Efficacy of use of forskolin plant extract in control of toxic effects of aflatoxicosis in food

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Abstract: The current study was undertaken to evaluate the efficacy of use of forskolin plant in control of the dangerous changes caused by aflatoxicosis. Forskolin has been isolated from the roots of Coleus Forskohllii, a plant rich in alkaloids which are considered to have a high probability of influence on the biological systems. Tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids are active principles of Coleus forskohlii. Forskolin has a unique property of activating almost all hormone sensitive adenylylcyclase enzymes in a biological system. Out of 150 samples of frozen, minced meat, raw milk, kareish cheese, wheat and yellow corn (25 of each), the obtained results revealed that aflatoxicogenic moulds of A. flavus and A. parasiticus were recovered from samples of cereals of wheat and yellow corn, respectively (72%, 12% and 60%, 16%), followed by samples of kareish cheese and frozen meat, respectively (44%, 4% and 40%, 4%). Whereas, the lowest level of isolation were detected in minced meat and raw milk, respectively (24%, 8% and 20%, 4%). The maximum levels of aflatoxins were obtained from A. flavus and A. parasiticus isolated from yellow corn and wheat, respectively (66.6%, 75% of isolates produced mean level of 600±6.2 ppb, 120±8.0 ppb in yellow corn and 66.6% of isolates produced mean level of 300±4.5 ppb, 75±0.3 in wheat). The isolates of A. flavus and A. parasiticus from frozen and minced meat were detected respectively in (50%, 100% and 66.6%, 50% with the mean level of 10.5±1.2 ppb, 12±0.0 ppb and 25±0.5 ppb, 22±0.0 ppb). On the other hand, the samples of raw milk and kareish cheese, showed a relatively lower levels of aflatoxins that produced by A. flavus and A. parasiticus which isolated from these samples. Whenever, 60, % of isolated A. flavus from raw milk produced mean level of aflatoxin 13±0.3 ppb and the A. flavus and A. parasiticus that recovered from 54.5%, 100% of kareish cheese produced 25±2.6 ppb and 15±4.0 ppb, respectively. For experimental evaluation the effect of forskolin against aflatoxicosis, forty rats were divided into 4 equal groups, Where, rats of the first group were given normal feed (free from mycotoxins and without any treatment) and kept as a negative control. While, rats of the other groups were given single dose of AFB1, intra-peritoneal at the rate of 1.5 ppm. Then on the second day, rats of the third and fourth were dosed orally by 50 and 100 mg of forskolin for 2 weeks, while those of the second group were left without any treatment and kept as positive control. The results showed significant elevation in the liver and kidney function enzymes and decrease in concentrations of serum total protein, albumin, alpha globulin, beta globulin and gamma globulin together with A/G ratio. The serum NO level significantly increased in AF B1 treated rats. Also, a significant decreased in catalase activity, GSH levels and increased TBARS levels were shown after Af treatment. However, supplementation of forskolin extract for toxicated rats with AFB1 increased CAT activities and TBARS and eliminates the possibility of oxidative stress due to the administration of AF B1 to rats. Histopathological changes of lung, liver, kidneys, spleen and skeletal muscle in aflatoxicated rats were discussed briefly. Receiving of forskolin to aflatoxicated rats decreased the destructive effect of AFB1 in the tissues examined specially liver, where, cytoplasmic regeneration of hepatocytes was detected and had a significant improvement in all lesions appeared in most organs which represented by minimizing of histopathological and biochemical alteration. Hence, the supplementation of forskolin in food and feed is valuable in reduction of the severity of the toxicity and the histopathological and biochemical alteration produced by aflatoxin B1.


Keyword: Efficacy, forskolin, plant extract, biochemistry, oxidative stress, histopathology.

1. Introduction

The increased population in the world requires a parallel raise in the production of food. Some countries as Egypt had to import many food and feeds. Majority of these foods may carry the dangerous factors for human and animal health. Fungal contaminations and their toxins represent the most significant contaminant of these foods (Hassan et al., 2011). Aflatoxins are a group of secondary metabolites produced by A. flavus and A. parasiticus in food and feed commodities (Hassan et al., 2010). The consumption of food contaminated with mould and their toxins induced food poisoning, hemorrhages,
hepatotoxicity, nephrotoxicity, neurotoxicity, dermatitis, carcinogenic, hormonal and immunospression effects (Hassan et al., 2010 and 2011). Livers characteristically are pale and enlarged as a result of aflatoxicosis, with microscopic changes including fatty change, hepatic necrosis, and biliary hyperplasia (Hassan et al., 2010). AFB1 is also biotransformed by P450 enzymes to yield an electrophilic epoxide, which attacks the DNA to initiate hepatotoxicity and genotoxicity via oxidative damage (Shen et al., 1996).

