

Ameliorative role of ethanolic extract of *Moringa oleifera* leaf on aflatoxin B₁-induced genotoxicity and biochemical alterations in rats

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Background/aim

The present study was conducted to assess the ameliorative role of *Moringa oleifera* leaf extract (MOLE) on genotoxicity and biochemical alteration of aflatoxin B₁ (AFB₁) in rats.

Materials and methods

The rat groups involved negative control, control of DMSO, positive control that received AFB₁ in DMSO (0.7 g/kg, body weight) four times weekly for 1 month, groups 4–6 that received the same dose of AFB₁ in DMSO at the same period plus MOLE doses (3.3, 4.0 and 4.7 g/kg) daily for 1 month, and groups 7–9 that received MOLE alone at the same doses for 15 days after cessation of AFB₁ treatment. Molecular genetic, cytogenetic, sperm, and biochemical studies were documented.

Results

Genetic and sperm results revealed that AFB₁ treatment induced significant elevation of genetic alterations and sperm abnormalities as compared with normal control. Biochemical studies showed that the treatment with AFB₁ disturbed the parameters of liver functions, where aspartate-transaminase, alanine-transaminase, and alkaline phosphatase were activated and bilirubin contents as well as the rate of malondialdehyde were increased significantly, but the endogenous antioxidative system (catalase, superoxide-dismutase activities and glutathione as well as total antioxidant capacity) and protein profile were reduced significantly. Moreover, kidney functions (urea, uric acid, and creatinine contents) were elevated under AFB₁ administration. The treatment with MOLE significantly minimizes the genetic alterations, sperm abnormalities, and biochemical destruction. These ameliorations were increased by increasing the dose level. Better findings were seen by using MOLE as a therapeutic agent than its using as a protective agent.

Conclusion

This study revealed that MOLE contains therapeutic factors used in curing of genotoxicity induced by AFB₁ in rats, and treatment of animals that were exposed to AFB₁ with MOLE significantly ameliorated the genetic, sperm, and biochemical parameters as compared with animals treated with AFB₁ alone.

Keywords:

aflatoxin B₁, biochemistry, genotoxicity, *Moringa oleifera*, rats

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Introduction

Aflatoxins (AFs) are considered to be a major class of mycotoxins. AFs are produced by filamentous fungi, especially the strains of *Aspergillus flavus* and *Aspergillus parasiticus* in feed stuffs [1]. AFs include approximately five types: B₁, B₂, G₁, G₂, and M₁. Aflatoxin B₁ (AFB₁) is a strong carcinogen and mutagen that induces hepatocellular carcinoma (HCC) and immunosuppression and causes also a lot of injuries in different organs and tissues. Testis and kidney as well as bone marrow cells were affected by exposure to AFB₁ [2,3]. The toxicity of AFB₁ might be owing to that during its metabolism in liver, reaction oxygen species (ROSs) are generated and formed. ROSs were

capable of attacking cell biomolecules including DNA, proteins, and lipids [4,5].

Darwish *et al.* [2] and Deabes *et al.* [6] detected high frequencies of chromosome aberrations in bone marrow and testis cells as well as spermatotoxicity in mice exposed to AFB₁. Moreover, Abdel-Rahim *et al.* [7] observed high rates of DNA damage, micronuclei, chromosome aberrations, and sperm abnormalities in

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mice treated with AFB₁ in comparison with untreated control. Eshak *et al.* [8] found in Japanese Quail that exposure to AFB₁ leads to significant DNA and micronuclei damage in comparison with normal control. Moreover, Eshak *et al.* [9] reported high frequencies of DNA fragmentation in mice receiving AFB₁ than those found in normal control. Induction of damage to the hepatic parenchyma might produce deleterious effects to the liver physiochemical functions [10,11]. Deabes *et al.* [6] observed that the treatment with AFB₁ significantly increased the liver tissue levels of malondialdehyde (MDA); however, the rate of glutathione (GSH) and activity of superoxide-dismutase (SOD) were significantly reduced in liver and kidney of mice. Moreover, Eshak *et al.* [9] reported a significant elevation of alanine-transaminase (ALT), aspartate-transaminase (AST), and MDA in liver tissues as well as significant increase of creatinine and serum uric acid concentrations in kidney tissues of mice exposed to AFB₁ compared with those found in normal control. Thus, AFB₁ induced DNA damage and mutation by oxidase systems which produce hydroxylated metabolites [5,7,12].

Therefore, the adverse effect of carcinogen (AFB₁) leads to increase of deterioration of metabolism of proteins, lipids, and carbohydrate [3,5], causing several health problems in humans and animals owing to the consumption of AF-contaminated diet [13]. To detoxify or eliminate the toxic effect of AFB₁ in the contaminated food, antioxidants were used without a reduction in the nutritional values. The nutritional additives were found to be important means for this purpose, where they contain a lot of natural antioxidants. These natural antioxidants were found to reduce the lesions induced by harmful toxicants [5]. *Moringa oleifera* plant, especially its leaves, was found to be an excellent source of natural antioxidants, which included, vitamins, essential sulfur-containing amino acids (cysteine and methionine), flavonoids, minerals, glucosinolates, kaempferol, sterols, and other compounds that are considered to have health beneficial effects [14–16]. These *Moringa oleifera* leaf extract (MOLE) antioxidants were also revealed to have strong properties as antimutagenic, anticarcinogenic, anti-inflammatory, and antifungal activity [16]. Rao *et al.* [17] observed that the percentages of micronuclei and chromosome aberrations were significantly minimized in the animals that are pretreated with *M. oleifera* leave extraction and then exposed to the radiation. Sathya *et al.* [16] reported that animals pretreated with MOLE and then injected with cyclophosphamide (CP) had

significantly decreased rates of micronuclei and DNA damage compared with those treated with CP alone. Radwan *et al.* [18] showed protective effects of MOLE against toxicity of CCL₄ to DNA, micronuclei, chromosome, and sperm shape compared with rats treated with CCL₄ alone. So, this study was carried out to evaluate the modulatory ameliorating role of MOLE on genotoxicity and cytotoxicity of AFB₁ in rats.

