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The Positive effects of vitamin E and selenium with activated charcoal against aflatoxin induced oxidative stress and cytotoxic damage in rabbits

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Abstract

A study was carried out to examine the hepatonephroprotective, chromosomal protective and antioxidant potential of vitamin E + selenium alone or with charcoal against aflatoxin B1 (AFB1) toxicity in rabbits. Fifty, New Zealand white rabbits were randomly allocated into five equal groups, each of ten. Group 1 was designed as the healthy control group. Rabbits in group 2, 3, 4 and 5 were received AFB1 orally at a dose of 0.05 mg /kg of body weight daily for 10 days. Group 2 was received AFB1 alone. Group 3 was treated with vitamin E + selenium at a dose of 0.05 ml / kg b.wt. intramuscularly twice with an interval of 7 days. Group 4 was treated with activated charcoal at a dose of 0.5gm/kg feed. While, group 5 was treated with both vitamin E + selenium and activated charcoal at the same doses. Blood and tissues samples were collected from rabbits of each group on the 1st and 21th day post-treatment. The results revealed that, rabbits received AFB1 alone showed significant decrease in the RBCs, Hb, TLC, lymphocytes, neutrophils and level of antioxidant (CAT and SOD) enzymes in liver tissues with significant increase in the liver function activities(ALT, AST, ALP and LDH), urea, oxidant marker (MDA) and total chromosomal aberrations compared with the other groups. Histopathological examination showed that the main affected organ due to AFB1 toxicity was liver. Hepatic degeneration with necrosis, hyperplasia of bile duct and infiltration of leucocytic inflammatory cells were showed severely in rabbits received AFB1 alone. These parameters approximated to control levels in case of rabbits received AFB1 and treated with vitamin E +selenium either alone or with activated charcoal. The study demonstrated that administration of vitamin E + selenium alone or with activated charcoal might be useful for the treatment of aflatoxicosis in rabbits. However, activated charcoal alone was of low value.

Key words: AflatoxinB1 - rabbits – Hematology- biochemical parameters-Oxidant and antioxidant markers-chromosomal aberration

Introduction

Aflatoxins (AFs) are metabolites produced by some fungi such as *Aspergillus spp.*, *penicillium spp.* and *Rhizopus spp*. They are considered to be among the most dangerous mycotoxins. Aflatoxicosis caused by aflatoxin (AF) B1 and related toxins represent one of the most serious diseases of rabbits and other animal species. Rabbits are considered one of the most sensitive animal to aflatoxicosis. Rabbits poisoned with AFs showed many effects including reduction of feed intake, poor efficiency of feed conversion, poor growth and malabsorption of various nutrients, decreased tissues integrity, increased susceptibility to infection, vaccine and drug failure and increased sensitivity to temperature extremes. Great pathological changes in most body organs are induced by AFs ingestion. Such changes are liver and kidney dysfunctions and genetic damage including carcinogenicity, teratogenicity and mutagenicity **(Lakkawar et al., 2004 and Marai &Asker, 2008).**

In susceptible animal species, aflatoxins especially AFB1 are metabolized by cytochrome P450 (CYP450) microsomal enzymes to aflatoxin-8, 9-epoxide, a reactive form that binds to albumin in the blood serum, forming adducts and hence causing DNA damage and cause acute toxicity (aflatoxicosis) or to DNA and induce liver cancer (**Wu and Khlangwiset, 2010).**

AFB1-mediated toxicity was also found to be related to its pro-oxidant potential. This is because reactive oxygen species (ROS) including superoxide anion (O-), hydrogen peroxide (H_2O_2), and hydroxyl radical (-OH) are generated during the metabolic processing of AFB1 by liver enzymes (**Preston and Williams, 2005).** ROS causes oxidative stress by damaging cellular membranes and components.

Both selenium and vitamin E are essential and highly efficient antioxidants which protect rabbits against lipid and protein oxidation **(Muller et. al., 2002).** They can act as immunostimulators and protect the broiler birds when challenged with aflatoxin **(Abeera et. al., 2009).**

Activated charcoal usage had a good effect as it prevented aflatoxin to accumulate in the organs **(Abdelhamid et.al., 1990).**

The present study was undertaken to examine the hepatonephroprotective, chromosomal protective and antioxidant potential of vitamin E + selenium alone or with charcoal against aflatoxin B1 toxicity in rabbits.

Materials and methods

Chemicals:

1. Aflatoxin B1 (AFB1): It was obtained from Biochemistry Department, Animal Health Research Institute, Dokki, Giza. It was given orally at a dose of 0.05 mg /kg of body weight daily for 10 days **(Clark et al 1982).**

2. Vitamin E + selenium (Vitesel® Emulsion for Injection). It was manufactured by Norbrook.co.Uk. Each 1 ml contain: Alpha-Tocopheryl Acetate 68mg, Selenium (as Potassium Selenate) 1.5mg and Benzyl Alcohol as antimicrobial preservative 20mg. The recommended dose is: 0.05 ml / kg b.wt intramuscularly twice with an interval of 7 days.