Because aflatoxins contamination of food cannot be avoided, numerous detoxification strategies have been proposed to alleviate its impact. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu et al.,1998). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa 2007). In general, there are two basic categories of antioxidants, natural and synthetic. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Gülçin et al., 2004 and Hassan et al., 2011). World Health Organization (2003) estimated that 80% of the world’s population depends on traditional medicine for their health needs. In many developing countries, traditional herbal remedies are making a comeback as alternatives to modern medicine and the existence of traditional medicine depends on plant diversity and the related knowledge of their use as herbal medicine. Medicinal plants are important for pharmacological research and drug development, not only when constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Mukherjee, 2003 ; Hassan et al., 2008; 2010 and 2011). It is reported that forskolin plant extract is useful in the treatment of congestive heart failure, glaucoma, asthma and certain type of cancers (Bhat et al., 1993). In addition, it has been shown to have anti-inflammatory property (Rupp et al., 1986).

Therefore, this study was undertaken to screen feeds, frozen meat and raw milk for contamination with aflatoxigenic A. flavus and A. parasiticus and detection their ability for aflatoxin B1 production. Also, evaluation the effect of forskolin plant extracts for ameliorating the toxic effect of aflatoxins.

2. Materials and Methods
Source of aflatoxigenic strains:

Out of 150 samples including frozen and minced meat, raw milk, kareish cheese, wheat and yellow corn (25 of each), the recovered isolates of A. flavus and A. parasiticus were selected for screening for AF. Production. The samples of feeds, meat and milk were obtained from animal’s farms which had a disease problems and markets of food and subjected for mycological and mycotoxicological investigations.

Origin of Forskolin:
It grows wild in the subtropical temperate climates as in Egypt, Arabia, Ethiopia, tropical East Africa and Brazil (Willemsen, 1985). The plant is found mostly on the dry and barren hills. The tuberous roots of the plant produce the active principle compound (labdane diterpenoid forskolin (7β-Acetoxyl-13-epoxy-1a, 6 β, 9 α-trihydroxy-labd-14-ene-11-one) (Shah et al., 1980).

Aflatoxins immunoaffinity columns:
Imunoaffinity columns of aflatoxins B1, B2, G1, G2 and other accessory materials for detection of mycotoxins by VICAM- fluorometric methods were purchased from sigma chemical company (USA).

Experimental animals:
Forty apparently healthy albino rats weighted (100-120 g) were housed under hygienic conventional conditions in suspended stainless steel cages. Prior to experiment rats fed on healthy basal diet free from any cause of disease. Drinking water was supplied in glass bottles, adlibitum.

Isolation and Identification of A. flavus and A. parasiticus :
Pure cultures of A. flavus and A. parasiticus were obtained from selected colonies for repeated sub-culturing according to (Smalla et al., 1998). After incubation of plates for 3 days at 30°C, from the grown fungi, hyphal tips or single spores were transferred to test tubes containing slant PDA medium. The purified fungi were identified by the author according to (Conner et al., 1992).

Cultivation and extraction of aflatoxins from isolates:
The isolated strains of A. flavus and A. parasiticus from the present samples were used for experimental production of aflatoxins and the produced toxins were extracted and measured as methods recommended by (Gabal et al., 1994).

Extraction of aflatoxins from frozen and minced meat, raw milk, kareish cheese, wheat and yellow corn samples:
The aflatoxins were extracted and determined according to the method described by Mazan et al. (2001) and Ozaslan et al. (2011) by using VICAM-fluorometric technique.

Extraction and Fractionation of forskolin:
Forskolin is extracted from tubers which were harvested at 75 to 85% moisture level on wet basis and stored at less than 12% moisture after drying. Tubers
mechanically dried at 40°C with tuber slice thickness of 0.5 cm and packed in polyethylene lined gunny bag retained the highest amount of forskolin. The quantification of forskolin is developed by thin layer and high performance liquid chromatographic (HPLC) methods are employed (Rajagam, 2005 and Saleem et al., 2006).

**Experimental design:**

Forty rats were divided into 4 equal groups. Rats of the first group were given normal feed (free from mycotoxins and without any treatment) and kept as a negative control. Rats of the other groups were given single dose of AFB1, intra-peritoneal at the rate of 1.5 ppm). Then on the second day rats of the third and fourth were dosed orally by 50 and 100 mg of forskolin for 2 weeks, while those of the second group were left without any treatment and kept as positive control. The period of feeding was continued for 4 weeks (Bao, 2002).

