BIO-PROTECTIVE EFFECT OF *ERUCA SATIVA* SEED OIL AGAINST THE HAZARDUS EFFECT OF AFLATOXIN B1 IN MALE – RABBITS

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ABSTRACT

The objective of this study was to investigate the efficacy of *Eruca Sativa* seed oil to ameliorate the hazardous effect of aflatoxin B1 in male rabbits with special reference to the reproductive performance. The current experiment was carried out on 40 adult White New Zealand bucks (3- 3.5 kg live body weight) during March – May. Rabbits were housed in an air-conditioned room at the Lab.Animal house of the National Research Center and had free access to water and pelleted diet (mycotoxin free).Bucks were proven to be fertile after examination of semen samples and were divided into four comparable groups, 10 bucks each. The first group (G1) was kept on basal diet without any additives, The second group (G2) was given 0.5 ml *Eruca sativa* seed oil (ESO)daily for 8 weeks, using an oral tube The third group(G3) was given 6 ppb of aflatoxin B1(AFB1; permissible concentration of aflatoxin in food stuff in Egypt.) dissolved in corn oil 3 times weekly for 8 weeks. The fourth group was treated similar to G3 in addition to 0.5ml of ESO daily starting two weeks before drenching AFB1.

The moisture content of the ration was determined and random samples were examined for the presence of fungus and/or toxins before the start of the experiment. ESO GC/MS was carried out. At the end of the experiment, animals were slaughtered. Blood and epididimal sperm samples were taken for evaluation. Samples of muscles were collected for analyzing the residual amount of aflatoxin B1, while specimens from testes, kidney and liver were sent for histopathological examination. Aflatoxin B1 administration resulted in suppressed immunity, oxidative stress and hepatic and renal ailment, oilgospermea, teratospermea and asthenozoospermia.

Results of the present study ascertained that *Eruca sativa* seed oil ameliorate the harmful effect of aflatoxine , It took all the disturbed values of blood and semen and pathological changes in liver,kidney and testes, due to aflatoxicosis back to normal. It is recommended to add ESO in drinking water in rabbit farms to control the problem of aflatoxicosis for the benefits of rabbit breeders and consumers.

Key words: Aflatoxin B1, Eruca sativa seed oil, reproductive disorders, male rabbits

1. INTRODUCTION

Aflatoxins are produced as secondary metabolites by *Aspergillus flavus* and *Aspergillus parasiticus* fungi when the temperatures are between 24 and 35°C, and the moisture content exceeds 7% . Aflatoxicosis is considered by the US Food and Drug Administration (FDA) to be an unavoidable contaminant of foods, especially in the developing countries, whereas food supplies are already limited, drastic legal measures may lead to lack of food and to excessive prices. (1).

Among all the mycotoxins, particularly aflatoxin B1 (AFB1) is the most toxic form for mammals and presents hepatotoxic, teratogenic, mutagenic and immunosuppressive properties (2).

Aflatoxicosis is one of the major problems facing animal breeders due to massive losses resulting from either lower productive performance of the animals or due to deaths (3, 4). In rabbit, it was recorded that aflatoxine causes significant reductions in the number of oocytes and large follicles in a dose dependent response with marked changes in reproductive blood hormone levels. (5). Moreover, aflatoxicosis results in a defective spermatogenesis and incomplete epididymal processing of spermatozoa, low sperm production, poor sperm motility or abnormal sperm morphology (4,6,7,8 and 9).

Eruca sativa is one of the medicinal plant known as an aphrodisiac, (10). It has several antioxidant constituents including glucosinolates, flavonoids,carotenoids, etc. (11). Also the nitrile 5-methylthiopentanenitrile has been detected as one of the constituent of the volatile fraction of Eruca sativa (Brassicaceae) by headspace analysis (12). Nitriles, thiocyanates and isothiocyanates are degradation products of glucosinolates. (13). Several reports are found in the literature about the antifungal activity of these compounds on postharvest pathogens (14).

The current study aimed first, to study the ability of *Eruca sativa* seed oil in counteracting the deleterious effects of aflatoxin in rabbit farm with emphasis on the reproductive performance. Second, to improve the animal productivity and meat quality, get meat free from aflatoxins or drug or pesticide residues for the safety of consumers and profit of rabbit breeders.

