Protective Effect of Curcuma Longa or Nigella Sativa on Aflatoxin B1-Induced Hepato-Toxicity in Rats in Relation to Food Safety on Public Health

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Abstract

Mycotoxins are naturally occurring substances produced by fungi growing on food and animal feed. Aflatoxins are the most toxic group of mycotoxins, and they are produced by two species of the Aspergillus. Aflatoxin constitutes a real threat to the health of livestock as well as humans. Aflatoxins especially aflatoxin B1 are known to be genotoxic and carcinogenic, can produce acute necrosis, cirrhosis and carcinoma of the liver. Curcuma longa (curcumin), Nigella sativa (black seed) are extensively used in cuisine and in traditional medicines so we tried to investigate their role as hepatoprotective agents from natural products (origin) against AFB1-induced hepatotoxicity in male Sprague-Dawley rats. Eighty male Sprague-Dawley rats weighing 200-239gm (mean: 215.5 ±1.27) divided into 4 groups (20 rats each). G1-G2 was fed normal control diet, G3 & G4: Fed normal diet supplemented with Curcuma longa (curcumin), Nigella sativa (black seed) respectively. G2, G3 and G4 were given single intraperitoneal injection of AFB1 at the beginning of the experiment. The experiment lasted for 6 weeks after injection. After that rats were scarified. Organs as liver, kidney, heart, spleen, testes and heart were removed, washed, weight, put into formalin for histopathological examination. Blood was drawn on plain tubes and tubes with anticoagulant, centrifuged to get serum and plasma respectively. Serum and plasma were subjected to biochemical analysis (ALT, AST, Alk Phos, LDH, urea, creatinine, uric acid, total protein, malondialdehyde (MDA) in plasma and liver and some immunoglobulin biomarkers as IgG, IgM, IgA).

The increased levels of serum enzymes (ALT, AST, Alk Ph, and LDH), and urea, creatinine, uric acid, total protein observed in rats treated with AFB1 were greatly reduced in the rats treated with N. sativa or Curcuma longa along with AFB1. The immunoglobulin biomarkers as IgG, IgM, IgA, were decreased in rats treated with AFB1, but they increased after treatment with N. sativa or Curcuma longa. These biochemical observations were supported by histopathological examination of liver sections.

Treatment with N. sativa or Curcuma longa along with aflatoxin ameliorates aflatoxin-induced changes in serum parameters as liver and kidney function tests returning their level to near normal.

Key Words: Aflatoxin – Curcumin – Nigella sativa – Creatinine – Protein.

Introduction

AFLATOXINS are a group of naturally occurring, highly toxic mycotoxins. These fungal metabolites are produced by specific strains of Aspergillus flavus and Aspergillus parasiticus [1], a fungus which is especially found in areas with hot and humid climates. The climatic conditions of the Mediterranean countries favours mould infestation [2]. The most common form of aflatoxins is aflatoxin B1 (AFB1). Aflatoxin constitutes a real threat to the health of livestock as well as humans by their continuing intermittent occurrence in both feeds and foods [3]. Aflatoxins especially aflatoxin B1 are known to be genotoxic and carcinogenic, can produce acute necrosis, cirrhosis and carcinoma of the liver [4,5]. IARC 1987 [6] has classified aflatoxin as a group one carcinogen. Mixtures of aflatoxins and aflatoxin B1 have been tested for carcinogenicity in several strains of mice and rats, and other species. Oral administration of mixtures of aflatoxins and/or aflatoxin B1 to different species caused hepatocellular and/or cholangiocellular liver tumours, including carcinomas in almost all species except mice. Additionally, in some species, they produce tumours at other sites in the body as kidney and colon.

Aflatoxicosis is poisoning that result from ingestion of aflatoxins in contaminated food or feed. For aflatoxins, liver is the primary target organ for toxicity in all species studied. The precise manifestations of toxicity depend upon a number of factors, including dose and duration of exposure. AFB1 is
also biotransformed by P450 enzymes to yield an electrophilic epoxide, which attacks the DNA to initiate hepatotoxicity and genotoxicity via oxidative damage [7], and also induction of mutations by interchelating into DNA, through forming an adduct with guanine moiety in the DNA [8].

Black seed is used for treating digestive tract conditions including gas, colic, diarrhea, dysentery, constipation, and hemorrhoids. It is also used for respiratory conditions including asthma, allergies, cough, bronchitis, emphysema, flu, and swine flu, con- gestion, high blood pressure, cancer, parasitic worms of intestine, and boosting the immune system [9,10].