**Blood samples:**

At the end of the experiment, blood samples were collected from each group into small labeled dry and clean vials. The 1st were collected with anticoagulant for lysated Red blood cells (RBCs). Preparation of hemolysate according to Tietz (1996). Catalase activity; lipid peroxidation as malonaldehyde (MDA) and reduced glutathione (GSH) in lysated rbc were determined according to Aebi (1974); Okhawa et al. (1979) and Ellman (1959), respectively.

The 2nd blood samples were without anticoagulant in centrifuge tube, allowed to clot and then centrifuged at 3000 rpm for 90 minutes for separation of serum which used to assay the biochemical parameters. The biochemical assays of serum lactic dehydrogenase (LDH) activities were determined according to methods of Szase et al. (1976), gamma glutamyle transferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities according to Reitman and Frankel (1957), serum urea according to Wyhenga et al. (1971), serum creatinine level according to Henry (1974). Serum NO levels of the samples were without anticoagulant in centrifuge tube, allowed to clot and then centrifuged at 3000 rpm for 90 minutes for separation of serum which used to assay the biochemistry parameters.

Detection of aflatoxins residues in the internal organs:

The extraction, purification and measurement of aflatoxins residues in liver, kidney and spleen of rats after experimental work was monitored according to the method described by Mazzani et al. (2001) and Ozaslan et al. (2011).

**Statistical analysis:**

The obtained data were computerized and analyzed for significance. Calculation of standard error and t-test according to (SPSS 14, 2006).

**3. Results and Discussion**

As shown in table (1) the maximum isolation of A. flavus and A. parasiticus were recovered from samples of cereals of wheat and yellow corn (72%, 12% and 60%, 16%), followed by samples of kareish cheese and frozen meat (44%, 4% and 40%, 4%). Whereas, the lowest level of isolation were detected in minced meat and raw milk (24%, 8% and 20%, 4%) respectively.

Table (1): Prevalence of A. flavus and A. parasiticus species in samples of frozen and minced meat, raw milk, kareish cheese, wheat and yellow corn.

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Prevalence of A. flavus and A. parasiticus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. flavus</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Frozen meat</td>
<td>10</td>
</tr>
<tr>
<td>Minced meat</td>
<td>6</td>
</tr>
<tr>
<td>Raw milk</td>
<td>5</td>
</tr>
<tr>
<td>Kareish cheese</td>
<td>11</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>15</td>
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<tr>
<td>Wheat</td>
<td>18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Prevalence of A. flavus and A. parasiticus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. flavus</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Frozen meat</td>
<td>7</td>
</tr>
<tr>
<td>Minced meat</td>
<td>5</td>
</tr>
<tr>
<td>Raw milk</td>
<td>4</td>
</tr>
<tr>
<td>Kareish cheese</td>
<td>4</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>6</td>
</tr>
<tr>
<td>Wheat</td>
<td>2</td>
</tr>
</tbody>
</table>

25 samples of each type were examined.
genus aspergillus (60%, 60% and 76%) with mean of count of (1.6 x 102 ± 0.1, 6.0 x 10 ± 0.23 and 3 x 102 ± 1.0), respectively, which were at the top of all isolated fungi. However, A. flavus was isolated from all kind of samples.

On the other hand, Hassan et al. (2007) reported that after the screening samples of meat, milk and its products for fungal contamination, yeast of C. albicans was recovered at the top rate of all isolated fungi, where it recovered from (75% of shawerma samples with the mean count of 1x10^3±0.3x10) followed by luncheon (60% with the mean count of 2x10^3±0.1x10), minced meat (40% with the mean count of 3x10^2±0.2x10). The incidence of C.albicans in samples of yoghurt was (62%) and soft cheese was (38%) which was higher than in raw milk (30%) with the mean of total colony count of (3x10^3±10x10) in yoghurt but in soft cheese total colony count was (2x10^2±2.0x10). The isolation of these fungi in collected samples may be due to the exposure to adverse environmental factors as high temperatures and humidity during preparation, and/or storage. Direct contamination for samples itself may be occur during handling, processing and transportation which help in all ways to initiate the fungal pollution Hassan et al. (2004) and El- Ahl et al. (2006).

As shown in table (2) significant levels of aflatoxin were produced by A. flavus and A.parasiticus isolated from collected samples, where, the maximum levels of toxin were obtained from A. flavus and A.parasiticus isolated from yellow corn and wheat (66.6%, 75% of isolates produced mean level of 600 ± 6.2 ppb, 120± 8.0 ppb for isolates of yellow corn and 66.6% of isolates produced mean level of 300±4.5 ppb, 75±0.3 ppb for isolates of wheat), respectively.

Table (2): Levels of aflatoxins produced by isolated A.flavus and A.parasiticus species from frozen and minced meat, raw milk, kareish cheese, wheat and yellow corn.