Materials and methods

Chemicals

Aflatoxin B₁

AFB₁ powder and DMSO (art no. 7029.1) were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Overall, 5 mg of AFB was dissolved in 250-ml DMSO [19].

Preparation of *Moringa oleifera* leaves extraction

Ethanolic extract of MOL was prepared according to Ugwu *et al.* [20] as following: the fresh leaves of *M. oleifera* plant were picked from trees grown on sand soil in El-Sharkia governorate, Egypt. The leaves were washed thoroughly with distilled water and dried at 29–35°C for 3 weeks, after which the leaves were pulverized into coarse form with acrestor high-speed milling machine. The coarse form (1000 g) was then macerated in absolute ethanol and was left to stand for 48 h. After that the extract was filtered through muslin cloth on a plug of glass wool in a glass column. The resulting ethanol extract was concentrated and evaporated to dryness using rotary evaporator at optimum temperature between 40 and 45°C to avoid denaturation of the active ingredients. The concentrated extract was diluted to 1000 ml using a polysaccharide as a carrier and stored in the refrigerator.

Experimental animals

Male albino rats of Sprague-Dawley strain weighing 120–150 g were obtained from the Animal House, National Research Centre, Egypt. Animals were housed in an ambient temperature of 25±3.2°C on light/dark cycle of 12/12 h. All rats were kept in clean polypropylene cages and administered food and water *ad libitum*. All animals were cared for, and experiments were carried out in accordance with the European Community guidelines for the use of experimental animals and approved by the Ethics Committee of the National Research Centre, Egypt [21].

Experimental design

A total of 72 rats were used and divided into nine equal groups:

Group 1 (control group) received saline intraperitoneal at dose of 0.7 g/kg four times (day by day) a week for 1 month.

Group 2 (DMSO group) received (intraperitoneal) DMSO at dose of 0.7 g/kg four times a week (day by day) for 1 month.

Group 3 (afla group) received (intraperitoneal) AFB₁ in DMSO (5 mg of AFB₁ in 250 ml DMSO) at dose of 0.7 g/kg four times (day by day) a week for 1 month.

Group 4–6 (afla+M1, afla+M2, and afla+M3) received (intraperitoneal) AFB₁ dissolved in DMSO at the same dose and way previously mentioned and for the same period. Starting on the first day of AFB₁ administrations, rats in 4–6 groups were treated (orally) daily for 1 month with MOLE (3.3, 4.0, and 4.7 g/kg, respectively) of the crude material that are equivalent to 561, 680, and 799 mg, respectively, of the extract, as each gram of the crude material yield contains 170 mg of the extract. These groups 4–6 were used to evaluate the protective role of MOLE against AFB₁.

Group 7–9 (M1, M2, and M3 groups) received (intraperitoneal) AFB₁ in DMSO in the same dose and way previously mentioned and for the same period, and then the rat groups were treated with MOLE (3.3, 4.0, and 4.7 g/kg, respectively) for 15 days. These groups 7–9 were used to evaluate the therapeutic effect of MOLE against AFB₁. Rats had free access to food and drinking water during the study. At the end of the experiment, blood samples were collected for biochemical study and then rats were killed by cervical dislocation for studying of the molecular genetics, cytogenetics, and sperm examination.

Evaluation of DNA damage

Assaying of DNA fragmentation using spectrophotometer

Liver samples were collected immediately after killing the animals. The tissues were lysed in 0.5 ml lysis buffer containing 10 mmol/l tris-HCL (pH, 8), 1 mmol/l EDTA, and 0.2% triton X-100 centrifuged at 10 000 rpm (Eppendorf) for 20 min at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and supernatant (S), 1.5 ml of 10% trichloroacetic acid was added, followed by incubation at 4°C for 10 min. The samples were centrifuged for 20 min at 10 000 rpm (Eppendorf) at 4°C, and the pellets were suspended in 750 µl of 5% trichloroacetic acid, followed by incubation at 100°C for 20 min. Subsequently to each sample, 2 ml of diphenylamine solution (200 mg diphenylamine in 10 ml glacial acetic acid, 150 µl of sulfuric acid and 60 µl acetaldehyde) was added, followed by incubation at room temperature for 24 h [22]. The proportion of

fragmented DNA was calculated from absorbance reading at 600 nm using the following formula:

DNA fragmentation

$$= \frac{\text{OD of fragmented DNA (S)}}{\text{OD of fragmented DNA (S)} + \text{OD of intact DNA (P)}} \times 100.$$

Cytogenetic analysis

Micronucleus test

Bone marrow slides were prepared according to the method described by Krishna and Hayashi [23]. The bone marrow was washed with 1 ml of fetal calf serum and then smeared on clean slides. The slides were left to air dry and then fixed in methanol for 5 min, followed by staining in May–Grunwald and Giemsa for 5 min, and then washed in distilled water and mounted. For each animal, 2000 polychromatic erythrocytes were examined for the presence of micronuclei.

Chromosome preparations

For chromosome analysis, both treated and control animals were killed by cervical dislocation at the end of experiment. An hour and a half or 2 h before killing, rats were injected intraperitoneally with 0.5 g colchicine/kg. Femurs were removed and the bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared using the method of Preston *et al.* [24]. Fifty metaphase spreads per animal were analyzed, for scoring the different types of chromosome aberrations.