Curcuma longa is used for treating biliary disease, anorexia, cough, hepatic disorder rheumatic pain [11]. A variety of pharmacological effects of curcumin have been reported, including anti-inflammatory, anti-oxidant, anticarcinogenic [12], hypolipidemic and antidiabetic (hypoglycaemic) activities [13]. Curcumin is thought to play a vital role against these pathological conditions [14]. It has been reported that the anti-cancer property of curcumin is mediated in part by its anti-angiogenic activity [15]. Components of Curcuma longa have been shown to be non-toxic and inhibit mediators of inflammation as NF-kappa B, cyclooxygenase-2 (COX-2), lipooxygenase (LOX), and inducible nitric oxide synthase (iNOS) [16].

Curcuma longa (curcumin), Nigella sativa (black seed) are extensively used in cousin and in traditional medicines so we tried to investigate their role as hepatoprotective agents from natural products against AFB 1-induced hepatotoxicity in male Sprague-Dawley rats.

Aim of the work: The present investigation aimed to evaluate the ameliorative effect of Curcuma longa or Nigella sativa on aflatoxin B 1-induced hepatotoxicity in male rats.

Material and Methods

Chemicals: All chemicals used were of high analytical grade, product of Sigma (USA), Merk (Germany) and BDH (England). AFB 1 was obtained from Sigma chemical company (St. Louis, Missouri, USA).

Animals:

Eighty male Sprague-Dawley rats weighing 200-239gm (mean: 215.5 ± 1.27) were housed individually under standard conditions and fed Standard rat diet for 10 days (adaptation period) and given water ad libitum.

Experimental design:

The rats were divided into four groups (20 rats each). Group 1, 2, 3 & 4 (G1-G4) were fed standard control diet for 10 days (adaptation period); after that G2, 3 & 4 will be infected with aflatoxin B 1 through single intraperitoneal injection (1.0mg AFB 1/kg body weigh, dissolved in 7% dimethyl-sulfoxide (DMSO)); G1 will be given single intraperitoneal injection of 7% DMSO. The 4 groups will continue feeding on their specific previous diet as shown below. The standard rat chow diet (AIN-93 M diet formulated for adult rodents) was prepared according to [17]. Rat diet and body weights were also recorded weekly.

1- Group 1 (Normal control, G1): Rats fed on standard rat diet; they were injected with single intraperitoneal injection of 7% dimethylsulfoxide (DMSO) and kept on standard diet [18].

2- Groups 2, 3 & 4: Aflatoxin B 1 treated rats, G2, G3 & G4: Rats infected with Aflatoxin B 1 through single intraperitoneal injection (1.0mg AFB 1/kg body weigh, dissolved in 7% dimethyl-sulfoxide (DMSO) [18], fed on standard rat diet supplemented with:
  • G2: No supplements (standard diet only).
  • G3: Curcuma longa (curcum, 1000mg/kg BW/day, modified from [19]).
  • G4: Nigella sativa (black seed, 500mg/kg BW/day, modified from [20]).

At the end of the experiment (6 weeks after injection), animals in all groups were fasted overnight, then sacrificed under ether anesthesia (sigma, USA). Blood samples were taken from hepatic portal vein. The organs as kidney, liver, spleen, brain, testis and the heart from different animal groups were immediately removed, washed with saline, dried then weighed. Part of the organs is kept in 10% formalin for histopathological examination. Relative liver weight (RLW) was calculated as follow:

\[
\text{RLW} = \left( \frac{\text{Liver weight (LW)}}{\text{Final body weight (FBW)}} \right) \times 100
\]

The protein, urea, creatinine and uric acid contents were estimated by the method of (21-24 respectively). Alkaline phosphatase (ALP) activities were measured by the methods of [25]. Alanine and aspartate transaminase (ALT & AST) activities were measured spectrophotometrically by the methods of [26]. Plasma Lactate dehydrogenase (LDH) was determined using kinetic endpoint kits (SGM Italia, Rome, Italy) according to the method of [27]. Plasma malondialdehyde (MDA) was determined according to the method of [28]. Immuno-globulins biomarkers were estimated using Rat
ELISA Kits supplied by Kamiya Biomedical Company (12779 Gateway Drive, Seattle, WA 98168) as Rat IgA ELISA for the quantitative determination of IgA in rat biological samples, Cat. No. KT-416; Rat IgM ELISA for the quantitative determination of IgM in rat biological samples, Cat. No. KT-419; Rat IgG ELISA for the quantitative determination of IgG in rat biological samples, Cat. No. KT-418.