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Levels of aflatoxins produced by (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. flavus</td>
</tr>
<tr>
<td></td>
<td>Total No.</td>
</tr>
<tr>
<td>Frozen</td>
<td>10</td>
</tr>
<tr>
<td>Minced</td>
<td>6</td>
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<tr>
<td>Raw milk</td>
<td>5</td>
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<tr>
<td>Kareish</td>
<td>11</td>
</tr>
<tr>
<td>Yellow</td>
<td>15</td>
</tr>
<tr>
<td>Wheat</td>
<td>18</td>
</tr>
</tbody>
</table>

The isolated A. flavus and A.parasiticus from frozen and minced meat were detected in (50%, 100% and 66.6%, 50% with the mean level of 10.5 ± 1.2 ppb, 12±00 ppb and 25±0.5 ppb, 22±00 ppb), respectively. On the other hand, the samples of raw milk and kareish cheese, showed a relatively lower levels of aflatoxins that produced by A. flavus and A.parasiticus which isolated from these samples. Wherever, ( 60% of isolated A. flavus from raw milk produced mean level of aflatoxin 13±0.3 and the A. flavus and A.parasiticus that recovered from 54.5% of kareish cheese produced 25±2.6 ppb and 15±00 ppb) respectively.

The results in (Table.3) showed a significant levels of aflatoxins was detected in feed and food samples. The maximum mean of aflatoxins level was detected in samples of wheat and yellow corn (20±1.5 and 35±2.6), respectively. Whereas, the other examined samples showed a relatively low levels of aflatoxins contamination. The significant levels of aflatoxins were also detected in other studies by isolated A. flavus and A.parasiticus that recovered from meat (Wafia and Hassan, 2000), milk (Hassan et al., 2002, 2004 and El- Ahl et al., 2006) and from meat and milk (Hassan et al., 2008, 2009 and 2010).

The most important mycotoxigenic fungi are those producing aflatoxins, which are structurally similar polysubstituted cumarine. Aflatoxins are a group of secondary metabolites produced by A. flavus and A. parasiticus in food and feed commodities (Oguz, 1997). Aflatoxins have received greater attention than any of the other mycotoxins because of their demonstrated carcinogenic effects in susceptible animals and their acute toxicogenic effects in human and as they are unique in being resistant to degradation under normal food processing conditions (Hassan et al., 2004; 2009 and 2010). This makes the selection of proper decontamination methods that will effectively decompose aflatoxins, while retaining the nutritive quality and palatability of the treated food a continuous challenge.

The results in (Table. 4) detected that aflatoxin B1 treatment alone caused severe liver and kidney damage in rats, as evidenced by increased serum ALT, AST, LDH and GGT activities, urea and creatinine concentrations (Table, 4). These elevations are indication of cellular leakage and loss of functional...
integrity of cell membrane in liver Eraslan et al. (2006 and Hassan et al., 2010). Also, it might be a marker of the activity of the nephrotic syndrome (Awad et al., 2011) and hepatic necrosis, thickness of bile duct and intrahepatic cholestasis (Ncibi et al., 2008 and Hassan et al., 2010). Also, the present study shows that serum NO level significantly increased in AFB1 treated animals (Table, 4). It has been reported that elevated levels of lipid peroxidation stimulates host cells, mainly monocytes/ macrophages, to produce and release NO by induction of inducible nitric oxide synthase (iNOS) protein, resulting in cytotoxicity and DNA damage (Shen et al., 1994 and Raso et al. 2001). The nitric oxide is naturally formed in activated macrophages and endothelial cells and is considered as an active agent in several pathologies based on inflammation. Ferrante et al., (2008).

Also, the administration of forskolin to aflatoxicated rats decreased the serum levels of ALT and AST towards their respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by aflatoxin B1,demonstrate that forskolin extract showed protection against AF B1-induced hepatotoxicity and nephrotoxicity due to the presence of Tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids as (Shankaragowda, 2000, Henderson et al., 2005 and Khatun et al., 2010).

