The Effect of Adding Natural Detoxifying Such as Dried Blueberries or Wheat Bran or Nano-Charcoal to Biscuits on Rats Infected with Aflatoxin

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ABSTRACT

Consumption of aflatoxin contaminated food are dangerous source to human health because of their toxic, carcinogenic, hepatotoxic and mutagenic characteristics. Due to their harmful effects concerned with serious health problems like hepatotoxicity and carcinogenicity (Kamkar et al., 2013 and Wacoo et al., 2014). Aflatoxins are carcinogenic and toxic mycotoxins produced by Aspergillus spp. They are classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC) (Ostry et al., 2017). The contamination of food with mycotoxins is a significant problem for the adverse effects on animals, humans and crops that result in sickness and economic losses (Reddy et al., 2009). For this reason, a number of methods have been studied related with their effectiveness to control aflatoxin in food and feedstuffs contaminated with toxin (Di Stefano et al., 2014).

Detoxification effects on mycotoxin-containing food and feedstuffs included physical procedures by mycotoxin binders in human intervention (Jans et al. 2014) or chemical approaches (Jouany, 2007). A practical approach for the detoxification aflatoxins as adsorbents have the potential to bind aflatoxin and prevent their absorption from gastrointestinal (Kannewischer et al., 2006).

Absorbent compound approach to decrease the risk of mycotoxosis in farm animals and to reduce the carry-over of mycotoxins (especially aflatoxin) from contaminated feeds into food of animal origin (Hasheminya and Dehghannya, 2013). The mycotoxin-sequestering capacity of activated charcoal depends on the specific surface area which is different in lignin-based and in activated charcoal (Ramos et al., 1996).

Nano-charcoal is an excellent adsorbent, and it has been widely used in, e.g feed additive, food processing, nutrition, medicines and cosmetics (Kittinaovarat and Suthamnoi, 2009). The unique composition of nano-charcoal are reducing the toxic effects of aflatoxin contamination (Shabani et al., 2010). A novel nano-additives of charcoal is studied to reduce the size of the adsorbents and their quantity due to they may change the materials organoleptic and nutritional values of foodstuff (Madrigal-Santillan et al., 2010).

Whole grains, bran brown rice, provide important detoxification enhancing substances such as fibre binds to toxins and decreases transit time an excellent source of dietary fiber to assist with detoxification. Because of mycotoxin concentrations in surface tissues of (grains, sorting, cleaning, dehulling and debranning) reduce mycotoxin contamination of the flour. A large fraction of mycotoxins can be destroyed with damaged kernels, fine material and dust (Cheli et al., 2013). Rice bran is therapeutic for detoxification for different reasons such as liver function as they assist in reduce inflammation and the healthy processing of fats.

Berries have the diversity and high concentration of antioxidants that dependent on the species and cultivar considered. Preharvest practices, environmental conditions, maturity at harvest, postharvest storage and processing operations are urgent determinants of the phytochemical profiles (Jimenez-Garcia et al , 2012). Natural substances that can prevent aflatoxin toxicity would be helpful to human and animal health with minimal cost in foods and feed. Traditional medicinal plants were used by some authors for their antifungal, anti-aflatoxigenic and antioxidant activity (Kumar et al., 2007). Carotenoids (carotenes and xanthophylls) found in fruits eg lutein and lycopene, carotene, β-cryptoxanthin, neoxanthin, 5,6-epoxylutein cis- and zeaxanthin and trans-violaxanthin have also been recognized in berries fruits (Szajdek and Borowska, 2008 and Lashmanova et al., 2012).

Chemoprevention has been intended as good strategy to decrease losses because of aflatoxin contamination in feed. Natural antioxidants : vitamins E and C, selenium, carotenoids, L-carnitine and melatonin were added for preventing mycotoxins because their effect on provoke oxygen free radical formation and the ability of these compounds to act as superoxide an ion scavengers (Guarisco et al., 2007 and Pál et al., 2009).