Sperm analysis

For sperm-shape analysis, the epididymis was excised and minced in ~8 ml of physiological saline, dispersed, and filtered to exclude large tissue fragments. Smears were prepared after staining the sperms with eosin Y (aqueous), according to the methods of Wyrobek and Bruce [25] and Farag *et al.* [26]. At least 3000 sperms per group were assessed for morphological abnormalities. The sperm abnormalities were evaluated according to standard method of Narayana [27].

Biochemical studies

At the end of the first stage and then the end of the second stage (15 days) of the present experiment (30 days), blood samples were collected and centrifuged at 3000g to obtain serum for the determination of total cholesterol according to Watson [28], high-density lipoprotein cholesterol according to Burstein *et al.* [29], low-density lipoprotein cholesterol according to Schriewe *et al.* [30], and triglycerides according to

Megraw *et al.* [31]. The levels of creatinine [32], urea [33] and uric acid [34] were determined as indicators for kidneys function, whereas the activities of ALT, AST, and alkaline phosphatase (ALP) as well as the bilirubin content were determined according to Reitman and Frankel [35], Belfield and Goldberg [36], and Walter and Gerarde [37], respectively, as indicators of liver function. MDA content and the activities of catalase (CAT) and SOD were determined according to Ohkawa *et al.* [38], Beers and Sizer [39], and Nishikimi *et al.* [40], respectively. Total antioxidant capacity (TAC) had been determined by ELISA technique (kit no MBS733414; My BioSource Co.). The GSH content was determined according to Beutler *et al.* [41]. Total protein and albumin were determined according to Gornall *et al.* [42] and Doumas *et al.* [43], respectively, but globulin content was calculated by the following equation:

$$\text{Globulin} = \text{Total protein} - \text{albumin}.$$

Statistical analysis

Statistical analysis was performed with SPSS software Inc., Chicago, IL, USA. Data were analyzed using one-way analysis of variance followed by Duncan's post-hoc test for comparison between different treatments. Results were reported as mean $\pm$ SE, and differences were considered as significant when *P* value of less than 0.05.

Results

Results of DNA fragmentation

The present data (Table 1) revealed that the percentages of DNA fragmentation significantly elevated in rats injected with AFB₁ with respect to untreated control. In the rats that received MOLE as a protective or therapeutic agent, the values of DNA fragmentations significantly reduced as compared with those received AFB₁ alone. This reduction of DNA fragmentation was increased by increasing the dose

Table 1 Shows the ameliorative role of *Moringa oleifera* leave extraction on aflatoxin B₁-induced DNA fragmentation in rats

	Range	Mean $\pm$ SE
Control group	2.65–3.62	3.12 $\pm$ 0.23 ^a
DMSO	3.78–4.66	4.19 $\pm$ 0.20 ^b
Afla	11.68–12.59	12.19 $\pm$ 0.20 ^f
Afla+M1	8.76–10.22	9.48 $\pm$ 0.35 ^e
Afla+M2	5.98–7.53	6.56 $\pm$ 0.34 ^c
Afla+M3	5.75–6.67	6.34 $\pm$ 0.22 ^c
M1	6.86–8.72	7.69 $\pm$ 0.46 ^d
M2	5.46–6.77	6.08 $\pm$ 0.36 ^c
M3	4.85–6.90	5.55 $\pm$ 0.46 ^c

Means with different superscript letters a, b, c, d, e, f are significantly different at *P*<0.05.

level. The highest dose caused amelioration of genetic material and gave the lowest percentages of DNA fragmentation in comparison with other doses of MOLE. Moreover, best results were occurred by using MOLE as a therapeutic agent rather than MOLE as a protective agent.

Results of micronucleated polychromatic erythrocytes

The present findings (Table 2) observed that the rate of induction of micronucleated polychromatic erythrocytes (MNPCE) significantly increased in the animals injected with AFB₁ as compared with untreated control. In rat groups that were injected with AFB₁ and received MOLE as a protective or therapeutic agent, the percentages of MNPCE significantly decreased in comparison with rat group injected with AFB₁ alone. This decrease of induction of MNPCE was increased by increasing dose. The treatment with the highest dose (4.7 g/kg) gave the lowest percentage of MNPCE. Furthermore, the use of MOLE as therapeutic agent especially by using the highest dose enhanced more the genetic material recovery (by reducing the rate of MNPCE) and gave much better results than the use of MOLE as a protective agent.

Results of chromosome anomalies

The data reported in Table 3 showed that the treatment with AFB₁ caused highly significant increases in the frequencies of chromosome aberrations as compared with untreated control group. The rat groups that were injected with AFB₁ and received the MOLE as protective or therapeutic agent had significant decrease of chromosome aberrations in comparison with rat group injected with AFB₁ alone. The decrease in chromosome aberrations was increased by increasing the dose level of MOLE. The highest dose of MOLE as protective or therapeutic agent resulted in the lowest percentages

Table 2 The ameliorative role of *Moringa oleifera* leave extraction on aflatoxin B₁-induced micronucleated polychromatic erythrocytes in rats

Treatment	Polychromatic erythrocytes/mice	Mean $\pm$ SE
Control group	2000	4.25 $\pm$ 0.75 ^a
DMSO	2000	5.00 $\pm$ 0.82 ^a
Afla	2000	31.75 $\pm$ 1.70 ^f
Afla+M1	2000	27.25 $\pm$ 1.0 ^e
Afla+M2	2000	21.50 $\pm$ 1.10 ^d
Afla+M3	2000	18.50 $\pm$ 1.30 ^d
M1	2000	20.75 $\pm$ 1.00 ^d
M2	2000	14.80 $\pm$ 1.00 ^c
M3	2000	8.50 $\pm$ 1.28 ^b

Means with different superscript letters a, b, c, d, e, f are significantly different (*P*<0.05).