Table (3): Levels of aflatoxins detected in frozen and minced meat, raw milk, kareish cheese, wheat and yellow corn.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Levels of aflatoxins (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total No.</td>
</tr>
<tr>
<td>Frozen meat</td>
<td>20</td>
</tr>
<tr>
<td>Minced meat</td>
<td>20</td>
</tr>
<tr>
<td>Raw milk</td>
<td>20</td>
</tr>
<tr>
<td>Kareish cheese</td>
<td>20</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>20</td>
</tr>
<tr>
<td>Wheat</td>
<td>20</td>
</tr>
</tbody>
</table>

Table (4): Chemoprevention of AFB1-induced hepatocarcinogenesis by Forskolin(n=10 for each group).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Gp1</th>
<th>Gp2</th>
<th>Gp3</th>
<th>Gp4</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (u/l)</td>
<td></td>
<td>46.11±4.06</td>
<td>90.45±7.21***</td>
<td>60.68±3.0*</td>
<td>57.32±4.1</td>
</tr>
<tr>
<td>ALT (u/l)</td>
<td></td>
<td>30.07±3.1</td>
<td>51.34±3.29***</td>
<td>43.33±3.1*</td>
<td>40.19±3.65</td>
</tr>
<tr>
<td>Urea (mg%)</td>
<td></td>
<td>37.48±2.17</td>
<td>68.86±3.79***</td>
<td>48.56±2.6**</td>
<td>40.21±2.11</td>
</tr>
<tr>
<td>Creatinin(mg)</td>
<td></td>
<td>0.66±0.05</td>
<td>1.14±0.06***</td>
<td>0.95±0.06**</td>
<td>0.78±0.07</td>
</tr>
<tr>
<td>LDH (u/l)</td>
<td></td>
<td>137.98±13.14</td>
<td>284.10±15.52***</td>
<td>183.53±10.82</td>
<td>149.42±12.5</td>
</tr>
<tr>
<td>GGT (u/l)</td>
<td></td>
<td>53.17±6.79</td>
<td>141.14±10.34***</td>
<td>93.73±9.86*</td>
<td>70.83±7.99</td>
</tr>
<tr>
<td>NO (u/l)</td>
<td></td>
<td>27.18±3.01</td>
<td>74.10±6.52***</td>
<td>43.53±4.12**</td>
<td>33.92±2.50</td>
</tr>
</tbody>
</table>

Significance at *p< 0.05 **p< 0.01 ***p< 0.001(ANOVA).

In present study as represented in table (5), there were a decrease in concentrations of serum total protein, albumin, alpha globulin, beta globulin and gamma globulin together with A/G ratio. AF B1 is also biotransformed by P450 enzymes to yield an electrophilic epoxide, which attacks the DNA to initiate hepatotoxicity and inhibition of protein synthesis via oxidative damage (Shen et al., 1996 and Hassan et al., 2004 ; 2009, and 2010). Aflatoxin is an electrophilic reactivity at the carbonyl carbon atom and could conceivably adduct amines, imidazoles and sulphhydryl groups on proteins and enzyme via the Michael carbonyl condensation reaction can cause conformational changes that interfere with their function (Lee et al., 2010 and Rawal et al., 2010).

Aflatoxin was associated with other alterations in serum protein sub-fractions fractions (table, 5). These include a significant increase in, beta-1 and gamma-1b globulins and a significant decrease in alpha-2, beta-1 and gamma-1a globulins. These results might be due to inflammation of liver and kidney tissues and immunosuppressive effect inhibit nearly cellular and humeral immunologic reaction induced by aflatoxin (Tietz, 1996 and Hassan, and mogda,2003 and Hassan et al. 1997; 2009 and 2010). The immunotoxic activity of aflatoxin probably results from degenerative changes and cell death following necrosis and apoptosis, in combination with slow replacement of affected immune cells, due to inhibition of protein synthesis (Al-Anati, and Petzinger,2006). TNF-α, a pleiotropic proinflammatory cytokines released from macrophages and other cell types in response to tissue
damage and evidence of hepatic necrosis (Petersen et al., 2004 and Lomborg et al., 2008).

Forskolin appears to exhibit potent immune system enhancement by activating macrophages and lymphocytes Ciotonea and Cernát (2010). It possesses anti-inflammatory and antioxidant actions, González-Sánchez et al.(2006). Forskolin is a unique diterpene derivative of the plant Coleus forskohlii that acts independently of cell surface receptors to increase intracellular levels of cyclic AMP (cAMP), Insel et al. (1982).

Free radical production and disturbance in redox molecular affecting certain cellular processes leading to inflammation process Evans, (1989). The antioxidants have been definitively linked to anti inflammatory and immunosuppressive properties and they may include superoxide dismutase, glutathione peroxidase, catalase and glutathione reductase (Lee et al., 1999). Reactive oxygen species (ROS) and lipid peroxidation (LPO) have been considered to be main mechanisms in the toxicity of AFB1 (Sohn et al., 2004). CAT is the main antioxidant enzyme in the body, which scavenge unwanted O2, H2O2, and ROOH produced by free radical. The decreased enzyme activities and increased TBARS levels produced by AFB1 can be attributed to lower ability of the tissue, which cannot scavenge free radicals and prevent the action of lipid peroxidation. In present study, reduction in GSH was shown after AF treatment. GSH play a critical role in the protection of tissues from deleterious effects of activated AFB1, Maslova and Boboriko (1990).