This study was investigated the effect of addition of some natural additives to biscuit such as dried berries or wheat bran and nano-charcoal on toxicity in rats fed diets contaminated with aflatoxins.
MATERIALS AND METHODS

Materials:
Natural additives: Blueberries, wheat bran and charcoal were purchased from local market of Mansoura city -El-Dakahlia Governorate.
Raw material: Peanut, Flour, Corn, Sugar ,Salt, Egg ,Vanilla and Baking powder from local market in Mansoura city - El-Dakahlia Governorate.
Experimental:
Preparation of nano-activated charcoal:
Samples were grinded then powdered and sieved through spheres of stainless steel balls were used as grinding media as (Amir et al., 2010).
Characterization of nano-activated charcoal
Size, surface area, shape, crystal structure and morphological data of the found nanoparticles were characterized using transmission electron microscopy, TEM (JEOL TEM-2100) connected to a CCD camera at an accelerating voltage of 200 KV.
TEM measurements were recorded at the Central Laboratory, Electron Microscope Unit, Faculty of Agriculture, Mansoura University, Egypt.

Preparation of biscuits:
The ingredients for control biscuits were: wheat flour 270 g; corn 135g butter 100 g; powdered sugar 140 g; salt 0.5 g; vanilla 0.5 g; 1 egg and baking powder 1g.
In other samples certain amount of the main raw material (wheat flour) was diminished on account of dried blueberries or wheat bran or nano-charcoal to obtain enriched biscuits. Namely, wheat flour was replaced by 60 gram dried blueberries or 15 gram wheat bran or 1 gram nano-charcoal. Dough was prepared by mixing all the ingredients, rolled out and shaped as round biscuits. The biscuits were baked in oven for 12 minutes at 180̊ C. After cooling, the biscuits were packaged into plastic bags and then stored at room temperature.

Experimental animals:
The experimental thirty male albino adult rats were obtained from Nicer Nile Academy of the Research, Mansoura, Egypt.

Experimental design:
Body weight of rats ranged from 160 ± 10 gram. A number of 30 rats were divided into 5 groups each contained 6 rats, were kept under normal healthy conditions.

Rat in each group was housed individually into separate stainless steel box in a room at temperature of 26°C. Water was admitted freely to rats from glass bottles mounted.

Then, rats of G1 to G5 were fed as follows:
G1(G-ve): Rats fed on basical diet (Commercial pelleted diet ) (from Ain 93, Modern Vet. Lab., contained 21% crude protein, 3% crude fat, less than 5% crude fiber, and 3190 Kcal/Kg) (BD). Was used also for feeding all rats for 6 days as acclimatization period.
G2(G+ve): Rats fed on toxic diet (Positive control)( peanut natural contaminated with toxin at the level of 20 ppb total aflatoxin (ng/g diet))
G3: Rats fed on 50% toxic diet (peanut natural contaminated with aflatoxin) + 50% biscuit with dried blueberries
G4: Rats fed on 50% toxic diet (peanut natural contaminated with aflatoxin) + 50% biscuit with wheat bran
G5: Rats fed on 50% toxic diet (peanut natural contaminated with aflatoxin) + 50% biscuit with nano-charcoal
An amount of food equal to 100 gram was weighed and hired in the dish inside the box. This was allowed for utilization over the day.

Animals were weighted once a week and the weights of the animal were recorded to follow their growth. The animal experiment lasted after 8 weeks.

Biochemical Analysis:
After 8 weeks (at the end of experiment) , rats were fasted overnight , blood samples were taken from three rats of each group. Blood samples were taken immediately after the slaughter of rats, to study the influence of aflatoxin on some blood parameters.
White blood cells count (WBCs), red blood cell count (RBCs) and hemoglobin concentration (Hb) were measured according to Cynthia et al., (1993)

Included blood plasma concentrations of glucose, total protein (TP), albumin, triglycerides (AL) and total cholesterol (TC) as well as activity of transaminases [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] in blood plasma were determined Three blood samples per treatment were obtained during slaughtering and kept in heparinized test tubes. They were immediately centrifuged at 3000 r.p.m for 15 minutes to separate blood plasma, which was frozen at –20°C until later study.

Serum liver enzymes including aspart aminotransaminase (AST), alanine aminotransaminase (ALT) and alkaline phosphatase (ALP) were determined according to (Reitman and Frankel, 1957 and Bowers and McComb, 1966). Serum total protein(TP), albumin(AL), globulin (GL), urea and creatinine were estimated according to (Doumas et al., 1981); Weissman et al., 1950; Kaplan and Tang , 1982 and Bartles et al., 1972 , respectively).

Triglycerides(TG), Total Cholesterol(TC), HDL-c, LDL-c and Glucose were determined by (Tietz (1986); Fossati and Prencipe (1982); Lopez ,1977 and Teuscher and Richterich, 1971), respectively.