Table 3 Shows the ameliorative role of *Moringa oleifera* leave extraction on aflatoxin B₁-induced chromosome aberrations in rats

Treatment	Gaps	Breaks	Frag.	Deletions	Centromeric attenuation	End to end association	Rings	Total structural aberrations	Peridiploidy	Polyploidy	Total numerical aberrations
Control group	1.0±0.4 ^{a,b}	0.25±0.25 ^a	0.25±0.25 ^a	0.25±0.25 ^a	2.50±0.28 ^{a,b}	0.00 ^a	0.00 ^a	4.25±0.47 ^a	1.25±0.47 ^a	0.00 ^a	1.25±0.47 ^a
DMSO	1.5±0.2 ^{a,b,c}	0.25±0.25 ^a	0.50±0.5 ^a	0.50±0.28 ^a	2.75±0.47 ^{a,b,c}	0.00 ^a	0.00 ^a	5.50±0.64 ^a	1.75±0.50 ^{a,b}	0.00 ^a	1.75±0.50 ^{a,b}
Afla	2.5±0.50 ^c	4.5±0.28 ^e	4.25±0.50 ^e	16.5±0.64 ^e	3.5±0.20 ^{b,c}	1.5±0.28 ^b	1.50±0.29 ^b	34.2±1.10 ^g	4.5±0.50 ^c	1.00±0.57 ^{a,b}	5.5±0.50 ^d
Afla+M1	2.5±0.29 ^c	3.75±0.25 ^{d,e}	4.25±0.25 ^e	15.0±1.10 ^{d,e}	4.00±0.58 ^c	1.00±0.04 ^c	0.50±0.29 ^a	31.0±0.5 ^f	3.0±0.40 ^{b,c}	1.25±0.25 ^b	4.5±0.28 ^{c,d}
Afla+M2	1.50±0.41 ^{a,b,c}	3.50±0.64 ^{c,d,e}	2.5±0.29 ^{b,c,d}	14.00±0.40 ^d	3.0±0.41 ^{a,b,c}	0.50±0.29 ^b	0.00 ^a	5.0±0.81 ^d	3.5±0.29 ^c	1.0±0.4 ^{a,b}	4.5±0.64 ^{c,d}
Afla+M3	1.00±0.40 ^{a,b}	2.5±0.29 ^{b,c,d}	2.00±0.40 ^{b,c}	11.0±0.41 ^c	3.00±0.39 ^{a,b,c}	0.00 ^a	0.00 ^a	19.5±0.64 ^c	3.0±0.40 ^{b,c}	0.50±0.29 ^{a,b}	3.5±0.29 ^{b,c}
M1	2.00±0.41 ^{b,c}	3.00±0.41 ^{b,c,d}	3.50±0.65 ^{d,e}	15.00±0.71 ^{d,e}	4.00±0.41 ^c	0.00 ^a	0.50±0.28 ^a	28.0±1.2 ^e	3.50±0.64 ^c	0.50±0.28 ^a	4.0±0.91 ^{b,c,d}
M2	1.00±0.38 ^{a,b}	2.00±0.40 ^{a,b,c}	3.0±0.50 ^{c,d}	10.00±0.4 ^c	2.00±0.40 ^a	0.00 ^a	0.00 ^a	18.0±0.91 ^c	2.25±0.25 ^{a,b}	0.25±0.25 ^a	2.5±0.28 ^{a,b}
M3	0.50±0.40 ^a	1.50±0.64 ^{a,b}	1.50±0.28 ^{a,b}	7.00±0.39 ^b	2.00±0.38 ^a	0.00 ^a	0.00 ^a	12.5±1.1 ^b	1.75±0.25 ^{a,b}	0.00 ^a	1.75±0.25 ^a

All data were expressed as mean±SE. Means with different superscript letters a, b, c, d, e, f, g are significant different at $P < 0.05$.

of chromosome aberrations. Moreover, the use of MOLE as a therapeutic agent gave more amelioration in genetic material by decreasing more the chromosome aberration than the use of MOLE as a protective agent.

Results of sperm abnormalities

The present investigation on sperm examination (Table 4) revealed anomalies in head and in tail of sperms. Head sperm abnormalities included amorphous and without hock, whereas tail sperm anomalies involved coiled tail, terminal droplet, and bent tail. The injection with AFB₁ induced high significant of sperm abnormalities as compared with untreated control group. However, the treatment with MOLE as a protective or therapeutic agent caused significant minimization of sperm-shape abnormalities. This amelioration of sperm shape is increased by increasing of MOLE. The treatment with the highest dose (4.7 g/kg) led to more amelioration in sperm shape by decreasing the head and tail abnormalities than other dose levels. The use of MOLE as therapeutic agent was revealed to give the best results.

Biochemical results

Biochemical results (Table 5) showed that AFB₁ administration significantly changed liver function enzymes actively, such as ALP, AST and ALT. These paralleled with significant elevation in bilirubin content under the same conditions of the serum of AFB₁-intoxicated rats relative to those of normal control. Moreover, induction significantly stimulated ALP, ALT, and AST activities. Bilirubin serum content was insignificantly increased compared with that of normal control. Rats subjected to AFB₁ developed significant hepatocellular damage as evident from the serum bilirubin content and activities of ALP, ALT, and AST as compared with normal control, and they have been used as reliable markers of hepatotoxicity.

