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Protective effect of some antioxidant compound on albino rats intoxicated with aflatoxin

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Abstract

Mycotoxins are unavoidable contaminants of animal and human feed and food respectively. This study was aimed to investigate the effect of Vitamin E, Vitamin C and Selenium Supplementation to drink water against aflatoxins B1 on the various serum parameters , liver tissue antioxidant and the associated pathological changes. Thirty five male albino rats weighing 120-150 g were used in the present study. Group1 (G1): rats served as control , Group2 (G2) orally administrated aflatoxin B1 dissolved in propylene glycol per Os as a dose of 50µg/kg body weight /week**,** for 10 weeks . Group3-7 : administrated with aflatoxin B1 50µg/rat/week for 10 weeks . These groups were treated as Group3 (G3):was supplemented with selenium (as sodium selenite was used as 4mg/l). Group4 (G4):was supplemented with vitamin E as 1000mg/l. Group5 (G5):was supplemented with vitamin E plus selenium. Group6 (G6): was supplemented with vitamin C as 1000mg/l. Group7 (G7):was supplemented with vitamin C plus selenium. The results showed significant elevation in the liver and kidney functions, alpha globulin and beta globulin and decrease in concentrations of serum total protein, albumin, and gamma globulin together with A/G ratio. Lipid peroxidase as MDA levels were shown after AFB1 treatment. pathological changes of liver in aflatoxicated rats was discussed briefly. It can be concluded that the hepatotoxicity induced by aflatoxin B1 seemed to be modulated effectively by the simultaneous use of antioxidants: vitamin E or C alone or incombination, with selenium. Moreover, they are more efficient modulating the biochemical alteration , liver antioxidant enzymatic system and pathological changes.

Introduction

Mycotoxins are produced mainly by the mycelial structure of filamentous fungi, or more specifically, molds. These secondary metabolites are synthesized during the end of the exponential growth phase and appear to have no biochemical significance in fungal growth and development. The contamination of foods and feeds with mycotoxins is a significant problem for the adverse effects on humans, animals, and crops that result in illnesses and economic losses. However**,** the fungi of Aspergillus and their toxins are widely distributed through the world where they occur in soil, on plants, plants debris and similar organic subtracts. They cause significant economic losses in agriculture, morbidity and mortality in animals and immunological compromised humans, where it

is capable of killing cells by causing extensive damage to cellular membrane **(Mogda et al., 2002 and Fraga et al., 2008).** The toxic effect of the ingestion of mycotoxins in humans and animals depends on a number of factors including intake levels, duration of exposure, toxin species, mechanisms of action, metabolism, and defense mechanisms. In general, the consumption of contaminated food and feed with mycotoxin induces to neurotoxic, immunosuppressive, teratogenic, mutagenic, and carcinogenic effect in humans and/or animals. Even it may lead to decreased immunity in animal. **(Fernandez et al., 2000).** The metabolism of AFB1 takes place by mixed-function oxidase system at the liver to AFB1 8, 9-epoxide and other hydroxylated metabolites. A metabolite form is binds to DNA and to albumin in the blood serum, forming adducts and hence causing DNA damage **Wu,& Khlangwiset, (2010)** and cause oxidative stress by damaging cellular membranes and components **Preston and Wiliams, (2005)**. Furthermore, effects of AF at the protein level were mirror to DNA damage which is the precursor to cell transforming from normal to malignant **(Shyamal et al., 2010).**

Because aflatoxin contamination of food cannot be avoided, numerous detoxification strategies have been proposed to alleviate its impact. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions **(Velioglu 1998).** The recent researches reported that biological decontamination of mycotoxins using microorganisms is one of the well-known strategies for the management of mycotoxins in foods and feeds.

Scientists have indicated that antioxidant obtained from daily diets such as non-enzymatic antioxidants vitamin E، vitamin C, carotenoids and polyphenols can scavenge the reactive oxygen species. These compounds may also be required as cofactors for antioxidant enzymes or be used by cells for up-regulating enzymatic antioxidants (**Eboh 2014)**. Vitamins C, E and selenium are known to be protective anti-oxidants **(Stoh et al., 2001; Das& King 2007).** They cause the inhibition of peroxidation, mopping up of free oxygen radicals and disorganization and breakage of peroxidation chain reactions **(Murray et al., 2000)** by an inhibition of glutathione peroxide, Protein Kinase C (PKC) inhibition and calcium metabolism **(Stoh et al., 2001; Das & King 2007)**.