3. Activated charcoal (charcoal activated powder): It is a pure lab. chemical manufactured by El NASSER Pharmaceuticals Co, Cairo A.R.E. The recommended dose is 0.2-0.5g/kg feed **(Jindal et al., 1994).**

Experimental animals:

Fifty New Zealand white rabbits (400-600 g body weight) were used in the present work. Rabbits were kept in battery cages. Both food and water were provided ad libitum. Standard rabbit feed free from aflatoxin was purchased for all groups.

Experimental design:

After an acclimation period of two weeks; rabbits were randomly allocated into five equal groups, each including 10 rabbits. Group 1 was designed as the healthy control group. Rabbits in the Groups 2, 3, 4 and 5 were received AFB1 orally at a dose of 0.05 mg /kg of body weight daily for 10 days. Group 2 was received AFB1 alone. Group 3 was treated with vitamin E + selenium at a dose of 0.05 ml / kg b.wt. Intramuscularly twice with an interval of 7 days. Group 4 was treated with activated charcoal at a dose of 0.5g/kg feed. Group 5 was treated with both vitamin E + selenium and activated charcoal at the same doses.

Blood and tissue samples were collected from 5 rabbits of each group on the 1^{st} and 21^{th} day post treatment.

Sampling:

a -**Blood samples:**

Two blood samples were collected from rabbits of each group through ear vein puncture. The first one was taken on heparin as anticoagulant for hematological examination. The second blood sample was left to clot at room temperature for about 2 hours and centrifuged

at 3000 rpm for 10 min. Serum samples were drawn in dry clean capped tubes and kept in deep freeze at -20ºC for biochemical analysis.

B-Tissue sample:

- 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 4ºC and the resultant supernatant was used for (enzymatic and non- enzymatic) antioxidant assays **(Chitra et al., 1999).**
- Specimens from liver and kidneys were collected from slaughtered rabbits for histopathological examination.
- Bone marrow was collected from femurs for chromosomal analysis.

Hematological study:

The erythrocytic count (RBCs), hemoglobin concentration (Hb gm/dl), packed cell volume (PCV %), the erythrocytic indices ["Mean corpuscular hemoglobin" (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC)] and the total leukocytic and differential counts were counted using CBC analyzer sysmex 2000 IV.

Biochemical analysis:

The biochemical assays of serum lactic dehydrogenase (LDH) activities was determined according to methods of **Szase et al. (1976),** aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities according to **Reitman and Frankel, (1957**), alkaline phosphatase (ALP) activity according to **Tietz, (1996)**, serum urea level was measured according to **Wybenga et al. (1971**) and serum creatinine level according to **Henry (1974).**

Oxidant/antioxidant markers including Malondialdehyde (MDA) ***(*Satoh, 1987)**, Catalase (CAT) **(Aebi, 1984)** and Superoxide dismutase (SOD) **(Misra and Fridovich, 1972)** were calorimetrically assayed using chemical kits (Biomed Egypt) and Shimdzu UV 240 spectrophotometer.

Histopathological examination:

Specimens from liver and kidneys were collected from slaughtered rabbits. They were fixed in 10 % buffered neutral formalin, prepared, embedded in paraffin sections of 5 micron thickness and stained by H&E to be examined microscopically according to **Bancroft et.al. (2013).**

Chromosomal analysis:

Rabbits were injected intramuscularly with 0.25 ml/100 gm b.wt. colchicine solution (0.5%) ninety minutes before slaughtering and both femurs were dissected for culturing the bone marrow according to **Yosida and Amano (1965).**

For analysis of chromosomal aberrations fifty metaphases per animal were selected **(Worton and Duff, 1979)** and described according to **Brusick (1980).**

Statistical Analysis:

The obtained data were computerized and analyzed for significance, calculation of standard error and variance according to **SPSS 14 (2006).**

Results and Discussion

The obtained results clearly demonstrated that, aflatoxin alone caused significant ($p < 0.05$) decrease in RBCs, Hb, PCV and TLC (Tables, 1&2). These findings agree with the other reports that explain the suppressive effects of aflatoxin on hematopoiesis and immune response such as the results observed in blood picture measurements of mature rabbits orally treated by AF B1 (**Ibrahim, 2000).**On similar ground, **Abdel-Wahhab et al. (2002)** reported a decrease in RBCs and Hb resulting in normocytic normochromic anaemia in aflatoxin application alone. This decrease in the hematological parameters may be due to many factors such as inhibition of protein synthesis as evidenced by lower serum albumin **(Kaneko,1989),** decrease of the total iron binding capacity **(Harvey et al., 1991)** and the hemopoietic cellular defects of AF **(Van Vleet and Ferrans,1992).**