Histological examination:

The organs were kept in 10% formalin for histological examination, dehydrated, cleared in xylol and embedded in parablast. Paraffin sections were cut serially at 6mm thickness and stained by hematoxylin and eosin (Hx & E) as described by [29].

Analysis of curcuma longa (curcumin), nigella sativa (black seed):

Curcuma longa (curcumin) and Nigella sativa (black seed) were subjected to chemical analysis for their nutritive value [30,31]. Also they were subjected to microbiological and Aflatoxin analysis before introducing in the diet.

1- Microbiological examination: Aerobic and anaerobic bacterial count was done according to [32]. Enumeration of Coliform, Fecal coliform, Staphylococcus aureus, mold and yeast count according to [33,34]. Enumeration of Bacillus cereus according to [35].

2- Aflatoxin content: Sample preparation procedure was performed according to the instruction of the test kit (Rida Aflatoxin column Art No: R 5001/5002, R-Biopharma, Darmstadt, Germany [36]). Test procedure of total Aflatoxins were according to Rida screen Aflatoxin total (Art No: 470 1) test kit [37]. Test procedure of AFB1, according to Rida screen Aflatoxin B1 30/15 (Art No: 1211) test kit [38].

Statistical analysis:

The results are expressed as Mean±SEM. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the SPSS software package for Windows. Post hoc testing was performed for inter-group comparisons using the Tukey. p<0.05 was considered significant.

Results

The average actual amount of C. longa or N. sativa (gm/day/kgm bw) eaten were 0.998±0.015 and 0.498±0.005 gm respectively which go with the proposed amount in the experimental design (1000 or 500mg/kgm bw/day of C. longa or N. sativa respectively).

1- Changes in body weight:

Initial body weights (IBW) were comparable between all studied groups. Results of our study (Table 1, Figs. 1,2) reveal significant reduction (p<0.01) of body weight in groups treated with AFB1 compared with normal control group. The reduction in body weight Gain reach-15.2% and the increase in RLW reach 3.75 vs 2.36, (AFB1 treated group vs normal control group respectively). Supplementation of diets with Curcuma longa (curcumin) or Nigella sativa (black seed) improved body weight gain and liver weight in comparison with AFB1 treated groups. Body weight gain (BWG) range was 64.27-76.13g.

2- Total protein and immunoglobulin (Table 2):

In this study, there was a significant decrease in the mean value of total protein and Immunoglobulin (IgG, IgM, and IgA) in AFB1 treated groups which agree with [39,40]. Our results showed that treatment of AFB1 infected rats with Curcuma longa or Nigella sativa at the same time ameliorates AFB1-induced changes in the protein content which agree with [41].

3- Kidney and liver function and mda in plasma and liver (Table 3):

The result of the study clearly indicates that the administration of aflatoxin caused, as compared to the normal controls, significantly higher level of serum urea, creatinine uric acid, alkaline phosphatase, AST and ALT of rats which agree with [42]. Our results showed that treatment of AFB1 infected rats with Curcuma longa or Nigella sativa at the same time ameliorates AFB1-induced changes in the level of serum urea, creatinine uric acid, alkaline phosphatase, AST and ALT of rats.

The result of this study reveal increase in MDA (index of lipid peroxidation, LPO) level in liver of AFB1-treated rats compared with normal control. The results of our study showed that there was a significant reduction in the serum enzymes represents liver function. The serum levels of the liver enzymes were also lowered in this group showing improvement of the liver functions which agree with [43]. In our study, the results of Curcuma longa or Nigella sativa are closely similar with no noticeable significant difference between them with the doses given here.

4- Histopathological examination:

- Kidneys: Histopathologically, kidneys of normal control group show normal histological structure of renal parenchyma (Fig. 1). Meanwhile, kidneys of AFB1 infected group revealed dilation, congestion of renal blood vessels (Fig. 2A) and
vacuolization of epithelial lining renal tubule (Fig. 2B). Kidney of AFB+ +C. longa or N. sativa treated group revealed no histopathological change (Figs. 3,4).