However co-supplementation of forskolin extract with AFB1 (Table 6) increased CAT activities and reduced lipid peroxidation, as measured by Malondialdehyde production, and eliminates the possibility of oxidative stress due to the administration of AFB1 to rats. It is also reported that forskolin exhibits an appreciable amount glutathione and both of these are known to be effective in direct scavenging of a wide variety of free radicals (Maslova and Boboriko, 1990). Also, GSH present in forskolin are potent lipid peroxidation chain-breaking agent and therefore further add to the protective role of the herbs against lipid peroxidation Gupta, and Sharma (2011).

Forskolin has inhibitory actions on the production of interleukins and the antioxidant and anti-inflammatory actions of forskolin are explained by an inhibitory action on macrophages, with subsequent decreases in the levels of thrombocyte B2 andsuperoxide radicals, González-Sánchez et al. (2006). From (Table, 7) it is detected that the residues of aflatoxins in the internal organs of aflatoxicated rats treated with high doses forskolin extract (100 mg) were completely eliminated. These findings was con in accord with findings of Hassan et al. (2010) who detected that the administration of dimethyl 4, 4-dimethoxy 5, 6, 5, 6-dimethylene dioxybiphenyl 2, 2-dicarboxylate (D.D.B.) in aflatoxicated rats (1.5 ppm of AFB1) resulted an improvement in the haematological picture and prevented serum biochemical changes, ameliorated, the toxic effect of aflatoxin B1 and completely eliminated the toxin residues in liver which caused hepatoprotective effect on AFB1 induced liver toxicity. Whereas, Awaad et al.(2011) obtained the protective effect of a Specific Combination of Mannan-Oligosaccharides and β-Glucans Extracted from Yeast Cell Wall on the health status and growth performance of ochratoxicated broiler chickens and degradation the toxin residues in internal organs of treated chickens.

Table (5) Chemoprevention of AFB1-induced alteration in total protein and its electrophoresis(g/dl) by forskolin (n=10 for each group).

<table>
<thead>
<tr>
<th>parameter</th>
<th>Group</th>
<th>Gp1</th>
<th>Gp2</th>
<th>Gp3</th>
<th>Gp4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alb</td>
<td></td>
<td>1.98±0.07</td>
<td>1.51±0.09*</td>
<td>1.81±0.05</td>
<td>1.84±0.09</td>
</tr>
<tr>
<td>T.alpha</td>
<td></td>
<td>1.03±0.07</td>
<td>0.76±0.06***</td>
<td>0.86±0.11</td>
<td>1.08±0.1</td>
</tr>
<tr>
<td>Alpha1</td>
<td></td>
<td>0.42±0.04</td>
<td>0.28±0.06</td>
<td>0.34±0.04</td>
<td>0.51±0.05</td>
</tr>
<tr>
<td>Alpha2</td>
<td></td>
<td>0.63±0.04</td>
<td>0.48±0.03*</td>
<td>0.52±0.07</td>
<td>0.57±0.09</td>
</tr>
<tr>
<td>t.beta globulin</td>
<td></td>
<td>1.51±0.06</td>
<td>1.51±0.08*</td>
<td>1.58±0.06</td>
<td>1.4±0.08</td>
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<tr>
<td>Beta1</td>
<td></td>
<td>0.88±0.04</td>
<td>1.07±0.05</td>
<td>1.00±0.07</td>
<td>0.72±0.06</td>
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<tr>
<td>Beta2</td>
<td></td>
<td>0.63±0.05</td>
<td>0.44±0.05</td>
<td>0.58±0.08</td>
<td>0.68±0.08</td>
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<td>Gamma globulin</td>
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<td>2.7±0.05</td>
<td>2.31±0.1</td>
<td>2.51±0.06</td>
<td>2.61±0.08</td>
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<tr>
<td>Gamma1</td>
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<td>2.29±0.08</td>
<td>1.66±0.06***</td>
<td>1.96±0.95**</td>
<td>2.03±0.11</td>
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<td>Gamma2</td>
<td></td>
<td>0.41±0.04</td>
<td>0.65±0.04***</td>
<td>0.55±0.09</td>
<td>0.58±0.06</td>
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<tr>
<td>T.elobulin</td>
<td></td>
<td>5.24±0.9</td>
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<td>4.95±0.12</td>
<td>5.09±0.27</td>
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<td>A/G ratio</td>
<td></td>
<td>0.38±0.03</td>
<td>0.33±0.02</td>
<td>0.37±0.01</td>
<td>0.36±0.02</td>
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<tr>
<td>T. protein</td>
<td></td>
<td>7.22±0.23</td>
<td>6.09±0.33**</td>
<td>6.76±0.26</td>
<td>6.93±0.23</td>
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</tbody>
</table>