Histological examination:
Rats fed on treated food and their controls were slaughtered, quickly anatomized and their kidney, liver are removed, sliced and fixed in 10% formalin suspension.
After three days, tissues were cleansed three times in 70% ethanol, dehydrated using a graded ethanol series and then inserted in paraffin wax. Paraffin sections were cut into (5-6) micrometers thick slices, stained with

![Image of TEM photographs](https://example.com/tem_photos.png)
Statistical Analysis

Data were subjected to statistical analysis using, one way classification, least significant differences (LSD) were determined at P value of < 0.05 level according to SAS, (2006).

RESULTS AND DISCUSSION

Data of addition of dried blueberries or wheat bran or nano-charcoal to biscuit on hematological parameters: hemoglobin, red blood cells, hematocrit and white blood cells of intoxicated rats fed aflatoxins contaminated diets are shown in Table (1). Hemoglobin (Hb) content showed significant reduction in (G2+ve) compared with control group (G1-ve). But, Hb showed significant increase in other groups (p< 0.05).

Red blood cells (RBCs) count significantly decreased in G3 (6.31), G4 (5.97) and G5 (5.90) in comparing with (G1-ve). (6.52) (p<0.05). Also, positive group (G2+ve) showed significant decrease in RBCs in comparing with (G1-ve). Hematocrit (Hct) showed significant reduction in G3 (40.29), G4 (39.60) and G5 (39.57) compared with (G1-ve). (40.31).

Table 1. Effect of biscuits with dried blueberries or wheat bran or nano- charcoal as detoxifying agents on hematological of intoxicated rats with aflatoxin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (mg/dl)</td>
<td>13.77a</td>
<td>12.47a</td>
<td>13.32b</td>
<td>13.07c</td>
<td>13.01d</td>
<td>0.031</td>
</tr>
<tr>
<td>RBCs (x 10^6/mm^3)</td>
<td>6.52a</td>
<td>5.85c</td>
<td>6.31b</td>
<td>5.97c</td>
<td>5.90c</td>
<td>0.038</td>
</tr>
<tr>
<td>WBCs (x 10^3/mm^3)</td>
<td>8.58b</td>
<td>8.95a</td>
<td>8.43d</td>
<td>8.29d</td>
<td>8.24d</td>
<td>0.023</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>40.31</td>
<td>41.13</td>
<td>40.29</td>
<td>39.60</td>
<td>39.57</td>
<td>1.167</td>
</tr>
<tr>
<td>MCV (µ3)</td>
<td>70.85</td>
<td>72.24</td>
<td>72.33</td>
<td>71.03</td>
<td>72.15</td>
<td>2.120</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>20.45</td>
<td>21.31</td>
<td>21.15</td>
<td>21.82</td>
<td>21.33</td>
<td>0.717</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.34</td>
<td>31.27</td>
<td>30.29</td>
<td>30.94</td>
<td>30.60</td>
<td>1.147</td>
</tr>
<tr>
<td>MCV/RBC</td>
<td>10.86b</td>
<td>12.35a</td>
<td>11.46d</td>
<td>11.90d</td>
<td>12.22a</td>
<td>0.362</td>
</tr>
</tbody>
</table>

Data are stated as means ± (SE).

Regarding white blood cells (WBC) count, there was significant increase in (G2+ve) (8.95) compared with (G1-ve) (8.58) (p< 0.05) and this elevation was significantly attenuated by using biscuit with berries in G3. So, this treatment recovered back the hemoglobin content, RBC and WBC cell count near to normal values.

Mahmoud et al. (1994) indicated that feeding animals contaminated food with mycotoxicosis influences their blood picture. On the other hands, The improvement happened in the results may be ascribed to berries fruits contents of antioxidants such as carotenoids and phenolic compound, vitamins and minerals which can prevent mycotoxicosis due to their effect on provoke oxygen free radical formation and act as superoxide an ion scavengers as represented by Szajdek and Borowska, 2008; Pál et al., 2009 and Lashmanova et al., 2012.

Table (2). Showed that several biochemical parameters are affected by aflatoxin exposure. Finally experimental, the positive group (G2+ve) fed on contaminated aflatoxin diet only without any additives achieved significantly (P<0.05) reduction in serum total protein, albumin and globulin by (23.48, 26 and 35.82%) compared with the control group (G1-ve). Reduction in serum globulin in toxic fed group might be due to the adverse effect of aflatoxin B1 on combination of total proteins and globulin (Agag, 2004).