Material and methods

Production and estimation of aflatoxins (Gabal *et al.*, 1994)*:* The strains of *Aspergillus flavus* were inoculated into flasks containing 50 ml of sterile yeast extract solution (2%): 20% sucrose (YES). Inoculated flasks were incubated at 25ºC for 10-15 days. At the end of the incubation period, 50 ml chloroform were added and the mixture was thoroughly mixed for one minute in electric shaker apparatus, then centrifuged (3000 r.p.m.) for 10 minutes after which the chloroform layer decanted. The

Chloroform extraction was repeated for more time. One ml ethanol, 3 gm copper – (III)-hydroxide carbonate and 5 gm anhydrous sodium sulphate were added to the chloroform extract, mixed well and filtered. The filtrate was then evaporated in a rotatory vacuum evaporator, the residue cooled and resuspended in 5 ml of chloroform.

Thin layer Chromatographic analysis of chloroform extract (Scott, 1990): The concentrated extract was spotted onto activated thin layer chromatography plates coated with silica gel of 0.25 mm thickness. Standard solutions of aflatoxins B1, B2, G1and G2 were spotted on the plate using 10-20 µl capacity pipettes. The spots were air dried and the TLC plates out in the developing tank containing the developing solvent system (5 Toluene: 4 ethyl acetate: 1 of 90% formic acid (V/V/V) .When the solvent travels about12 cm front, the plates were removed from the tank, air dried. The plates were inspected under UV light (256 nm and 365 nm) and the outline of each fluorescent was marked by a sharp pin. The RF values, colors and intensities of the unknown spots were compared with those of standard spots.

Quantitative estimation of aflatoxins by a fluorometeric method according to (Hansen, 1993): After calibration of the fluorometer by using specific FGis Afla test standards. At the end of the inoculation period, 25 ml of fungal culture filtrate were extracted three times with 50 ml chloroform. The chloroform extract was collected and evaporated in a rotatory flash evaporator. The residue of chloroform extract was dissolved in 100 ml methanol: water (80:20 V/V) and filter through fluted filter paper. Then, 10 ml of filtrate were diluted with 40 ml distilled water. The diluted extract was filtered through microfiber filter paper. 4 ml of filtered diluted extract were completely passed through AF- T-affinity column at a rate of 2 drops/second until air come through column. The column was washed twice with 10 ml distilled water at a rate of 2 drops/second. The affinity column was eluted by passing 1 ml HPLC grade methanol through column at a rate of 1-2 drops/second and the sample elute was collected in a glass cuvette. One ml of AF test developer was added to elute in the cuvette and mixed will. The cuvette was placed in a calibrated fluorometer and aflatoxin B1 concentration was read after 60 seconds.

***selenium** as sodium selenite was used as 4mg/l **. vitamin E** as dl-(α-Tocopherol acetate (15%) Adwia pharmaceutical Company was used as 1000mg/l.**. vitamin C** as ascorbic acid Memphis Company was used as 1000mg/l.

Animals:

Thirty five adult male albino rats weighing 120-150 g were used in the present study. The animals were obtained from the animal house in the Ophthalmology Research Center, Giza, Egypt. They were kept under observation for two weeks before

the onset of the experiment to exclude any intercurrent infection. The animals were kept at room temperature and exposed to natural daily light-dark cycles. Rats were fed adlibitum and clean water was continuously available

*Rats were randomly divided into equal seven groups:

Group1 (G1): rats served as control , Group2 (G2) administrated per Os by stomach tube with aflatoxin B1 dissolved in propylene glycol as a dose of 50µg/kg body weight /week**,** for 10 weeks **Ogido et al., (2004)** . Groups3-7 : administrated with aflatoxin B1 50µg/rat/week for 10 weeks . these groups were then treated as follows: Group3 (G3):was supplemented with selenium (as sodium selenite was used as 4mg/l). Group4 (G4):was supplemented with vitamin E. . Group5 (G5): was supplemented with vitamin E plus selenium. Group6 (G6): was supplemented with vitamin 7. Group (G7):was supplemented with vitamin C plus selenium.