- Significance at *p< 0.05 **p< 0.01 ***p< 0.001(ANOVA),
Histopathological findings in all groups of rats in the present study were observed. In lung, some alveolar wall showed alternative thinning of alveolar wall associated with emphysema, while, other areas revealed thickening of interalveolar septa with macrophage infiltration (Fig.9). Some pulmonary blood vessels showed thickened wall with hemolysed blood. Similar results was previously reported by Denli et al. (2009) and Hassan et al., 2011). Hepatic tissue showed preservation of hepatic cord pattern. One of the most commonly reported changes in is severe vascular degeneration of hepatocytes. Some hepatocytes lost their nuclei, while some had binucleation fig (1). Similar results were recorded Denli et al. (2009) and Hassan et al. (2011), where, liver tissue of broiler and sheep receiving aflatoxin B1 had peribular inflammation and vacuolar degeneration of hepatocytes, respectively. Cytoplasmic degeneration may cause organ malfunction. Gimeno and Martins (2006) recorded that aflatoxin B1, when consumed by rodent; expression of pathogenicity can be in the form of toxic abnormalities and organ malfunction including interference with the metabolism of amino acids and vitamin B complex. Hassan et al. (2003 and 2009) reported that aflatoxin bind to nucleic acids and also impairs protein formation in the body. Thus, they may cause organ damage and/or cancer from prolonged exposure. Moreover, Abou Rawash (1996) added that cytogenetical analysis clearly indicated that chronic aflatoxicosis had a damaging effect on the nucleus in the interphase stage. Jubb et al. (1993) and Hassan et al. (2010) added that protein and RNA synthesis are inhibited at higher dose rats, which probably laccouts for the necrotizing effects and fatty change seen at these rats. The endothelium of central veins of aflatoxicated group was swollen suggesting that aflatoxin B1 may have an irritative action on endothelium.

Kidney of aflatoxicated group showed Some glomeruli were atrophied while others showed moderate degree of periglomerular edema. Epithelial lining renal tubules (Proximal convoluted tubules) revealed advanced stage of granular degeneration with star-shaped lumen fig (4). Changes of renal tubules were nearly detected by Hassan et al. (2004) in bovine and Arafa et al. (2006) and Hassan et al. (2010) in goats and sheep and Denli et al. (2009) in hen. Some renal blood vessels engorged with hemolysed blood. In spleen, aflatoxicated group showed depletion of lymphoid cells in white pulp and congestion of spleenic vessels fig. (10). Few hemosidrin pigments were observed. This related to toxic effect of AFB1 which include immunosuppression. Hassan et al. (1997) and Yin et al. (2008) mentioned that noticeable clinical signs on rodents include renal, spleen, liver and pulmonary congestion. In skeletal muscles, aflatoxin B1 exposure showed in our study, loss of striation, zenker necrosis in some fibers and edematous reaction (Fig.,7). That is related to the binding of its toxic metabolites to macromolecules, in particular, to nucleic acids and nucleoproteins (Jubb and Hassan et al., 2004). Smith et al. (1975) recorded that skeletal muscle showed toxic myositis in chick embryo treated with aflatoxin B1.

Treatment of aflatoxicated rats with low dose of forskolin revealed mild and focal areas of emphysema in lung. Hepatocytes showed granular degeneration with reduction of binucleation (around central vein) (Fig., 2). Vacuolar degeneration changes were more evident at area adjust to portal area. In kidneys, Moderat improvement of glomeruli appeared in less frequency of atrophied glomeruli; while renal tubules

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control(10)</th>
<th>Aflatoxicated gp (10)</th>
<th>Aflatoxicated +50mg fors.gp(10)</th>
<th>Aflatoxicated+100mg fors.gp gp(10)</th>
</tr>
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<tbody>
<tr>
<td>Liver</td>
<td>0</td>
<td>1.5</td>
<td>1.0</td>
<td>0</td>
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<tr>
<td>Kidney</td>
<td>0</td>
<td>1.5</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>Spleen</td>
<td>0</td>
<td>1.5</td>
<td>0.05</td>
<td>0</td>
</tr>
</tbody>
</table>

- Significance at *p< 0.05 **p< 0.01 ***p< 0.001(ANOVA),

Table (6): Chemoprevention of AFB1-induced oxidative stress by Forskolin.