However, the experimental groups of rats fed biscuit with dried blueberries or wheat bran or nano-charcoal showed that the reduction in plasma total protein, albumin and globulin concentration were descended for (G2+ve) by (9.11%, 3.7% and 13.43% for group (G3), 12.9%, 0.3% and 23.38% for group (G4), 10.49, 8.69 and 11.69 for group (G5), respectively, compared to negative group (G1-ve). such effect may be because of the metabolism of aflatoxins (in the liver) where it interferes with protein synthesis and RNA, this resulted from the damage occured in liver by aflatoxins. There were significantly increased (P<0.05) in creatinine and urea in (G2+ve).

Data are in agreement with (Mogda, et al., 2015) who found that low total protein level acts as an indicator of the toxic effect of aflatoxin b1 in serum and creatinine and urea in positive group (G2+ve) compared with control(G1-ve) because anhydride of creatinine, being formed when water is removed. Hence, plasma creatinine rises in renal disease.

Table 2. Effect of biscuits with dried blueberries or wheat bran or nano- charcoal as detoxifying agents on protein fractions (g/dl) and kidney functions (mg/dl) of intoxicated rats with aflatoxin:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total proteins (TP) (g/dl)</td>
<td>7.24a</td>
<td>5.54a</td>
<td>6.58c</td>
<td>6.30b</td>
<td>6.48c</td>
<td>0.03</td>
</tr>
<tr>
<td>Albumin (AL) (g/dl)</td>
<td>3.22a</td>
<td>2.96c</td>
<td>3.10b</td>
<td>3.21b</td>
<td>2.94c</td>
<td>0.03</td>
</tr>
<tr>
<td>Globulin (GL) (g/dl)</td>
<td>4.02d</td>
<td>2.58a</td>
<td>3.48b</td>
<td>3.08c</td>
<td>3.55b</td>
<td>0.06</td>
</tr>
<tr>
<td>Albumin / Globulin ratio (A / G ratio)</td>
<td>0.81c</td>
<td>1.15a</td>
<td>0.89c</td>
<td>1.04b</td>
<td>0.83c</td>
<td>0.03</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.22a</td>
<td>1.55b</td>
<td>1.30c</td>
<td>1.37b</td>
<td>1.39c</td>
<td>0.02</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>36.33bc</td>
<td>47.08a</td>
<td>33.37c</td>
<td>38.36bc</td>
<td>42.70b</td>
<td>2.27</td>
</tr>
</tbody>
</table>

Data are stated as means ± (SE).

Data at the same row with different letters are significantly different at P<0.05
Total lipid, triglycerides and total cholesterol were determined in blood serum experimental rats to assess the effect of natural additives such as dried blueberries, wheat bran and nano-charcoal to biscuit on aflatoxin contaminated diets. The obtained data are given in Table (3). The level of total cholesterol (TC), total triglycerides (TG), LDL and HDL were significantly increased (P<0.05) in (G+ve).

Dietary supplementation with dried blueberries, wheat bran and nano-charcoal resulted in a significant increased (P<0.05) in AST and ALT level as compared with control group (G1Pve) as showed in (Table 4).

Aflatoxin has a harmful and stressful effect on liver. AST, ALT and ALP are cytosolic enzymes, famous biochemical indicators for hepatic damage. The results of the current study suggested that exposure to aflatoxin resulted in a significant increased (P<0.05) in AST and ALT level as compared with control group (G1-ve) as showed in (Table 4).

These results are in agreement with Sherif et al., (2009) who found that most increases in ALT activity are associated with hepato cellular damage, when cellular degeneration happens in liver because liver cell death and liver injury. Moreover, treated groups fed on biscuit with wheat bran or nano-charcoal at the tested level significantly decreased (P<0.05) the elevated levels of serum ALT and AST compared to (G+ve). The best results of liver functions were documented for the group fed on biscuit with dried blueberries in group (G3).

Similar results have been reported by Sahin and Sehu, (2007) who showed that serum biochemical values (glucose, protein, Ca and P content) were reduced.

### Histological Investigations

#### Liver:
Liver plays a great role in detoxification any injury to it or impairment of its functions may lead to many modulation on one’s health (Subramaniam et al., 2015).