- Liver: Microscopically, liver of normal control group shows normal histological structure of hepatic lobule (Fig. 1). Examination of sections from AFB+ infected group showed Kupffer cells activation (Fig. 2A) and vacuolization of hepatocytes (Fig. 2B) and fibroplasias in the portal triad (Fig. 2C). Moreover, liver of AFB+ +C. longa treated group showed cytoplasmic vacuolization of hepatocytes (Fig. 3,3A,B). Normal histological structure (normal hepatocytes) was noticed in AFB+ +N. sativa treated group (Fig. 4).

- Brain: Microscopically, brain of normal control group shows normal histological state in normal control group (Fig. 1). Examination of sections from AFB+ infected group showed pyknosis of neurons (Fig. 2A), neuropathia of pyknotic neurons (Fig. 2B), intracellular aedema (Fig. 3B). Brain of AFB+ +C. longa or N. sativa treated group revealed no histopathological change (Figs. 3,4).

Heart: Microscopically, heart of normal control group shows normal histological structure of normal cardiac myocytes (Fig. 1). Examination of sections from AFB+ infected group showed intermuscular leucocytic cells infiltration (Fig. 2A) and intramuscular aedema (Fig. 2B). However, heart of AFB+ +C. longa or AFB+ +N. sativa treated group revealed no histopathological changes (Figs. 3,4).

Testis: Microscopically, Examination of testis of normal control group showing normal seminiferous tubules (Fig. 1). Meanwhile, testis of AFB+ infected group showed degeneration of spermatogoneal cells lining seminiferous tubules (Fig. 2). However, testis of AFB+ +C. longa or AFB+ +N. sativa treated group revealed no histopathological changes (Figs. 3,4).

Spleen: Microscopically, Examination of spleen of normal control group showing normal lymphoid follicles (Fig. 1). Atrophy of lymphoid follicles was noticed in Spleen of AFB+ infected group (Fig. 2). However, Spleen of AFB+ +C. longa or AFB+ +N. sativa treated group revealed no histopathological changes (Figs. 3,4).

Table (1): Initial, final body weight (IBW, FBW), body weight gain (BWG), liver weight (LW), relative liver weight and actual amount of C. longa or N. sativa eaten (gm/day/kgm bw).

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<thead>
<tr>
<th></th>
<th>IBW (gm)</th>
<th>FBW (gm)</th>
<th>BWG (gm)</th>
<th>LW (gm)</th>
<th>RLW (gm)</th>
<th>C. longa or N. sativa eaten (gm/day/kgm bw)</th>
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<tr>
<td>G1: Normal control</td>
<td>215.93±2.47</td>
<td>285.33±2.73</td>
<td>69.40±1.03</td>
<td>6.74±0.12</td>
<td>2.36±0.04</td>
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<tr>
<td>G2: AFB+ treated</td>
<td>216.20±2.15</td>
<td>201.00±2.42</td>
<td>1.15±2.14</td>
<td>7.51±0.15</td>
<td>3.75±0.10</td>
<td>0.98±0.015</td>
</tr>
<tr>
<td>G3: AFB+ +N. sativa treated</td>
<td>200.00±3.37</td>
<td>292.00±3.66</td>
<td>76.13±2.20</td>
<td>6.92±0.08</td>
<td>2.37±0.04</td>
<td>0.98±0.015</td>
</tr>
<tr>
<td>G4: AFB+ +C. longa treated</td>
<td>214.07±2.23</td>
<td>278.33±1.97</td>
<td>64.27±0.73</td>
<td>6.96±0.15</td>
<td>2.50±0.05</td>
<td>0.98±0.015</td>
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Table (2): Effect of curcuma longa or nigella sativa on t. protein, IgG, IgM and IgA.

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<th>T. Protein</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
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<td></td>
<td>gm/dl</td>
<td>gm/dl</td>
<td>gm/dl</td>
<td>gm/dl</td>
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<tr>
<td>G1: Normal control</td>
<td>6.18±0.07</td>
<td>723.69±7.82</td>
<td>177.47±1.84</td>
<td>48.33±1.19</td>
</tr>
<tr>
<td>G2: AFB+ treated</td>
<td>3.98±0.03a</td>
<td>576.72±11.43a</td>
<td>154.93±1.83a</td>
<td>31.43±0.49a</td>
</tr>
<tr>
<td>G3: AFB+ +N. sativa treated</td>
<td>5.70±0.01ab</td>
<td>655.75±7.29ab</td>
<td>166.02±1.95ab</td>
<td>40.53±0.70ab</td>
</tr>
<tr>
<td>G4: AFB+ +C. longa treated</td>
<td>5.36±0.00abc</td>
<td>656.20±6.82abc</td>
<td>165.43±2.31abc</td>
<td>39.94±0.53abc</td>
</tr>
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Table (3): Effect of curcuma longa or nigella sativa on liver and kidney function tests, LDH, P. MDA and L. MDA.