<table>
<thead>
<tr>
<th>Group parameter</th>
<th>Gp1</th>
<th>Gp2</th>
<th>Gp3</th>
<th>Gp4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAD nmole/mg Hb</td>
<td>8.6±0.63</td>
<td>14.78±1.03***</td>
<td>11.13±0.83</td>
<td>9.49±0.74</td>
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<tr>
<td>Catalase u/mg Hb</td>
<td>187.18±10.04</td>
<td>100.98±8.98***</td>
<td>143.17±12.02*</td>
<td>164.22±12.33</td>
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<tr>
<td>GSH nmole/mgHb</td>
<td>276.98±12.14</td>
<td>157.81±17.62***</td>
<td>198.56±16.82***</td>
<td>229.81±13.58</td>
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</tbody>
</table>
still suffering from severe degree of granular degeneration in association with intratubular hyaline casts deposition (Fig., 5). Some renal blood vessels engorged with hemolysed blood. Demarcation of red and white pulps in spleen well observed. Few cells of skeletal muscles showed loss of striation with mild edematous reaction was observed (Fig., 8).

With high dose no marked changes were detected than treatment in lung. While, marked regeneration of hepatocytes was more detected at centrilobular area and extended peripherally. Mild granular degeneration was detected than previously described (Fig., 3). In kidneys no distinct pathological lesions in the most of glomeruli were noticed. However, few glomeruli still showing atrophy. Some epithelial lining renal tubules showed mild degree of granular degeneration (Fig., 6) with disappearance of most hyaline casts. No marked changes was detected than treatment with low doses in spleen .Muscle fibers have no pathological alteration. Receiving of foroskolin, reduced the destructive effects in tissues examined. Which are represented by minimizing of histopathological alteration in the form of mild and focal areas of emphysema in lung tissues of both groups of rats received forskolin. The cytoplasmic regeneration of hepatocytes with reduction of bionucleation (around central vein) with low dose . While high dose revealed marked regeneration of hepatocytes which more detected at centrilobular area and extended peripherally which noticed by mild hepatocytic degeneration (granular type instead of severe vacuolar type which previously detected. Denli et al. (2009) reported that cytoplasmic visualization with disseminated necrotic cells were observed in the experimental groups, chicks treated with aflatoxin and afla Detox (Adi, Agro-Res, Reus, Tarragona, Spain). Whereas, Hassan et al. (2011) and Awad et al. (2011), used herbal extracts and AGRIMENOS compound to decreased the histopathological changes which occurred due mycotoxicosis. The low dose of forskolin resulted of a moderate improvement of glomeruli in less frequency of atrophied glomeruli. No distincted pathological lesion of glomeruli except few still showed atrophy with disappearance of most hyaline casts and few epithelial lining renal tubules showed mild degree of granular degeneration indicated the improvement of histopathological alteration with high dose. Well demarcation of red and white pulps of spleen indicated a good immunological response by receiving foroskolin (Shankaragowda, 2000).

De Souza and Shah, (1988); Bhat et al. (1993); Shankaragowda (2000) and Khatun et al. (2010), recorded that it is an important plant used against various disorders in indigenous systems of medicine such as antioxidant, as remedy for heart, abdominal and respiratory disorders in addition, it has been shown to have anti-inflammatory property, where forskolin in our study minimized the swollen of endothelium of central vein in liver tissue and periglomerular edema in renal tissue and decreased markedly edematous reaction in skeletal muscles caused by aflatoxin B1.

In conclusion, the supplementation of foroskolin extract in food and feed is valuable in reduction the severity of the toxicity and the histopathological alteration produced by aflatoxin B1. Hence, the presence of fungi and their toxins in feed and food reflected unhygienic measure during cultivation, irrigation harvesting transportation, handling, and storage and processing of feed and food. Also, the fungal inhibitors may be added if the level of contamination over the limited level. In addition, continuous investigations for finding new safe methods for controlling the growth of fungi and mycotoxins production to keep the health of human and animals consumer are critical demand.
Fig. (3): Liver of rats received aflatoxin B1 and high dose of forskolin. (H & E X 400).

Fig. (4): Kidney of aflatoxicated rats. (H & E X 200).

Fig. (5): Kidney of rats received aflatoxin B1 and low dose of forskolin. (H & E X 200).

Fig. (6): Kidney of rats received aflatoxin B1 and high dose of forskolin. (H & E X 200).

Fig. (7): Skeletal muscles (cross section) of aflatoxicated rats. (H & E X 400).

Fig. (8): Skeletal muscles of rats received aflatoxin B1 and low dose of forskolin, (H & E X 400).
Acknowledgment

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References

Fig. (9): Lung of aflatotoxicated rats . (H & E X 400). Fig. (10): Spleen of aflatotoxicated rats. (H &E X 200).
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