2. MATERIALS AND METHODS

Animals:

The current experiment was carried out on 40 adult White New Zealand bucks (3- 3.5 kg live body weight) during March – May. Rabbits were housed in an air-conditioned room at the Lab.Animal house of the National Research Center and had free access to water and pelleted diet (mycotoxin free).

Aflatoxin *:*

Aflatoxin B1 desiccate from *Aspergillus flavus* [1162-65-8] EEC no 214-603-3 was purchased from Sigma (St. & Louis, USA) for laboratory use.

Eruca sativa seeds oil:

The essential oil of *Eruca sativa* seeds used in this study was provided by the Medicinal and Aromatic Plants Division, Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. It was obtained by steam-distillation for 3 hr using a Clevenger-type apparatus (15) The obtained essential oil was closed under nitrogen gas and stored in airtight glass vials covered with aluminum foil at -20°C.

Experimental design:

Bucks were proven to be fertile after examination of semen samples using artificial vagina. and were divided into four comparable groups, 10 bucks each. .

The first group (G1) was kept on basal diet without any additives

The second group(G2) was given 0.5 ml *Eruca sativa* seed oil (ESO)daily (16) for 8 weeks, using an oral tube

The third group (G3) was given 6 ppb of aflatoxin B1(AFB1; permissible concentration of aflatoxin in food stuff in Egypt.) dissolved in corn oil 3 times weekly for 8 weeks.

The fourth group (G4) was treated similar to G3 in addition to 0.5ml of ESO daily starting two weeks before drenching AFB1

Sampling :

The moisture content of the ration was determined and random samples were examined for the presence of fungus and/or toxins before the start of the experiment.

At the end of the experiment, animals were slaughtered. Blood and epididimal sperm samples were taken for evaluation.

Samples of muscles were collected for analyzing the residual amount of aflatoxin B1, while specimens from testes, kidney and liver were sent for histopathological examination.

Analysis:

Ration:

Moisture content (MC) in the ration was determined by using a vacuum oven, and the presence of moulds and fungi was examined according to AOAC (17). Aerobic Plate Count (APC) was carried out as outlined by Barnett and Hunter. (18).

The concentrations of AFB1in the ration were determined according to the procedure of AOAC (17)

***Eruca sativa* seed oil**:

1-Eruca sativa seed oil antifungal activity was determined using diffusion plate method against *Aspergillus flavus* NRRL 5906 (Mycotoxigenic fungi) obtained from Standard Association of Australia. North Sudnay. It was maintained on Potato Dextrose Agar (PDA, OXOID) at 28°C.The diffusion plate method was used with slight modification as reported by Terras *et al*. (19), 0.1 ml of fungal spore suspension (grown for 7 days in 10 ml of Potato Dextrose Agar) was thoroughly mixed with 20 ml of melted PDA and poured into sterilized Petri plates. When the agar was set one hole of 8 mm. diameter bore was made on the plate. The hole filled with 100ul of the ESO. Plates were performed in triplicate. The Petri plates were incubated at 25°C for 7 days. The zone of inhibition produced by the extract was compared with control.

2-Gas chromatographic analysis was performed by using Hewllet-Packard model 5890 equipped with flame ionization detector (FID).according to Adams (20).

Blood samples were centrifuged and serum samples were collected for analysis of total protein, albumin, urea, creatinine ,cholesterol , Aspartate amino transferase (AST), alanine amino transferase (ALT) as outlined by Henery(21). γ glutamyl transferase (γGT; 22). Immunoglobulin (IgG;23) and interleukin 6 (IL-6) using Eliza Kit for rabbit Cusabio Biotech Co.,Ltd. Oxidant/ antioxidant markers including malondialdehyde (MDA) (24), superoxide dismutase; (SOD) (25), reduced glutathione; R-GSH(26) were calorimetrically assayed using chemical kits from Bio Diagnostic, Egypt

Semen samples were diluted (1:1)immediately with warmed (37^{o}C) 0.9%Nacl (27) and evaluated for motility, live/dead ratio and morphologically abnormalities in smear stained with eosin-nigrosin by counting 500 cell/slide (28).The concentration of sperm was measured by haemocytometer following 1/400dilution of the sample in formol physiological saline(29) .