<table>
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<tr>
<th></th>
<th>Urea</th>
<th>Creatinine</th>
<th>Uric</th>
<th>Alk phos</th>
<th>AST</th>
<th>ALT</th>
<th>LDH</th>
<th>P. MDA</th>
<th>L. MDA</th>
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<tr>
<td></td>
<td>mg/dl</td>
<td>IU/ml</td>
<td>Nmol/ml</td>
<td>Nmol/gm tissue</td>
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<tr>
<td>G1: Normal control</td>
<td>37.01±0.56</td>
<td>1.00±0.02</td>
<td>2.65±0.01</td>
<td>60.71±1.30</td>
<td>35.32±1.63</td>
<td>38.39±1.24</td>
<td>712.21±17.10</td>
<td>63.84±0.86</td>
<td>66.01±1.19</td>
</tr>
<tr>
<td>G2: AFB+ treated</td>
<td>60.65±0.58a</td>
<td>2.74±0.03a</td>
<td>5.54±0.11a</td>
<td>84.24±0.78a</td>
<td>116.05±1.27a</td>
<td>51.99±1.04a</td>
<td>887.64±8.74a</td>
<td>134.36±2.60a</td>
<td>139.17±2.92a</td>
</tr>
<tr>
<td>G3: AFB+ +N. sativa treated</td>
<td>47.34±1.14ab</td>
<td>1.56±0.03ab</td>
<td>3.05±0.12ab</td>
<td>71.11±1.41ab</td>
<td>85.25±0.82ab</td>
<td>44.61±1.04ab</td>
<td>785.16±8.94ab</td>
<td>89.47±0.97ab</td>
<td>86.58±1.04ab</td>
</tr>
<tr>
<td>G4: AFB+ +C. longa treated</td>
<td>46.64±0.73ab</td>
<td>1.49±0.04ab</td>
<td>2.99±0.03ab</td>
<td>72.02±0.60ab</td>
<td>63.93±0.70ab</td>
<td>42.12±0.20ab</td>
<td>778.60±5.81ab</td>
<td>85.03±1.99ab</td>
<td>82.37±1.81ab</td>
</tr>
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</table>

Fig. (1): Effect of *Curcuma longa* and *Nigella sativa* on initial body weight (IBW), final body weight (FWG) and body gain (BWG).

Fig. (2): Effect of *Curcuma longa* and *Nigella sativa* on liver weight (LW (gm)), and relative liver weight (RLW).

Histopathological Figs. (1-6): Histopathological examination of different organs under study: Kidney, Liver, Brain, Heart, Testis and Spleen

I- Kidney

Fig. (1): Kidney of normal control group shows normal histological structure of renal parenchyma (H & E X 400).

Fig. (2A): Kidney of AFB\(_1\) infected group showing dilation and congestion of renal blood vessels (H & E X 400).

Fig. (2B): Kidney of AFB\(_1\) infected group showing vacuolization of epithelial lining renal tu bule (H & E X 400).

Fig. (3): Kidney of AFB\(_1\) + *C. longa* treated group showing no histopathological change (H & E X 400).

Fig. (4): Kidney of AFB\(_1\) + *N. sativa* treated group showing no histopathological change (H & E X 400).
II- Liver

Fig. (1): Liver of normal control group showing normal histopathological structure of hepatic lobule (H & E X 400).

Fig. (2A): Liver of AFB1 infected group showing Kupffer cells activation and vacuolization of hepatocytes (H & E X 400).

Fig. (2B): Liver of AFB1 infected group showing vacuolization of hepatocytes (H & E X 400).

Fig. (2C): Liver of AFB1 infected group showing fibroplasias in the portal triad (H & E X 400).

Fig. (3A): Liver of AFB1 + C. longa treated group showing cytoplasmic vacuolization of hepatocytes (H & E X 400).