The AFB₁-intoxicated rats were treated with MOLE, which significantly alleviated the harmful effects of AFB₁. This effect was dose dependent, but the values were still more than healthy controls. In case of groups 7–9, MOLE treatments were continuously ingested into intoxicated rats with AFB₁. The aforementioned enzyme activity was more reduced companied with the plant extract ingestion. Their activities were around those of the normal health control. It means that the ingestion of MOLE with AFB₁ was able to decrease AFB₁ harmful effects. Regarding AFB₁ effects on rat kidneys function, results in Table 5 showed that urea, uric acid, and creatinine levels were unchanged for groups of DMSO relative to that of normal control, but

Table 4 Shows the ameliorative role of *Moringa oleifera* leave extraction on aflatoxin B₁-induced sperm abnormalities in rats

Treatments	Amorphous	Without hook	Total head	Coiled tail	Terminal droplet	Bent tail	Total tail	Total sperm abnormalities
Control	3.25±0.47 ^a	1.0±0.4 ^a	0.25±0.4 ^a	2.5±0.64 ^a	2.25±0.25 ^a	2.75±0.48 ^a	7.5±0.64 ^a	11.75±0.94 ^a
DMSO	3.75±0.62 ^a	2.25±0.47 ^{a,b}	6.0±0.40 ^{a,b,c,d}	2.5±0.50 ^a	2.75±0.62 ^a	3.50±0.64 ^{a,b}	8.75±0.7 ^{a,b}	14.75±1.0 ^{b,c}
Afla	5.5±0.64 ^b	5.0±0.7 ^c	10.5±0.28 ^f	5.0±0.7 ^c	7.0±0.91 ^b	9.0±0.4 ^e	21.0±0.7 ^f	31.5±0.86 ^f
Afla+M1	4.0±0.4 ^{a,b}	5.0±0.91 ^c	9.0±0.7 ^{e,f}	3.0±0.4 ^b	7.0±0.64 ^b	7.5±0.6 ^{d,e}	17.5±1.5 ^e	26.5±1.5 ^e
Afla+M2	3.5±0.65 ^a	4.0±0.71 ^{b,c}	7.5±0.5 ^{d,e}	2.0±0.4 ^{a,b}	6.75±1.0 ^b	5.0±1.0 ^c	13.75±0.94 ^d	21.25±1.2 ^d
Afla+M3	4.0±0.57 ^{a,b}	3.25±0.75 ^{b,c}	7.25±0.85 ^{c,d,e}	1.25±0.25 ^{a,b}	3.5±0.86 ^a	6.5±0.65 ^{c,d}	11.25±0.5 ^c	18.5±0.87 ^d
M1	2.75±0.47 ^a	4.0±0.4 ^{b,c}	6.75±0.86 ^{b,c,d}	2.75±0.48 ^{a,b}	3.0±0.7 ^a	5.0±0.4 ^{b,c}	10.75±0.47 ^c	17.5±0.64 ^{c,d}
M2	3.0±0.41 ^a	2.5±0.64 ^{a,b}	5.5±0.64 ^{a,b}	2.75±0.25 ^{a,b}	2.75±0.47 ^a	4.2±0.62 ^{a,b}	9.75±0.62 ^{b,c}	15.25±1.1 ^{b,c}
M3	3.0±0.39 ^a	2.0±0.4 ^{a,b}	5.0±0.4 ^a	1.25±0.62 ^a	2.75±0.4 ^a	3.5±0.64 ^{a,b}	7.5±0.47 ^a	12.5±0.64 ^{a,b}

All data are expressed as mean±SE. Means with different superscript letters a, b, c, d, e, f are significantly different at $P<0.05$.

Table 5 Liver and kidney functions of serum aflatoxin B₁-intoxicated rats treated with *Moringa oleifera* leaf extract

Treatments	ALT (IU/l)	%	AST (IU/l)	%	ALP (IU/l)	%	Bilirubin (mg/dl)	%	Urea (mg/dl)	%	Uric acid (mg/dl)	%	Creatinine(mg/dl)	%
Control	31.99±2.27 ^e	100	60.44±4.01 ^g	100	90.11±5.21 ^e	100	0.91±0.49 ^e	100	15.12±1.01 ^g	100	0.42±0.03 ^g	100	0.50±0.03 ^h	100
DMSO	33.00±194 ^e	103	64.10±3.87 ^g	106	96.02±6.00 ^e	107	1.00±0.62 ^{d,e}	102	17.00±0.89 ^f	112	0.45±0.03 ^g	107	0.53±0.03 ^g	106
Aflatoxin	55.21±3.07 ^a	173	112.16±7.21 ^a	186	140.07±b.41 ^a	155	1.79±0.69 ^a	106	47.77±3.32 ^a	316	1.89±0.10 ^a	450	2.01±0.18 ^a	402
Afla+M1	50.00±3.11 ^b	156	104.26±6.73 ^b	173	124.64±7.77 ^b	138	1.31±0.80 ^b	104	40.97±2.97 ^b	271	1.11±0.06 ^b	264	1.32±0.078 ^a	264
Afla+M2	47.21±2.86 ^{b,c}	148	97.00±6.66 ^c	160	116.33±6.21 ^{b,c}	129	1.22±0.79 ^c	98	35.11±2.79 ^c	232	0.90±0.06 ^c	214	1.09±0.06 ^c	218
Afla+M3	44.12±2.94 ^c	138	91.00±6.74 ^d	151	108.08±6.43 ^c	120	1.15±0.64 ^c	98	29.99±2.01 ^d	198	0.71±0.04 ^d	169	0.86±0.05 ^d	172
M1	40.21±3.00 ^d	126	82.17±6.0 ^e	136	98.00±6.01 ^e	109	1.02±0.62 ^d	104	22.78±1.78 ^e	151	0.60±0.03 ^e	143	0.73±0.04 ^e	146
M2	37.46±3.08 ^d	117	75.34±5.02 ^f	125	96.02±5.83 ^e	107	0.97±0.61 ^{d,e}	102	18.21±1.02 ^f	120	0.52±0.03 ^f	124	0.64±0.04 ^f	128
M3	33.00±2.65 ^e	103	66.17±4.07 ^g	109	92.00±5.77 ^e	102	0.93±0.57 ^e	98	16.97±0.98 ^f	112	0.46±0.03 ^g	110	0.57±0.04 ^g	114