Residue anaysis of aflatoxin B1:

Rabbit flesh was examined for the residue of Aflatoxin B1. Extraction and Quantitation by Enzyme-linked Immunosorbent Assay (ELISA) were carried out as those described by Bintvihok et al (30).

Histopathological examination: After scarification of bucks, the testis, epididimis, liver and kidney were collected and examined grossly for pathology then fixed in 10% formol saline, processed by conventional parafine embedding technique, sectioned (3-4mμ) and stained by hematoxylene and eosin(31) for routine histopathological examination..

Data were statistically elaborated with Analysis of Variance (32) using Mc Graw Hill software (33).

3. RESULTS

Examination of the commercial ration used for feeding of rabbits in the current experiment revealed that it contained moisture percent of 8.91 However, it was found that this ration contained *Aspergillus Niger* and few Pencillium and Alternaria spp.

Aspergillus Niger was found to be unable to produce any traces of aflatoxin B1 and proved to be non toxigenic species. *Eruca sativa* seed oil was found to have antifungal activity ,whereas it inhibited the growth of fungus on potato dextrose agar. (Fig M)

GC-MS analysis of the ESO (Tables 1,2) showed the presence of the bio-active isothiocyanates (ITCs) beside free fatty acids. Erucic acid was by far the most represented in both the free form and triglycerides, being >50% (w/w) in each fraction.. Other long-chain mono- and poly-unsaturated acids were also abundant, among them oleic (14.9% w/w) and cis- 11-eicosenoic (12.3% w/w). Among short chain fatty acids (entries 1–5), dicarboxylic acids (4, 7) and odd-carbon acids (9, 12).

Table1. HS/SPME/GC–MS analysis of volatile isothiocyanates in Eruca sativa seed oil

R.t.(min)	compound	M.W.	Amount (µg/g)	Characteristic ions[a] (m/z)
6.237	Allyl isothiocyanate	99.15	40.3	41, 72, 99 (bp)
9.632	3-Butenyl isothiocyanate	113.18	259.6	55,72 (bp), 85,113
22.450	2-Phenylethyl isothiocyanate	163.24	158.5	91 (bp), 105, 163
28.373	Sulforaphane	177.29	743.1	55,72 (bp),85,115,160
29.558	Bis(4-isothiocyanatobutyl)disulphide		~5000[b]	55,72,85,86 (bp),114

The base peak (100%) is indicated as bp, while the molecular ion (M+–), when visible in the spectrum, is indicated in bold face.The actual amount determined, using sulforaphane as reference, was 5015.1 µg/g.

Table 2. Free and total fatty acid content in Eruca sativa seed oil, as obtained from GC–MS analysis

No.	Compound	Free faty acids	Total fatty acids
		%w/w	%w/w
1	Caproic acid methyl ester	0.009	0.005
2	Caprylic acid methyl ester	0.033	0.009
3	Nonanoic acid methyl ester(Pelargonic)	0.069	0.004
4	Adipic acid dimethyl ester	0.021	0.003
5	Capric acid methyl ester	0.017	0.007
6	Lauric acid methyl ester	0.041	0.016
7	Azelaic acid dimethyl estere	0.095	0.013
8	Myristic acid methyl ester	0.257	0.079
9	Pentadecanoic acid methyl ester	0.083	0.009
10	Palmitoleic acid methyl ester	0.212	0.12
11	Palmitic acid methyl ester	3.471	2.37
12	Heptadecanoic acid methyl ester	0.053	0.18
13	Linoleic acid methyl ester	6.576	6.93
14	Oleic acid methyl ester	13.036	14.89
15	Elaidic acid methyl ester	1.16	1.21
16	Stearic acid methyl ester	1.13	0.94
17	*cis*-11,14- -Eicosadienoic acid methyl ester	0.32	0.19
18	*cis*-11-Eicosenoic acid methyl ester	12.41	12.32
19	*cis*-11,14,17-Eicosatrienoic acid methyl ester	0.788	0.86
20	*trans*-11-Eicosenoic acid methyl estere	1.04	1.07
21	Arachidic acid methyl	0.66	0.61
22	Erucic acid methyl ester	50.71	50.55
23	Behenic acid methyl ester	0.715	1,27
24	Nervonic acid methyl ester	1.18	0.94
25	Lignoceric acid methyl ester	0..34	0.21

Bucks administered aflatoxinB1 (G3) showed loss of weight , reduced food intake and low sexual desire when females were introduced to their cages as compared to their pair-fed control group(G1) meanwhile, those given ESO and AFB1 (G4) showed much better general health condition as well as sexual desire if compared to G3.