Samples: Individual blood samples were obtained at the end of experiment from rats of each group, left to clot and sera were separated for biochemical analysis.
Liver was removed and washed with saline solution, then minced and homogenized (10% w/v) in ice-cold normal saline. The homogenate was centrifuged at 10,000xg for 20 min at 4C° and the resultant supernatant was used for (enzymatic and non-enzymatic) antioxidant assays ***Chitra et al., (1999).***

Pathological examination. (a) Postmortem examination was done immediately after slaughtering. **(b)** Tissue specimens from liver was collected and fixed in 10% neutral buffered formalin. They were routinely processed by standard paraffin embedding technique. Section at 4 micron, stained with Hematoxylin and Eosin (**Bancroft and Gamble, 2002).**

Serum biochemical studies: The biochemical assays of serum gamma glutamyle transferase (GGT) and lactic dehydrogenase (LDH) activities were determined according to methods of **(Szase et al., 1976),** alkaline phosphatase (ALP) activity according to **Tietz,(1996)**, aspartate amino transferase (AST) and alanine amino transferase (ALT) activities **(Reitman and Frankel, 1957)**, serum urea level according to **Wybenga et al.,. (1971**), serum creatinine level according to **Henry (1974).**

Estimation of serum total protein and electrophoretic pattern were carried out after **SonnenWirth and Jaret, (1980), Davis, (1964)** respectively and calculated according SynGene S. No. 17292*14518 sme*mpcs**.**

lipid peroxidation as malonaldehyde (MDA) level and Catalase (CAT) and SOD activities homogenate liver tissues were determined according to **Aebi (1974); Ohkawa et al., (1979) and Ellman (1959),** and **Nishikimi et al., (1972)** respectively. The

protein content of tissues samples were measured by the method of **Bradford (1976)** by using bovine serum albumin as a standard.

Statistical Analysis: The obtained date were computerized and analyzed for significance, Calculation of standard error and variance according to ***(SPSS 14, 2006).***

Results and Discussion

It is well known that, aflatoxin has a harmful and stressful effect on liver tissue. AST, ALT, ALP, GGT and LDH are cytosolic enzymes and are famous biomarkers of liver damage. The results of the current study revealed that exposure to AFs resulted in a significant increase in AST, ALT, ALP, GGT and LDH, urea and creatinine level were comparable to the control (Table 1). AST, ALT and ALP have been used as the biochemical indicators for hepatic damage.

AST and ALT are cytoplasmic in location and the increased serum levels may be due to leakage of these enzymes into blood stream as a result of autolytic breakdown or cellular damage **(Gaskill et al., 2005).** Elevated levels of both ALP and GGT are suggestive of liver or bile duct disease, and GGT is a prime marker of bile duct epithelial proliferation that is typical of aflatoxicosis **Kramer et al., (1989).**

The levels of serum urea and creatinine were significantly high in aflatoxicated groups as compared to healthy. This increase in concentrations in toxicated animals might be due to nephrotoxic action, which causes renal impairment by destruction of epithelial cells of proximal and distal convoluted tubules and alteration in tubular function **(Nashwa et al., 2008).**

However, administration of vitamins and/or Se along with AFB1 showed marked recovery compared with control groups (Table1). Regarding the ameliorative effect of vitamin and/or Se against AFB1 toxicity, previous reports **He et al., (2013)** showed a significant hepatoprotective activity of vitamins and/or Se by lowering the level of serum biomarker enzymes in AFB1 intoxicated rats.

Addition of Se may alleviate the aflatoxicosis by increasing GSH – Px activity and eventual changes of toxic substances to inert metabolites **Weiss et al., (1990) and Shlig (2009).** They also reported that the Vit. E reduced the formation of AFB1 adducts in the liver.

Table1: Effect of aflatoxin B1 and some antioxidant agents on serum biochemical parameter of male rats.

Groups / Parameter	control	AFB1	AFB1 + Se	AFB1 + vit E	AFB1 + Se+ vit E	AFB1 +vitC	AFB1 + Se+ vit C
AST u/l	88.6± 3.26	136.25± 3.76***	97.5± 4.18	106.12± 3.27***	100.13± 3.67	102.02± 3.01**	96.29± 3.25
ALT u/l	35.54± 2.45	51.5± 2.96***	42.57± 2.41	44.25± 2.85	40.21± 2011	43.95± 2.85	46.48± 2.49*
ALP u/l	215.2± 9.69	375.74± 5.31***	269.0± 19.6	274.75± 14.57**	262.08± 13.88*	297.6± 14.76***	270.8± 15.86*
GGT u/l	13.73± 2.81	32.16± 1.64***	24.76± 1.79**	25.88± 2.29**	20.22± 3.16	19.93± 2.32	22.79± 3.39
LDH u/l	478.14± 26.14	680.013± 22.13***	581.61± 22.51*	597.35± 22.1**	575.03± 25.26*	604.33± 25.97**	604.71± 21.24**
Urea mg/dl	31.6± 2.07	60± 5.43	37.94± 3.33***	45.94± 3.73**	38.1± 1.69	42.74± 2.34**	39.25± 1.64*
Creatinine mg/dl	0.56± 0.04	1.14± 0.05	0.79± 0.08***	0.82± 0.053**	0.74± 0.04***	0.84± 0.08*	0.7± 0.04