In each column the same superscript letters mean insignificant differences, while the different superscript letters mean significant differences among the tested groups at $P=0.05$. ALP, alkaline phosphatase; ALT, alanine-transaminase; AST, aspartate-transaminase; %, parameters in treated groups to control group.

in AFB₁-intoxicated rats, creatinine, uric acid, and urea levels were highly significantly increased compared with normal control. All the treatments with MOLE ameliorated the undesirable effects of AFB₁ on kidneys. The same trend of liver function improvement was observed because of the continuous ingestion of MOLE without AFB₁. These effects were dose dependent. The best improvement was found for the ninth group indicate.

Concerning serum protein profile, Table 6 illustrates the total protein, albumin, and globulin in serum of the experimental rat groups. Rats of the DMSO group showed that the three parameters of protein profile were not significantly changed relative to control, but these fractions were significantly decreased under the ingestion of AFB₁ at normal control levels. The induction of AFB₁ with different doses of MOLE reduced disturbance in serum protein profile. In addition, more treatments with MOLE without AFB₁ removed the effects of AFB₁ ingestion in which serum protein profile (total protein, albumin, and globulin) was around to the control value, especially in the last two groups (G8 and G9). All the aforementioned results were dose dependent.

In case of endogenous peroxidation (MDA) and antioxidative system (SOD, CAT, and GSH) in the present experiment rats, results are shown in Table 2; DMSO (G2) induction insignificantly changed MDA and GSH contents as well as SOD and CAT activities in serum relative to normal content (G1). In contrast, AFB₁ ingestion (G3) elevated significantly the level of MDA, but reduced the GSH content. Activities of SOD and CAT were decreased significantly under the induction of AFB₁. The treatments by MOLE together with AFB₁ slightly significantly ameliorated the adverse effects of AFB₁-induced toxicity, such that MDA was decreased but GSH was increased. Moreover, SOD and CAT activities were stimulated relative to intoxicated control (G3). For the TAC (Table 2), the value had similar trend that TAC was unchanged by DMSO induction, but the other treatments significantly decreased by AFB₁ ingestion. The intoxicated control group (G3) showed the lowest value of TAC, and this value was elevated and improved gradually by treatment with MOLE together with AFB₁ ingestion. The data were dose dependent. The continuous treatments with MOLE without AFB₁ (G7, G8 and G9) into intoxicated rats resulted in more amelioration and improved the endogenous oxidative and antioxidative effects, such as the MDA value which decreased significantly at intoxicated control (G3), but was still higher than that of normal control. However, GSH and TAC were improved and increased

Table 6 Serum protein profile as well as endogenous peroxidation and antioxidant system of serum in aflatoxin B₁-intoxicated rats treated with *Moringa oleifera* leaf extract

Treatments	Total protein (g/dl)	%	Albumin (g/dl)	%	Globulin (g/dl)	%	MDA (nmol/ml)	%	SOD activity (U/ml)	%	CAT activity (U/ml)	%	GSH (μM/ml)	%	Antioxidative capacity (ng/ml)	%
Control	7.01±0.42 ^a	100	3.68±0.21 ^a	100	3.33±0.24 ^a	100	2.50±0.18 ^g	100	349.07±21.13 ^a	100	8.09±0.53 ^a	100	0.52±0.03 ^a	100	1.10±0.06 ^a	100
DMSO	6.90±0.39 ^a	98	3.60±0.19 ^a	98	3.30±0.21 ^a	99	2.69±0.19 ^g	108	333.09±20.71 ^a	95	8.00±0.50 ^a	99	0.48±0.030 ^a	22	1.11±0.07 ^a	101
Aflatoxin	5.62±0.32 ^b	80	3.00±0.17 ^b	82	2.62±0.18 ^b	79	5.00±0.28 ^a	200	242.10±18.11 ^c	69	4.63±0.30 ^c	57	0.27±0.019 ^d	52	0.80±0.05 ^c	73
Afla+M1	6.01±0.40 ^b	86	3.21±0.20 ^b	87	2.80±0.20 ^b	84	4.61±0.30 ^b	184	256.12±19.99 ^c	73	5.19±0.36 ^c	64	0.31±0.021 ^c	60	0.815±0.05 ^{b,c}	74
Afla+M2	6.30±0.37 ^b	90	3.34±0.22 ^{a,b}	91	2.96±0.19 ^{a,b}	89	4.22±0.29 ^c	169	272.02±21.11 ^{b,c}	78	5.82±0.38 ^{b,c}	72	0.3±0.030 ^{b,c}	69	0.864±0.06 ^{b,c}	78
Afla+M3	6.47±0.47 ^a	92	3.40±0.18 ^a	92	3.07±0.22 ^a	92	3.79±0.28 ^d	152	285.73±17.87 ^b	82	6.39±0.44 ^b	79	0.39±0.029 ^b	75	0.90±0.05 ^b	82
M1	6.54±0.45 ^a	93	3.46±0.22 ^a	94	3.08±0.17 ^a	92	3.38±0.27 ^e	135	295.00±19.51 ^b	85	6.82±0.42 ^b	84	0.43±0.030 ^b	83	0.94±0.06 ^{a,b}	86
M2	6.68±0.52 ^a	95	3.55±0.24 ^a	96	3.13±0.27 ^a	94	3.19±0.21 ^{e,f}	128	302.07±20.12 ^b	87	7.30±0.48 ^a	90	0.47±0.031 ^a	90	0.97±0.07 ^{a,b}	89
M3	6.89±0.51 ^a	98	3.64±0.27 ^a	9	3.25±0.26 ^a	98	3.00±0.20 ^f	120	310.78±21.21 ^b	89	7.61±0.43 ^a	94	0.49±0.034 ^a	94	1.00±0.07 ^a	91