Serum chemistry of bucks given aflatoxin B1 and /or ESO are shown in Table (3). It was clear that bucks in G3

showed hypercholesterolemia if compared to the control group (G1) while bucks in G4 showed significant (P<0.05) decrease in serum cholesterol value if compared to G3.

Bucks suffering from aflatoxicosis showed decreased total protein, albumin, and globulins if compared to their pair-fed control (G1) while the condition in those groups supplemented with ESO (G2 and G4) was much better.

Table 3. Serum analysis of bucks challenged with Aflatoxin B1 and supplemented with Eruca sativa seed oil.(Means ± SE) Different superscript letters means significant difference within rows (p< 0.05)

	Normal healthy (G1)	ESO (G2)	AFB1 (G3)	ESO +AFB1 (G4)
Total protein (g/dl)	7.06±0.16 ^{a}	7.80±0.08 ^{a}	5.16±0.09 ^{b}	6.36±0.05^{d}
Albumin (g/dl)	3.08± 0.05 ^{d}	3.96± 0.04^{b}	2.06±0.05 a	3.46±0.05 ^{d}
Globulin (g/dl)	3.97±0.08 ^{c}	4.02±0.09 ^{c}	2.05±0.10 ^{a}	3.20±0.10 ^{b}
IgG (mg/dl)	515.46±13.20 ^{b}	570.64±7.33^{c}	340.55±8.22 ^{a}	513 ± 15.2 ^{b}
IL-6 (pg/ml)	240.76± 9.99^{b}	286.87 ± 6.99^{c}	154.24 ± 9.80^{a}	250.78 ± 8.99^{b}
Cholesterol (mg/dl)	86.40±5.55 ^{b}	81.00±3.37 ^{b}	130.20±1.20 ^{a}	98.00±0.70 ^{d}
δGT (U/L)	10.07±0.017 ^{a}	11.24±.037 ^{a}	25.89±.050 ^{b}	17.20±.017 ^{b}
ALT (IU/ml)	23.80±1.46 ^{a}	27.80±4.02^{a}	45.40±1.12 ^{c}	36.60± 3.37 ^{b}
AST (IU/ml)	22.00±.057 ^{b}	19.90±1.98^{b}	28.40±1.60 ^{a}	25.80±0.58 ^{ab}
Urea (mg/dl)	29.20±0.37^{b}	27.20±2.26 ^{b}	41.60±1.16^{a}	29.60±0.50^{b}
Creatinine (mg/dl)	0.68±0.06 ^{a}	0.88±0.06 ^{c}	1.22±0.07 ^{b}	0.68±0.04^{a}
R-GSH (mmol / L)	18.00±0.18 ^{a}	22.00±0.54 ^{a}	7.00±1.30 ^{b}	15.40±0.30 ^{d}
SOD (U/ml)	26.00±0.83 ^{c}	38.40±0.12 ^{b}	18.40±0.50 ^{a}	24.80± 0.15 ^{a}
MDA (mmol/ml)	28.60±0.14 ^{d}	14.80±1.32 ^{b}	40.80±0.26 ^{a}	21.00±0.34 ^{c}

Aflatoxicosis suppressed the immune status in bucks of G3, whereas the immunoglobuline (IgG) and cytokine (IL-6) showed low values if compared to the control group ESO reversed the condition back towards normal in G4 if compared to the control group G2 and G1. Aflatoxicosis resulted in significant (P<0.05) elevation of liver enzymes in serum of G3 wheras γGT, ALT and AST showed higher values if compared to the control group (G1). In the same time, serum urea and creatinine took the same line suggesting involving of hepatic and renal ailments.