- **Results are expressed as mean ± SEM, student "t" test.**
- **Significante at *p<0.05 **p<0.01 ***p<0.001**

Table2: Effect of aflatoxin B1 and some antioxidant agents on antioxidant parameter on homogenate liver of male rats.

Groups / Parameter	control	AFB1	AFB1 + Se	AFB1 + vit E	AFB1 + Se + vit E	AFB1 +vitC	AFB1 + Se + vit C
SOD u/mg protein	6.9± 0.59	2.53± 0.21***	4.00± 0.48*	3.33± 0.42**	5.03± 0.32*	3.82± 0.24***	5.38± 0.31
CAT u/mg protein	12.88± 0.83	6.57± 0.56***	9.48± 0.95*	9.01± 0.91*	11.24± 0.17	8.42± 0.59***	12.01± 0.65
MDA nmol /mg protein	1.09± 0.54	4.74± 0.41***	2.64± 0.24*	2.78± 0.25*	2.33± 0.15	2.66± 0.15*	1.61± 0.24

Results are expressed as mean ± SEM, student "t" test.
Significante at *p<0.05 **p<0.01 ***p<0.001

SOD and CAT showed significantly decreased activity while significant increase in level of MDA in the AFB1 treated group with respect to controls (**Table 2**). This observation is in accordance with other studies reported that AFB1, significantly induced free radical production **(Marin and Taranu 2012)**. **Mogda et al., (2014**) reported that AFB1 decreased the antioxidase activities SOD and CAT in rabbits. The decreased level of the antioxidant enzymes may result from the oxidative stress induced by AFB1 exposure **(Guindon et al., 2007).** The antioxidant enzyme levels decrease as a

result of the consumption of enzymes to neutralize free radicals generated by pesticides, **(Amer et al., 2002).**

In contrast to the results obtained in this study, **Machlin and Bendich, (1987)**found that increase in antioxidant enzymes might provide protection against AFB1-induced free radical stress because it is known that peroxidation is reduced by the antioxidant enzymes **.** That is the efforts of the endogenous antioxidant enzymes to remove the continuously generated free radicals initially increase due to an induction but later enzyme depletion results, resulting in oxidative cell damage **(Kalra *et al.*, 1994).**

It is clear that mycotoxins stimulate the lipid peroxidation directly through the increase of the ROS synthesis, or the increase of the tissue susceptibility to the peroxydation is the result of the compromised antioxidant defense. Initiation of lipid peroxidation is caused by attack of any species that has sufficient reactivity to abstract a hydrogen atom from a methylene group upon a PUFA **(De Zwart et al., 1999 and Halliwell, 1999).** It was shown that also 8, 9-epoxide increases lipid peroxidation, followed by loss of membrane stability and the blockage of the membrane bound enzyme activity **Rastogi et al., (2007).**

Vitamins and/or Se co-administration with aflatoxin produced a significant reduction in tissue malondialdehyde (MDA) and ameliorated the lowering of SOD and CAT enzymes in the liver tissues as compared with those treated with aflatoxin B1 alone. These results are in agreement with those obtained by ***Ding et al., (2010)*** the authers suggested that vitamin or Se increased antioxidative function resulting from an increase in activity of GSH-Px whose center is Se**.** As previous study revealed, Se can inhibit lipid peroxidation **Battin et al., (2006).** Furthermore, Se also has an anti-apoptotic property involved with ROS and mitochondria linked signal pathway **Zhou et al., (2009).** Also vitamins C were shown to play a great role in reducing the oxidative stress induced by aflatoxins including AFB1 **Alpsoy and Yalvac (2011).**