In each column, the same superscript letters mean insignificant differences, whereas the different superscript letters mean significant differences among the tested groups at $P=0.05$. CAT, catalase; GSH, glutathione; MDA, malondialdehyde; %, parameters in treated groups to control group.

compared with G3, but their values were more than those of the normal control. The SOD and CAT activities, which were inhibited under the ingestion of AFB₁, were stimulated again by MOLE treatments relative to intoxicated control (G3), either with or without AFB₁ ingestion. The continuous treatments by MOLE without AFB₁ induction resulted in more improvements in the both activities. The aforementioned results were dose dependent. The best treatment results were seen in G8 and G9 in which MOLE was administrated with the highest doses without AFB₁.

Discussion

In the present study, the mutagenic effect of AFB₁ in rat cells was observed by inducing high rates of DNA damage, micronuclei, chromosome aberrations, and sperm-shape abnormalities. These findings were similar with that observed in previous studies: concerning the inducing of DNA damage, the exposure to AFB₁ had induced DNA fragmentation in different animal species [8,9]. Moreover, high frequencies of micronuclei and chromosome aberrations were observed in animals treated with AFB₁ as compared with untreated controls [2,6,7,44]. Moreover, sperm-shape abnormalities were found to be significantly increased in animals receiving AFB₁ in comparison with normal control groups [2,6,7].

The genotoxic effect of AFB₁ might be owing to that this carcinogen when metabolized in the liver and activated by the system of cytochrome P450 enzyme causes production of AFB₁-8,9 epoxide. This epoxide binds with nucleophilic sites in DNA producing AFB₁-DNA adduct and consequently leads to induction of DNA damage, micronuclei, chromosome aberrations, and sperm abnormalities [4,45]. On the contrary, several studies concluded that during the metabolic processes for AFB₁ in liver, the oxidative stress was induced by forming intracellular ROSs that include hydroxyl radical ($^{\bullet}\text{OH}$), hydrogen peroxide (H_2O_2) and superoxide anion ($\text{O}^{\bullet -}$). These radicals attack cell components (DNA, proteins, and lipids) and cellular membrane causing DNA lesions, cytotoxicity, and impairment of cell functionality [46–48]. Moreover, Alvarez *et al.* [49], Saradha and Mathur [50], and Fsieh *et al.* [51] reported that ROSs can cause peroxidation of fatty acids in the sperms inducing lipid peroxidation, and this lipid peroxidation causes damage to phosphatides of cell membrane and consequently leads to sperm-shape abnormalities and decline of sperm count.

Fenske and Fink-Gremmels [52] reported that this toxin (AFB₁) has the ability to accumulate in germinal cells leading to impaired spermatogenesis. Kumari and

Sinha [53] emphasized that AFB₁ is considered to be a reproductive toxicants. Furthermore, Bose and Sinha [54], Solti *et al.* [55], and Darwish *et al.* [2] concluded that mycotoxin (AFB₁) has strong adverse effect on spermatogenesis or spermiogenesis.

From the present findings, it was found that the animals that were treated with AFB₁ and received MOLE as a protective or therapeutic agent have significant reduction of rates of DNA fragmentation, micronuclei, chromosome aberrations, and sperm-shape abnormalities as compared with animals treated with AFB₁ alone. These results prove the ameliorate effect of MOLE against the lesion effect of AFB₁. These ameliorations of MOLE on genetic materials and sperms might be owing to its containing high percentages of natural antioxidants as polyphenols, which have important properties as antimutagens or antigenotoxins and anticarcinogens [16–18,56,57]. The action mode of antioxidants might be owing to its binding with the mutagen (AFB₁) or its suppression to the activation of cytochrome enzyme system leading to reduction of DNA adduct and consequently causing minimization of abnormalities in genetic materials and sperms [8,16,18]. The decrease of DNA lesions leads to reduction of micronuclei and chromosome aberrations [58,59] as a result of a decrease in suppression of DNA replication and consequently cause a decrease of sperm abnormalities [60,61]. Moreover, the antioxidants of MOLE have been found to have potent strategies by scavenging the ROS or free radicals causing reduction of oxidative stress on cellular components including proteins, DNA, and lipids [17,62,63], leading to decrease of genotoxicity and cytotoxicity.

In previous studies, the antimutagenic effects of MOLE phytoconstituents were revealed by minimization of genotoxicity and cytotoxicity that were induced in different animal species exposed to radiation [8,17] and treatment with each of CP [16,18]. Moreover, Prasanna and Sreelatha [62] detected that the treatment with MOLE in cells of *Saccharomyces cerevisiae* reduced the oxidative stress (that induced by ROS H_2O_2) and enhanced the levels of antioxidant enzymes, SOD and CAT, and this led to decrease of cytotoxicity and formation of lipid peroxidation. Moreover, other studies [64–66] reported that the antioxidant properties of MOLE played an important role as anticarcinogens by reducing the risk of cancer in lung, prostate, and ovarian tissues in human and mice.