Fig. (3B): Liver of AFB1 + C. longa treated group showing cytoplasmic vacuolization of hepatocytes (H & E X 400).

Fig. (4): Liver of AFB1 + N. sativa treated group showing normal hepatocytes (H & E X 400).

III- Brain

Fig. (1): Brain of normal control group showing no histopathological change (H & E X 400).

Fig. (2A): Brain of AFB1 infected group showing pyknosis of neurons (H & E X 400).

Fig. (2B): Brain of AFB1 infected group showing neurophagia of pyknotic neurons (H & E X 400).
**IV- Heart**

Fig. (1): Heart of normal control group showing normal cardiac myocytes (H & E X 400).

Fig. (2A): Heart of AFB infected group showing intramuscular leucocytic cells infiltration (H & E X 400).

Fig. (2B): Heart of AFB infected group showing intracellular edema (H & E X 400).

Fig. (3): Heart of AFB + C. longa treated group showing no histopathological change (H & E X 400).

Fig. (4): Heart of AFB + N. sativa treated group showing no histopathological change (H & E X 400).
V- Testis

Fig. (1): Testis of normal control group showing normal seminiferous tubules (H & E X 400).

Fig. (2A): Testis of AFB$_1$ infected group showing degeneration of spermatogonial cells lining seminiferous tubules (H & E X 400).

Fig. (2B): Testis of AFB$_1$ infected group showing degeneration of spermatogonial cells lining seminiferous tubules (H & E X 400).

Fig. (3): Testis of AFB$_1$ + C. longa treated group showing no histopathological change (H & E X 400).

Fig. (4): Testis of AFB$_1$ + N. sativa treated group showing no histopathological change (H & E X 400).

VI- Spleen

Fig. (1): Spleen of normal control group showing normal lymphoid follicle (H & E X 400).

Fig. (2): Spleen of AFB$_1$ infected group showing atrophy of lymphoid follicle (H & E X 400).

Fig. (3): Spleen of AFB$_1$ + C. longa treated group showing no histopathological change (H & E X 400).

Fig. (4): Spleen of AFB$_1$ + N. sativa treated group showing no histopathological change (H & E X 400).
Discussion

Aflatoxin especially AFB1 are the most common mycotoxin to which humans and animals are exposed through food and feed.

Little information is available concerning effect of N. sativa on AFB1-induced toxicity especially on some parameters as LDH, Immunoglobulins and ALk Phos.

1- Safety of Curcuma Longa L:

Curcumin, demethoxycurcumin, bisdemethoxycurcumin and cyclocurcumin are the four principal curcuminoiids obtained from the coloured extracts of dried roots from turmeric [44,45]. One of the most prominent features of curcumin is its extremely good tolerance and its very low toxicity and side effects. However, although turmeric and curcumin are natural products used in the diet, the doses used in clinical trials exceed those consumed in the diet; therefore, systemic toxicity studies are needed. Curcumin is Generally Recognized As Safe by the Food and Drug Administration (FDA), and this compound has been granted an Acceptable Daily Intake level of up to 3mg/kg bw by the Joint FAO and WHO Expert Committee on Food Additives, 1996 [46]. No studies in either animals [47,48] or humans [49] have found any toxicity associated with the consumption of curcumin even at very high doses. Pharmacologically, curcumin has been found to be safe. Human clinical trials indicated no dose-limiting toxicity when administered at doses up to 10g/day [12].

The bioavailability of oral curcumin is low because 40-65% of curcumin passes through the gastrointestinal tract unchanged. Most of the absorbed curcumin is metabolized via glucuronidation to glucuronide and glucuronide/sulfate metabolites in the intestinal mucosa and liver.

• Safety of Nigella Sativa L:

There is insufficient reliable information available about the safety of black seed for its other uses and it is likely safe when used orally in amounts found in foods. However, the mutagenic effect of aqueous extract in primary rat hepatocyte culture was recently reported [50]. Also A minimal cytotoxicity was reported for ethyl and butanol extracts toward normal human peripheral blood mononuclear cells. Thymoquinone (TQ, one of the reactive components of black seed) demonstrated cyto-and genotoxic effects. It could be possibly due to the metabolic conversion of TQ to reactive species thereby increased oxidative stress, which contributes to the depletion of antioxidant enzymes and damage to DNA in hepatocytes treated with high thymoquinone concentrations [51].