ESO supplementation could take the levels of the enzymes back to normal and ameliorates the effect of aflatoxicosis in G4 if campared to control group G2 and G1.

The oxidative status of animals given AFB1 showed decreased level of R-GSH and SOD and increased MDA while the reverse was recorded with those groups given ESO.

Histopathological investigation of liver in G3 showed sporadic necrotized heptocytes with mononuclear cells infiltration, mostly lymphocytes with mild activation of Van-Kupffer cells. Moreover, most of hepatocytes showed vacuolar degeneration and most of the portal areas revealed mononuclear cell infiltration mostly lymphocytes. Congestion of the portal veins and mild fibrous connective tissue proliferation were obvious (fig A). Meanwhile, liver of buck orally administered Eruca sativa seed oil and AFB1 (G4) showed dilated portal veins with blood and mild fibroblastic proliferation in the portal areas and vacuolar degeneration of the hepatocytes (fig B).

Kidneys of bucks in G3 showed marked shrinkage and atrophy of most of glomerular tuft. (fig.C). In the same time, intertubuler tissue revealed focal infiltration with mononuclear cells mostly lymphocytes and severely congested blood vessels. In addition some of the tubular epithelium showed more advanced necrotic changes especially in the convoluted tubules of the cortex (fig D)

Histopathological investigation of testes in G4 that given ESO and AFB1 revealed focal areas of mild degenerative changes, thickening of the basement membrane and depletion of the spermatozoa. Interstitial edema was traced and leydig cells appeared low in number (fig F) On the other side, testes in G3 given AFB1 showed multifocal areas of degenerated tubules with marked hypospermatogenesis. (fig E)

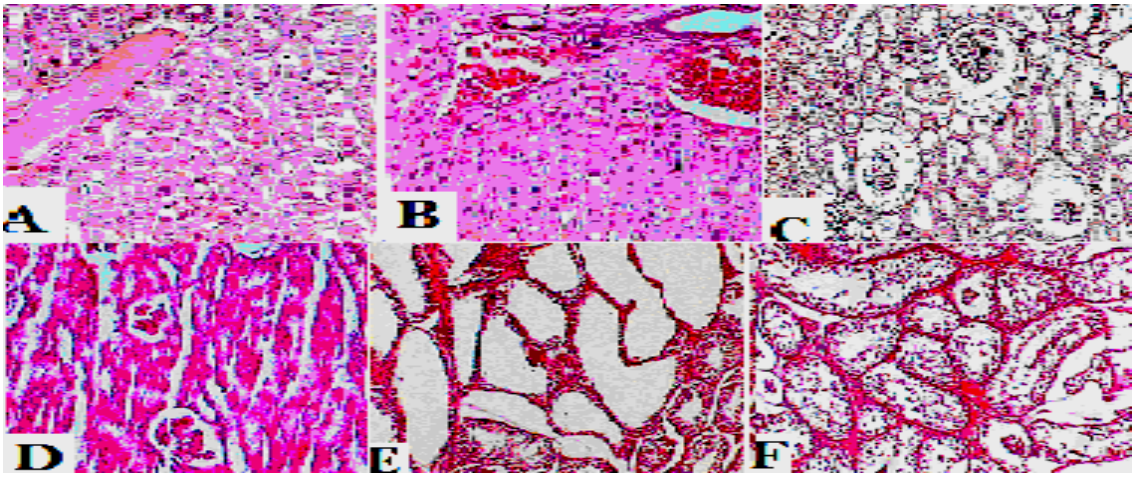

Table 4. Effect of of Eruca sativa seed oil on semen characteristics of white rabbits challenged with aflatoxin B1(Means ± SE)

	Normal healthy (G1)	ESO (G2)	AFB1 (G3)	ESO +AFB1 (G4)
Sperm Motility (%)	78.83 ±3.00^{b}	88.67±2.77^{b}	61.67±1.99^{a}	86.66±2.00^{b}
Dead Sperm (%)	16.12 ±1.30^{b}	14.33±1.67^{b}	36.00±1.40^{a}	14.00±1.65^{b}
Abnormal Sperm (%)	14.54 ±0.80^{b}	12.00±0.90^{b}	22.00±0.87^{a}	14.33±0.76^{b}
Acrosomal defects (%)	6.22±0.21^{b}	4.3± 0.087^{b}	17.66±0.96^{a}	5.00 ± 0.77^{b}
Concentration (10^{6} /ml)	280.6 ±19.2^{b}	280.00±13.8^{b}	68.00±12.54^{a}	277.33±11.87^{b}