As shown in Fig.2 (A-B), the histopathological profile of the liver of control group (G1) showed the normal architecture of the classic hepatocytes. The hepatocytes from branching cords radiating from the central vein. Normally, they showed vesicular nuclei. The cells appeared to be separated by the blood sinusoids that arrangements by endothelial cells (Fig.2.A).

Treated group with aflatoxin contaminated diet (positive group) (G2+ve) showed degenerative changes in the hepatocytes. Cells all over the hepatic lobules were noted to have fat vacuoles (Fig.2.B). The most significant improvement in the histological structure was found in group G3 fed on biscuit enriched with dried blueberries (Fig.2.C) which showed normal designed structure of hepatic which was almost similar to that of (G-ve). (Fig.2.A).

Treated group with biscuit addition of wheat bran(G4) showed apparently normal hepatocytes. Also, they showed few mononuclear infiltrations and degenerated leukocytes. Moreover, normal appearing round nuclei is also noticed. It is illustrated in (Fig. 2.E) shows hepatic tissues of rats fed biscuits addition of nano-charcoal (G5) with no necrotic hepatic and nearly normal hepatic strands.

Similar results were shown by Abdelhamid (1995) who found that feeding animals food contaminated with mycotoxin caused pathological findings, particularly in heart, liver, kidney, and spleen. The changes involve (hepatic round, cell infiltration, focal necrosis, irregularities of lobular plats and periporal fibrosis).

Berries consumption could invert most of the histological and biochemical changes in the liver of the group fed aflatoxin contaminated diets because its hypoglycemic and antioxidant effects. The histological investigation provide clear confirmation for hepatoprotective effect of berries in positive group which is in close agreement with the information presented by Mogda et al., (2015) who found that antioxidants typically boost the liver's health and can dismiss any excess damage done to the liver and may even hurry liver recovery.
Fig. 2. A. Photomicrographs of histological section of control group (G1) of liver showing normal hepatocytes with normal nucleus and naturally hepatocyte arrangements with intact blood sinusoids. BV, Blood vessel; N, nucleus. (H&E. X400).

Fig. 2. B. Photomicrographs of histological section of liver of positive group (G2) showing increased leukocyte severe infiltration between hepatocytes and hepatocellular degenerated (abnormal nucleus), ballooning degeneration inflammation and loss of cellular boundaries leukocy infiltration (LI), Focal leukocyte aggregation (FLA), BV, Blood vessel; N, nucleus. (H&E. X400).

Fig. 2. C. Photomicrographs of histological section of liver group (G3) showing nearly normal hepatocyte arrangements with intact blood. (H&E X400).

Fig. 2. D. Photomicrographs of histological section of liver group (G4) showing nearly hepatic cell but also degenerated leukocyte. (H&E. X400).

Fig. 2. E. Photomicrographs of histological section of liver group (G5) showing nearly normal hepatocytes but showing leukocyte infiltration. (H&E. X400).

Kidney:
A comparative histopathological investigation of kidney of all groups are illustrated in Fig.3(A:E). Normal renal structure with controlled nuclear arrangement of uriniferous tubules and collecting tubules is observed. The renal corpuscles are formed of glomeruli(G) and Bawman’s Capsules. Renal tubules are lined by healthy epithelial cells around the lumina as shown in Fig. 3(A).
The renal tubules appeared irregular, dilated, and atrophied of the glomerulus.

Fig. 3 (B) section of kidney positive group (G2+ve) shows renal tubules with certain arranged collecting tubules. Degeneration of the lining epithelium of renal tubules with dilatation in renal tubules. Degeneration of renal corpuscles, degeneration of the lining of renal tubules. With abnormal swelling lumina, besides interstitial leukocyte infiltration were detected.

Treated group fed on biscuits addition of dried blueberries (G3) is shown in Fig. 3(C). The sections looked to be returning the normal appearance and evidence nearly normal renal structure including renal corpuscles and renal tubules. In Fig. 3 (D and E), treated groups fed on biscuits with wheat bran or nano-charcoal showed that distortion of renal corpuscles, exhibiting some degeneration of renal tubules, slight damage of epithelium lining the tubules and interstitial leukocyte infiltration are observed.

These findings were in parallel with those of (Nashwa et al., 2008) who told that the levels of serum urea and creatinine were significantly high in aflatoxicated groups as compared to healthy. This increase in concentrations in toxicated animals might be due to nephrotoxic action, which affects renal impairment by destruction of epithelial cells of proximal and distal convoluted tubules and alteration in tubular function.
REFERENCES


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