• Mechanism of Aflatoxin Toxicity:

Many approaches have been investigated trying to figure out and understand Aflatoxin toxicity. Aflatoxins B1 are the most one that has been extensively studied. Other aflatoxins have not been so extensively investigated, but in a variety of studies aflatoxin B2, G1, G2, and M1 have all shown evidence of genotoxicity. One approach is that Aflatoxin B1 has shown to possess genotoxic potential in a variety of test systems, inducing DNA damage, gene mutation and chromosomal anomalies in human and animal cells’ culture, while in other systems as insects, bacteria, it induces DNA damage and/or gene mutations through many investigations. Second approach through its metabolism in the body (liver) where aflatoxin B1 is metabolised to a highly reactive chemical compound, called the 8, 9-epoxide where it binds very rapidly to protein, DNA and other important constituents of living cells’ culture, forming ‘adducts’. Formation of these adducts disrupts the normal working processes of the cell, and in the case of DNA adducts, can ultimately lead to a loss of control over cellular growth and division. Humans metabolise aflatoxin B1 to the major aflatoxin B1-7-guanine adduct at levels comparable to those in species which are susceptible to aflatoxin-induced hepatocarcinogenicity, such as the rat. However, both humans and animals possess enzyme systems which are capable of reducing the damage to DNA and other cellular constituents caused by the 8, 9-epoxide, as glutathione S-transferase which mediates the reaction (termed conjugation) of the 8, 9-epoxide to the endogenous compound glutathione. This essentially neutralises its toxic potential.

Humans have less glutathione S-transferase activity for 8, 9-epoxide conjugation than rats or mice, suggesting that humans are less capable of detoxifying this important metabolite. Humans possess the biochemical processes necessary for aflatoxin-induced carcinogenesis. Thus, presence of DNA and protein aflatoxin adducts urinary excretion of aflatoxin B1-7-guanine adducts and the ability of tissues to activate aflatoxin B1 have all been demonstrated for humans [52-58].

2- Body weight:

Results of our study reveal reduction of body weight in groups treated with AFB1 and this may be due to anorexia, inhibition of protein and DNA synthesis and lipogenesis. Lower body weight Gain (-15.2%) and higher RLW (3.75 vs 2.36, AFB1 treated group vs normal control group respectively)
are in agreement with [39,40]. Supplementation of diets with Curcuma longa (curcum), Nigella sativa (black seed) improved body weight gain and liver weight in comparison with AFB \(1\) treated groups suggesting antioxidant protection. Curcuma longa contain curcumin, the active ingredients, which is known to inhibit the biotransformation of AFB \(1\) to aflatoxicol in liver and also is responsible for its anti mutagenic and anticarcinogenic action [52-59].

3- **Total protein and immunoglobulins:**

Little work is done regarding effect of Curcuma longa (curcum), and Nigella sativa (black seed) on immunoglobulins. In this study, there was a significant decrease in the mean value of total protein and Immunoglobulin (IgG, IgM, and IgA) in AFB \(1\) treated groups, which consider as indicative of the toxic effect of AFB \(1\) in liver. These results agree with [39,40] who stated that curcumin has the ability to induce choleretic hepatoprotection and it is considered as a potent immunomodulatory agent. Aflatoxin is known to impair protein biosynthesis by forming adducts with DNA, RNA and proteins inhibit RNA synthesis, DNA-RNA polymerase activity and causes degranulation of endoplasmic reticulum [60]. Also the reduction may be due to increased necrosis in the liver. Our results showed that treatment of AFB \(1\) infected rats with Curcuma longa or Nigella sativa at the same time ameliorates AFB \(1\)-induced changes in the liver protein content which agree with [41]. This change may be due to increased DNA synthesis and reduction in harmful adduct formation. Curcuma longa, and Nigella sativa are powerful antioxidant agent, scavenge or neutralize the free radicals, inhibits oxidative enzymes as cytochrome P450, and inhibit peroxidation of membrane lipids and maintain cell membrane integrity and their function in the liver and kidney. Li and Liu, 2005 [61] stated that curcumin can regulate immune function of mice in a dose dependent manner. The possible underlying mechanism might be its ability to suppress the activity of NF kappa B P65.