Among the vitamins, vitamin E rapidly reacts with the peroxide radicals in order to form tocopheroxyl, a form of stable radicals, capable to generate α-tocopherol through the reaction with the ascorbate **(Atroshi et al., 2002).** A study realized in AFB1 intoxicated rats, **Cassand et al.,. (1991),** showed that dietary vitamin E protects directly the membrane against damage induced by lipid peroxidation, and indirectly the hepatic microsomal monooxygenase activities. On the other hand, vitamin E increases the activity of biomarkers associated with the oxidative stress **(Alpsoy & Yalvac, 2011)** and was not able to reduce AFB-epoxide formation **(Yu et al., 1994).**

Low total protein level acts as an indicator of the toxic effect of AFB1in serum **Mogda, et al., (2014).** Aflatoxin is known to impair protein biosynthesis by forming adducts with DNA, RNA and proteins, inhibits RNA synthesis, DNA-dependent RNA polymerase activity and causes degranulation of endoplasmic reticulum **(Cullen and Newberne 1995).** The present data observed that aflatoxin B1 given to the rats inducing significantly decreased values in serum total protein, albumin, and gamma globulin with increase in alpha globulin and beta globulin, (table3a). Decrease in serum globulin in toxin fed group might be due to the adverse effect of aflatoxin B1 on synthesis of total proteins and globulin.

The present results showed that, vitamins and/or Se treatment along with AFB1 ameliorates AFB1-induced changes in protein contents in the serum of rats (Table). The amelioration in protein contents might be due to increased DNA synthesis and reduction in harmful adduct formation **(Cheng et al., 2003).**
Dietary vitamin E decreases the genotoxic effects of AFB1 through the alteration of the activities of hepatic microsomal cytochrome P-450 activities, **Cassand et al.,. (1993).** Selenium can also prevent from oxidative damage to mitochondria DNA **Battin et al., (2006).**

Table 3a: Effect of aflatoxin B1 and some antioxidant agents on serum total protein and its main fraction fractions of male rats.

Parameter / groups	T. protein and main fraction g/dl						
	t.protein	albumin	T.Alpha	T.beta	T.gamma	T.globulin	A/G ratio
control	6.97± 0.17	2.25± 0.05	1.21± 0.02	1.18± 0.03	2.33± 0.10	4.72± 0.11	0.47± 0.01
Aflatoxin (AFB1)	6.01± 0.14***	2.00± 0.07*	1.35± 0.03***	1.24± 0.02	1.42± 0.12***	4.01± 0.13***	0.49± 0.01
AFB1 + Se	6.59± 0.16	2.15± 0.06	1.24± 0.02	1.21± 0.02	1.98± 0.11	4.44± 0.12	0.48± 0.02
AFB1 + vit E	6.39± 0.16	2.11± 0.05	1.2± 0.04	1.11± 0.04	1.97± 0.10*	4.28± 0.10	0.49± 0.02
AFB1 + Se+ vit E	6.68± 0.24	2.22± 0.05	1.21± 0.05	1.19± 0.03	2.06± 0.09	4.46± 0.12	0.49± 0.02
AFB1 + vit C	6.49± 0.29	2.18± 0.08	1.17± 0.03	1.19± 0.03	1.95± 0.10*	4.31± 0.11*	0.5± 0.02
AFB1 + Se+ vit C	6.73± 0.24	2.26± 0.06	1.14± 0.05	1.21± 0.04	2.12± 0.08	4.47± 0.11	0.5± 0.02

Results are expressed as mean ± SEM, student "t" test.
Significante at *p<0.05 **p<0.01 ***p<0.001

Globulins is connected with the reactive irritation of reticulendotel system as a result of toxic damage of the liver cells **Hristev et al., (2008)** Reduction in protein content and alteration on its fractions levels observed in the current study may be attributed to increase in the rate of degeneration of liver tissues as underlined by

increasing activities of ALT and AST (Table1). The injured liver logically is unable to maintain vital biochemical processes particularly protein biosynthesis. Prolonged excessive ROS/RNS production can trigger chemical chain reactions with all major biomolecules such as DNA, proteins, and membrane lipids. DNA is affected with a variety of lesions like oxidized bases, stand brakes, as well as DNA–DNA and DNA–protein cross-links **Barker et al., (2005).**These results are in accordance with previous work **(Quezada et al., 2000)** which reported a decrease in protein content in skeletal muscle, heart, liver and kidney of aflatoxin-fed animals. At the table (3b) the globulin component showed drop in α1, β1 and γ1 globulin in all the experiment while increase α2, β2 and γ2globulin as compared with control gp. The results coincided with the tune of total proteins and albumin. This may be attributed to that AFB1 causes hepatotoxic, nephrosis, hemorrhages (liver and kidneys) **(Tietz, 1996)**. In addition, AFB1 has immunosuppressive effect inhibit nearly cellular and humeral immunologic reaction **(Tietz, 1996, and Hassan, and Mogda 2003).** Decrease in serum globulin in toxin fed group might be due to the adverse effect of aflatoxin B1 on synthesis of total proteins and globulin **Agag (2004).**