Semen evaluation revealed that aflatoxicosis caused a defective spermatogenesis at the level of the testis. The epididimal semen evaluation showed low sperm production (oligozoospermia) and poor sperm motility (asthenozoospermia). Sperms showed round movement if compared to its pair- fed control group (G1) where the sperms showed progressive movement. Abnormal sperm morphology (teratozoospermia) was also demonstrated in G1 The most frequent abnormalities were loose head, thick neck, incomplete head, bent or coiled tail and detachment of the head from flagellum.

Eruca sativa seed oil ameliorated the harmful effect of aflatoxin on semen as shown in table (4) where no marked differences were traced between G1and G2 or G4.

Residue analysis of flesh in the experimental rabbits was found to be free from AFB1 in the control and ESO groups. On the other side, AFB1 was detected at concentration of 0.931 ppb in AFB1 group and 0.726ppb in AFB1+ESO group.

4. DISCUSSION

Aflatoxin B1, a potent mycotoxin elaborated by Aspergillus group of fungi has a wide range of hazardous biological activities, whereas it was recorded to induce acute toxicity, teratogenicity, mutagenicity and carcinogenicity.There are≈ 4.5 billion persons living in developing countries (at latitudes between 40°N and 40°S of the equator) chronically exposed to largely uncontrolled amounts of aflatoxins. (34), there are global wide variations in national aflatoxin standards in food stuff. ie,15 – 20 pbb in Canada and the United States 4 pbb in France and the Netherlands 30pbb in India and 20pbb in Egypt(34). Therefore, the main goal of the investigators is to minimize the risk of aflatoxins and its health–hazardous effects in human beings and animals (35,36).

A growing body of evidence suggests the role of oxidative stress in the pathogenicty of many diseases (37,38) and several plant extracts/compounds have been shown to alleviate several ailments by their antioxidant property(16). A number of investigations in recent years has dealt with antioxidant, detoxifying potential of Brassicaceae (family which Eruca sativa belongs to).

In the present study the GC/MS of ESO showed the presence of the bio-active isothiocyanates (ITCs) which results from glucosinolate (GLs) upon myrosinase hydrolysis., previous studies recorded that GLs and ITCs showed antibacterial and antifungal effect (39) . and induce phase-II metabolizing enzymes such as glutathione-S-transferase, epoxide hydrolase, NAD(P)H:quinone reductase, which plays a vital role in detoxification of electrophiles and protection against oxidative stress(40). Beside ITCs, the free fatty acids particularly in erucic found here, >50% (w/w), is suggested to contribute to the antimicrobial activity of the seed oil. (39). The antioxidant activity of glucoerucin plausibly implicates free radical scavenging activity and an ability to induce phase-II metabolizing enzymes (41).

The present study revealed significant decrease in sexual desire of bucks administered AFB1 (G3) together with a significant decrease in serum protein level. Previous investigators recorded that Aflatoxin B1 is metabolically biotransformed in the liver by Cytochrome P450 enzymes, to a highly reactive electrophilic compound which is capable of binding to both DNA and proteins (42). This means that aflatoxin impairs protein biosynthesis by forming adducts with DNA, RNA and protein, inhibits RNA synthesis, DNA-dependent RNA polymerase activity, and causes degranulation of endoplasmic reticulum (43,44). Reduction in protein content has also been reported in the testis of aflatoxin-treated mice (45), which could be responsible for reduced enzyme activities responsible for steroidogenesis. The oxidative DNA damage and lipid peroxidation are previously observed in the testis (46) and this could be responsible for the degenerative changes observed in this study in Leydig cells. Similar results were recorded earlier by (46). Thus, the reduced sexual desire in bucks given aflatoxin B1 may be explaned in the light of reduced steroidogenesis in Leydig cells.

