunately aflatoxin B1 stand on the opposite to the toxoplasmocidal effect of TNF-γ by lowering it and many other major cytokines produced by macrophages through ingestion of AF at a rate of 0.03;0.145 and 0.7 mg/kg body weight for 2 weeks to male CD-1 mice, (43).

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