Table (3b): Effect of aflatoxin B1 and some antioxidant agents on serum protein sub-fractions fractions in aflatoxin B1 intoxicated rats.

Parameter / groups	Protein Sub-fraction g/dl					
	Alpha1	**Alpha2**	**Beta1**	**Beta2**	**Gamma1**	**Gamma2**
control	0.47± 0.04	0.74± 0.05	0.71± 0.03	0.47± 0.03	2.08± 0.11	0.25± 0.01
Aflatoxin (AFB1)	0.26± 0.05**	1.09± 0.03**	0.5± 0.06**	0.74± 0.03***	1.12± 0.14***	0.29± 0.02
AFB1 + Se	0.3± 0.06	0.94± 0.04*	0.67± 0.03	0.54± 0.02	1.73± 0.11	0.25± 0.01
AFB1 + vit E	0.29± 0.04*	0.91± 0.03	0.62± 0.04	0.49± 0.04	1.71± 0.11	0.26± 0.01
AFB1 + Se+ vit E	0.32± 0.04	0.89± 0.05	0.68± 0.04	0.51± 0.02	1.79± 0.10	0.27± 0.02
AFB1 + vit C	0.29± 0.05*	0.88± 0.03	0.69± 0.03	0.5± 0.04	1.66± 0.11*	0.29± 0.01
AFB1 + Se+ vit C	0.32± 0.05	0.82± 0.06	0.67± 0.05	0.54± 0.04	1.86± 0.12	0.26± 0.01

- **Results are expressed as mean ± SEM, student "t" test.**
- **Significante at *p<0.05 **p<0.01 ***p<0.001**

PM or gross examination:

Histopathological profile of the liver of aflatoxin treated rats showed vacuolar degeneration of hepatocytes, congestion of hepatic sinusoids and hepatic necrosis with inflammatory cell infiltration (fig, 2) compared with finding of control (fig, 1) this

finding well agree with **Devendran and Balasubramanian (2011)**. The pretreatment of rats receiving AFB1 with Vitamin E or C showed extended portal tract infiltrated with few mononuclear inflammatory cells, less fibrosis, and degeneration of some hepatocytes Furthermore, selenium showed minimal, vacuolatiof hepatocytes on and fibrosis compared to aflatoxin treated group (Fig 3).These observations were well correlated with the biochemical findings and gives clear evidence that there was not only improvement in the liver functions with the treatment of antioxidant agent , but also in the hepatic architecture **(Fig 4,5 and 6). Vayalil PK. (2002)** showed that the induced liver protection against aflatoxicosis occurred via decreased the level of liver enzyme activity as well as decreased the free radical propagation, also besides its lowering the pathological lesions resulted from AFB_1.

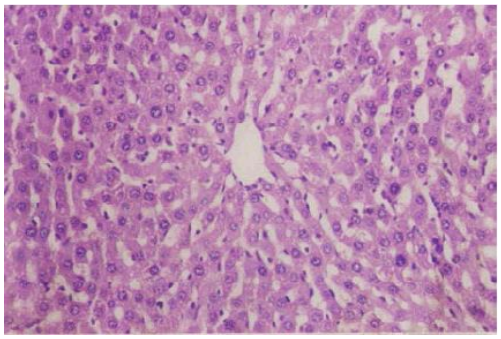


Fig1: Liver of control (-ve) rat group showing the normal histological structure X 200)