From the present study, it could be reported that liver and kidneys being their target organs respectively especially during the protective stage, but after the therapeutic stage of experimental periods, values

were around those of the normal state in the highest treatment (ninth group). The intoxicated rats treated with the different doses of MOLE showed different improvements in dose-dependent manner than intoxicated control rats.

These results are in agreement with Corcuera *et al.* [67] who stated that there was statistically significant stimulation in ALP and ALT activities under the induction of AF relative to those of normal control. After AFB₁ induction, histology and biochemistry of liver showed necrosis, focal inflammation, and an increase in liver function enzymes. The AFB₁ induction produced the same trend in kidneys function like those of liver in which kidney biomarkers (urea, uric acid and creatinine levels of serum) had the same manner of liver parameters. Mansour *et al.* [68] reported that MOLE showed protective influences against AFB₁ hepatotoxicity and nephrotoxicity (ALT, AST, urea and creatinine). Moreover, Sheikh *et al.* [69] found that *M. oleifera* leaves abrogated the xenobiotic induced harm in liver function (ALP, ALT and AST) and kidneys function (urea). It also improved the contents of glucose, triglycerides, cholesterol, high-density lipoprotein, and low-density lipoprotein as serum lipid profile.

Hepatic tissues of the liver absorb toxic substances from the blood stream and thus from circulation. AF, specifically AFB₁, is eventually secreted in the liver where it has been shown to be toxic to cell. AF in the liver is degraded in two phases [1] biotransformation to a more toxic product [2] and detoxification to a less toxic and easily excretal product [12,70]. Ingestion of AFB₁ is a major risk factor for HCC and is an immune stimulant [5]. In liver, AFB₁ is biotransformed to various metabolites especially the active AFB₁-exo-8,9-epoxide (AFBO).

DNA adduct formation by the AFBO can be diminished by formation of AFB₁-glutathione conjugates, mediated by glutathione-S-transferase [12,71]. The accumulation of AFB₁ and AFBO depletes the GSH owing to the formation of high amount of epoxides and other ROS. These could activate and deactivate the various epigenetic mechanisms leading to development of various cancers [72,73].

The MOLE treatments for AFB₁-intoxicated rats against the AFB₁ harm may re-adjust the levels of protein profile by stimulation of protein biosynthesis. It may be possible that MOLE treatments led to enzymes induction necessary to detoxify the toxicant (AFB₁ and its metabolites), which play an antagonistic effects against the present xenobiotic. AFB₁ induction

stimulates cellular metabolism to generate ROS and hydroxyl radical which can endogenously and exogenously attack lipid, protein, nucleic acids and other compounds simultaneously in the living cells [5]. AFB₁ and AFBO damaged DNA that occurred from forming DNA by glutathione-S-transferase adduct (oxidase systems) [12]. Forth with, AFB₁ and AFBO interact with DNA, RNA, and protein which cause breakages in these molecules, especially DNA, and disturb DNA-replication. These result in chromosomal aberrations and inhibit the protein biosynthesis system [7,64,73,74].

AFB₁ induction produced a complex significant metabolic disorder and disturbance of organs function and endogenous oxidative and antioxidative system against xenobiotic harm. The accumulation of AFB₁ and its metabolites depletes the GSH due to the formation of high amounts of epoxides and other ROS that could activate and deactivate the various epigenetic mechanism leading to development of various cancers [72,73]. The main biological influences of AFB₁ and its oxidative metabolites are carcinogenicity, immuno-suppression, and teratogenicity and also cause injuries in body animal organs with production DNA mutation, and damage by oxidase systems results in hydroxylated metabolites [7,71]. These hydroxylated metabolites with ROS radicals can attack the endogenous biological structure such as lipid, protein, and nucleic acids (DNA and RNA) [75] and also deteriorate the different metabolic pathways in the living cells [5,76] in which MDA content was increased but GSH was decreased, and also SOD and CAT activities were decreased.

M. oleifera attenuates the oxidative stress and enhances the levels of activity of antioxidative enzymes such as SOD and CAT. Moreover, it caused inhibition of the extent of lipid peroxidation (MDA) and stimulated the hydroxyl radical scavenging activity. *M. oleifera* leaves alcoholic extracts suppressed the effects against AFB₁ and its metabolites induced HCC or apoptotic cellular changes [62]. The plant as a herbal drug has been discussed in terms of redox imbalance and oxidative stress and may be useful in efficient killing of tumor cells leading to establishment of improved protocol in patients with cancer [77,78]. *M. oleifera* leaves are renewable source of chlorophylls, tocoferol, phenolics, and β -carotene, saponin, tannins, and other compounds [79]. Chlorophylls can act as an interceptor molecule through the formation of tight molecular complexes with AFB₁ antioxidant to inhibit lipid peroxidation (MDA), and it is a potent inhibitor of cytochrome p450 enzymes [80].

Administration of MOLE as antioxidant agents ameliorated the hepatotoxicity and nephrotoxicity [68] induced by AFB₁ [75] and enhancement of the antioxidant defense mechanisms [9,18,78].

Conclusion

This study revealed that MOLE contains therapeutic factors used in curing of genotoxicity induced by AFB₁ in rats. The treatment of animals exposed to AFB₁ with MOLE significantly ameliorated the genetic, sperm, and biochemical parameters as compared with animals treated with AFB₁ alone.

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Conflicts of interest

There are no conflicts of interest.

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