4- **Kidney function:**

Administration of aflatoxin caused, as compared to the normal controls, significantly higher level of serum creatinine of rats (Fig. 3) which agree with [42] (they reported the occurrence of nephrotoxicity and the elevation of creatinine in serum and urine of rabbits receiving aflatoxin-contaminated feed (15mg/kg) for 60 days) and agree also with [62] (they reported rises in creatine and creatinine concentrations in the serum and urine of aflatoxin-fed rabbits). Creatine is synthesized in the liver, passes into circulation and is taken up almost entirely by skeletal muscle for conversion to creatine phosphate, and then both creatine and creatine phosphate are converted spontaneously into creatinine [63]. Both are filtered at glomerulus. Although there may be some additional secretion of creatinine by renal tubules, creatine is reabsorbed by the tubules at low plasma concentration. This ensures that there is little or no creatine in urine [63]. The appearance of creatinine in the serum of aflatoxin-fed rabbits indicates the increased transformation of phosphocreatine to creatinine in muscle which might be due to lesser utilization of phosphocreatine in muscular contraction. Histopathological studies revealed dilation, congestion of renal blood vessels (Fig. 2A) and vacuolization of epithelial lining renal tubule (Fig. 2B) in the kidney of aflatoxin-fed rats. Thus significant increase in creatinine concentration in serum could be due to increased release from muscles and/or decrease excretion from the kidney. Curcumin when given along with aflatoxin, ameliorates aflatoxin-induced effects in the serum parameters as compared to the aflatoxin alone treated rats. These changes could be due to amelioration in aflatoxin-induced histopathological changes in kidney.

5- **MDA and liver function:**

The result of the study clearly indicates that the increase in MDA (index of lipid peroxidation, LPO) in liver of AFB \(1\)-treated rats compared with normal control suggesting liver oxidative damage. The level of MDA return to near normal after treatment with Curcuma longa (curcum), and Nigella sativa (black seed). AFB \(1\) is well known as a hepatotoxin. The AFB \(1\) induced hepatotoxicity has been referred to the excessive formation of free radicals and nitric oxide (NO.) formed during its detoxification in the hepatocytes by the cytochrome P450. Such reactive oxygen species cause the exhaustion of the natural antioxidants in the body tissues. They initiate lipid peroxidation thus increasing the malondialdehyde products of these reactions. Several pathological mechanisms are then triggered causing damage to parenchymal and non-parenchymal tissues in the liver. The uncontrolled, prolonged and/or massive production of nitric oxide radicals (NO.) could further lead to the later development of hepatic carcinogenesis. Chan et al., 1998 [64] reported that in vivo oral treatment of curcumin reduces iNOS mRNA expression in the liver of lipopolysaccharide (LPS)-injected mice by 50-70% i.e. suppression of NO production. Nigella sativa L has been encountered among the hepatoprotective herbs. Its active con-
stituent thymoquinone (TQ), considered as a potent antioxidant. The results of our study showed that there was a significant reduction in the serum enzymes representing liver function. The enzymes AST and ALT are present in the cytosol of the hepatocytes. The AST is also localized in the mitochondria. Whenever liver hepatocytes are damaged, these enzymes are released into the blood. A significant increase in AST and ALT activities indicates the damage to the cytosol and also to mitochondria. The results obtained in the present study indicate significant increase in ALT and AST activities in the aflatoxin treated rats (Fig. 4). On the other hand, aflatoxin treatment with curcumin showed marked recovery. Similar results have been reported by [65] with CCL4. Nigella sativa preserve the natural antioxidants in the cells by scavenging the superoxide anions [66,67]. The serum levels of the liver enzymes (AST, ALT, Alk phos) were also lowered in this group showing improvement of the liver functions [43]. The result of the study clearly indicates an increase in LDH activity in AFB1 treated rats. These results disagree with [68]. In our study, the results of Curcuma longa or Nigella sativa are closely similar with no noticeable significant difference between them with the doses given here. As mentioned before TQ of black seed has cyto-and genotoxic effects if taken in excessive amounts. Zaoui et al., 2002 [69] reported that aqueous extracts of Nigella sativa seeds were implicated in the induction of hepatocyte damage and liver dysfunction. Also curcumin protection of liver from inflammatory condition may be due to its anti-inflammatory effect through inhibition of expression of cyclo-oxygenase-2 [70].

Conclusion:

Treatment with N. sativa or Curcuma longa along with aflatoxin ameliorates aflatoxin-induced changes in serum parameters as kidney and liver function tests (urea, creatinine, uric acid, t. protein, immunoglobulin, AST, LDH, Alk Phos and MDA in plasma and liver).

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