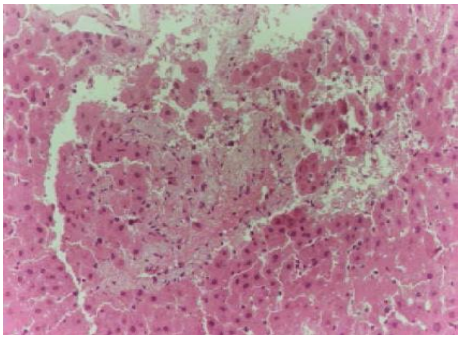
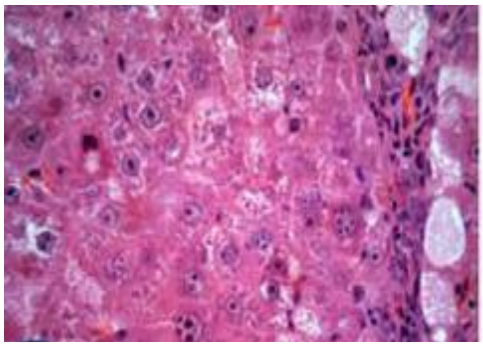
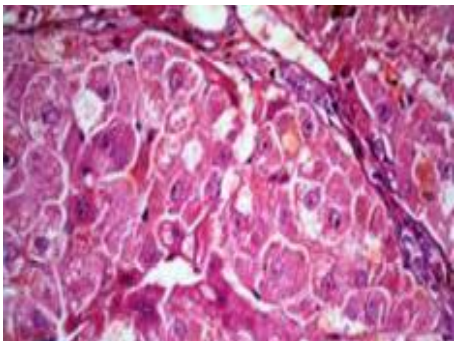


Fig2: Liver of control (+ve) rat group showing vacuolar degeneration of hepatocytes, (H and E congestion of hepatic sinusoids and hepatic necrosis with inflammatory cell infiltration (H and E X 200).



Aflatoxin with vitamin E



Aflatoxin with vitamin C

Fig 3: Aflatoxin and vitamin E or C showed extended portal tract infiltrated with mononuclear inflammatory cells, less fibrosis, less disarrangement and degeneration of hepatocytes(HE X 400).

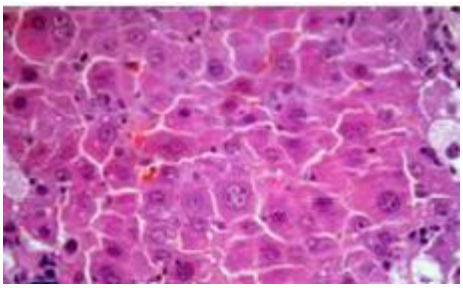


Fig4 : aflatoxin with selenium showed minimal minimal, vacuolation and fibrosis (HE X 400).

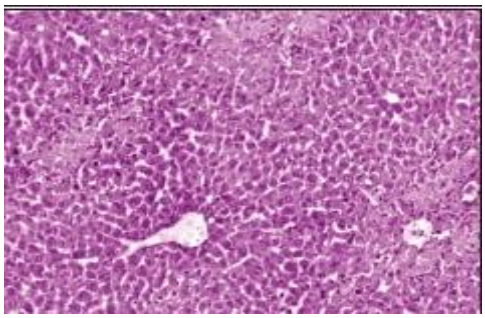


Fig5 :Aflatoxin and Vitamin E+ Se induced hepatotoxicity showing partial protection of liver with amelioration of necrosis with fatty changes (H and E, ×100).

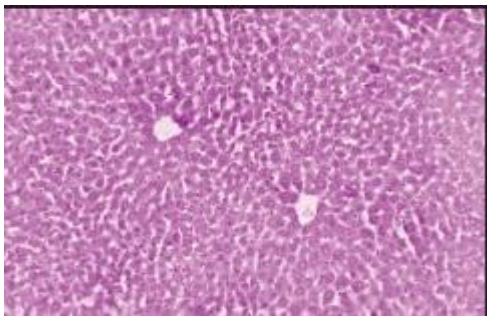


Fig 6: Vitmin C+ Se in aflatoxin induced hepatotoxicity showing normalization of hepatic architecture without any necrotic changes (H and E, ×100). mild

It can be concluded that the hepatotoxicity induced by aflatoxin B1 seemed to be modulated effectively by the simultaneous use of antioxidants: vitamin E or C alone or incombination, with selenium. Moreover, they are more efficient modulating the biochemical alteration , liver antioxidant enzymatic system and pathological changes. It is highly recommended to eat well-balanced and nutritious diets that contain sufficient amounts of natural antioxidants as a way to counteract the deleterious effects of the environmental hepatotoxins, including aflatoxin B1. These dietary antioxidants typically boost the liver's health and can minimize any excess damage done to the liver and may even expedite liver recovery.

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