This study revealed obvious increase in serum cholesterol value in animals given aflatoxin (G3). It is well known that cholesterol and the key steroidogenic enzymes such as 3b- and 17b- hydroxysteroid dehydrogenase, are responsible for the synthesis of testosterone in steroidogenic process (45). found that oral administration of aflatoxin for 45 days caused significant decease in these enzymes in rats and reduces the utilization of cholesterol in stroidogenesis. The exact mechanism of aflatoxin action is not clearly understood, but it could be due to alterations in mitochondria, inhibition of protein synthesis/enzyme activity and/or alteration in plasma membrane of Leydig cell due to lipid peroxidation. (47) and Verma and Metha (48), reported mitochondrial swelling and dysfunction due to accumulation of calcium during aflatoxicosis that could impair steroidogenesis. The traced pathological changes in the present study in

testes of animals administered AFB1 confirm this suggestion whereas hypospermatogenesis was clearly demonstrated in testes of bucks administered AFB1.On the other side ESO took the cholesterol and protein level back towards normal.

The present study showed significant depression in the level of immunoglobulins (IgG) as well as cytokine(IL-6) in serum of bucks given oral aflatoxin B1 (G3). This can be explained in the light of previous finding of Pier (49) that AFB_1 induces thymic aplasia, reduce T-lymphocyte function and number, suppress phagocytic activity and reduce complement activity (49,50). Many studies conducted in poultry, pigs, and rats showed that exposure to aflatoxin in contaminated food results in suppression of the cell-mediated immune responses (51,52). Splenic CD4 (helper T) cell numbers and interleukin 2 (IL-2) production decreased significantly when mice were treated with AFB_1 at a dose of 0.75 mg/kg (53). Other reports suggest that aflatoxin impairs the function of macrophages in animal species (54,55). If given in a concentrations ≥100 pg/mL because it becomes cytotoxic to the monocytes, and concentrations of 0.5 to 1 pg/mL inhibits monocyte phagocytic activity. (56).

ESO stimulates the production of the reactive glutathione *S*-transferase (one of the phase-II metabolizing enzymes) system found in the cytosol and microsomes catalyzes the conjugation of activated aflatoxins with reduced glutathione, leading to the excretion of aflatoxin (57). Moreover, ESO with its potent free radical scavenging ability was expected to inhibit Fe3+–ascorbate induced damage to lipids and proteins. Since Fe3+ is a Fenton catalyst accelerating ROS formation, ESO might also be expected to exclude Fe3+ as a strategy to control ROS. (39)

Liver and kidney function test showed significant elevation in serum level of enzymes and this coincide with the results of (58) who stated that aflatoxin causes hepatocellular degenerative changes with elevated serum levels of ALT,AST, urea and creatinine. The histopathological finding of this study confirm the condition whereas G3 showed sporadic necrotized hepatocytes, mononuclear cell infiltration and mild activation of Van- Kupffer cells. On the other side the group of buck given ESO with AFB1 showed better condition.

The oxidative status of the animals in G4 showed better condition and less oxidative stress than those administered AFB1 (G3). This can be explained in the light of the previously mentioned antioxidant efficiency of ESO.

The present investigation of semen picture and pathological finding in testis showed a defective spermatogenesis at the level of the testis represented by oligozoospermia, asthenozoospermia or teratozoospermia Similar findings were reported by (6, 7, 8) .Microscopical examination of the testes of this group showed thickening of the basement membrane of the somniferous tubules and marked hypo spermatogenesis. Significant improvement in the picture was traced in the group of bucks given ESO and AFB1.

Results of the present study ascertained that Eruca sativa seed oil ameliorate the harmful effect of aflatoxine by reduction of both oxidative damage and bioavailability of aflatoxin as mentioned above. Previous studies in multiple animal species, reported that the aflatoxin toxicities may be modified by the dietary intakes of antioxidant vitamins, such as vitamins A, C, and E(59,60).

Our final recommendation is to add Eruca Sativa seed oil in drinking water in rabbit farms. This leads to better reproductive performance of animals, less mortalities, better kind of meat with less residual toxins. This will make benefit for the animal breeders and the consumers.

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