Rabbits treated with vitamin E + Se evoked a significant increase in TLC. Vitamin E and Se played a significant role in neutralization of aflatoxin effects. This observation was in line with **Flora and Malathy (2004)** who reported this effect in case of the combination of both vitamin E and Selenium rather than using each of them alone.Vitamin E converts arachiodonic acid into prostaglandin, which plays an important role in enhancement of immune response **(Balker, 1993).** Selenium has also been reported to improve the immune response in broilers fed with AF contaminated diets **(Perozo and Rivera, 2003).**

Data presented in table (3) showed significant increase in the activity of AST, ALT and ALP of the liver enzymes in rabbits received AFB1 alone. This result is in agreement with the observation of **Abdel-Wahab et al. (2006); Barbour et al. (2014) and Onyegeme-Okerenta and Enyadike (2015)** who found that an increase in the levels of serum enzymes measured is interpreted as a consequence of hepatocyte degeneration and subsequent leakage of enzymes. Such hepatic toxic effect of AF could be attributed to its

active metabolite in liver as epoxide**.** These elevations indicate cellular leakage and loss of functional integrity of cell membrane in liver **(Darwish et al., 2011).**

Significant increase in the level of LDH in our study is in agreement with the result of **Rizvi and Shakoori (2000).** Increased LDH activity was regarded as an indication of hepatic damage.

In the present investigation, it has been demonstrated that, aflatoxicosis could elevate serum urea, this indicated presence of kidney dysfunction (Table, 3). This result was in agreement with **Nowar et al. (1992; EI-Zahar et al., (1996) and Orsi et al. (2007)** who revealed that serum urea was found to increase in calves, rats, broilers and rabbits as a result of aflatoxicosis.

These biochemical changes in liver and kidney functions were due to some pathological changes in liver and kidney tissues. On the 1st day post treatment, the liver of rabbits received AFB1 alone was enlarged, congested and friable. Kidneys were swollen and had pinpoint hemorrhages on its surface. The histopathological alteration observed in liver of rabbits received AFB1 alone showed congestion of hepatic B.VS. with perivascular leucocytic cells infiltration (Fig1),hyperplasia of epithelial lining of the bile duct with periductal fibrosis and edema (Fig2), in addition to Focal areas of coagulative necrosis of hepatic cells in the hepatic parenchyma (Fig3). Kidneys revealed cystic dilatation of some renal tubules (Fig4), accompanied with peritubular leucocytic cells infiltration (Fig.5). However, the histopathological study on the 21th day post treatment revealed that the liver of rabbits received AFB1 showed hyperplasia of epithelial lining of bile ducts with periductal fibrosis (Fig.6). Also, the Kidneys showed hypo cellularity of glomeruli (Fig.7). Similar results were observed by **Cam et al (2008)**; **Ortatatin and Oquz (2011); Sawarkar et al (2011); Pathod et al (2013)** and **Prabu et al. (2013)**.

Our results confirmed also by **Sepahdari et al. (2009)** and **Varior and Philip (2012)** who recorded that AFB1 significantly changed the stability of the lysosomal membrane, leading to a disorder of hepatocyte permeability and pathological changes in the liver .This effect confirmed by high levels of ALT and AST enzymes in the serum**.**

Furthermore, the histopathological alteration observed in the liver of rabbits received AFB1 and treated with charcoal showed mild congestion of the hepatic B.Vs with perivascular leucocytic infiltration (Fig.8). The kidney showed cloudy swelling of some renal tubules with focal infiltration of leucocytic cell to renal tubules (Fig.9). Similar results were recorded by **Mohamed and Mokhbatly (1997) and Amany et al. (2009)**.

The Liver of rabbits received AFB1 and treated with vit E+ selenium showed normal hepatic architecture with minimal cellular details abnormalities (Fig10). Mild

degenerative changes in both liver and kidneys may be due to the immunomodulatory effect of vit.E + selenium. These results were supported by **Pathod et al (2013)** who mentioned that a combination of vit.E and selenium can act as immunomodulator in chicken previously given aflatoxin B1.

In view of oxidative stress, the present results indicated that the activities of antioxidant biomarkers (Catalase and Superoxide dismutase) were significantly decreased while the content of oxidative product (Malondialdehyde) showed significant increase in rabbits received AFB1 alone if compared to the other groups (Table, 4).

A significant increase in Malondialdehyde (MDA) level demonstrated in rabbits received AFB1 alone had been investigated by **Eraslan et al. (2005).** Malondialdehyde is considered to be the most significant indicator of membrane lipid peroxidation, arising from the interaction of reactive oxygen species (ROS) with cell membranes. The induced increase in lipid peroxidation after aflatoxins ingestion may be due to the fact that onset of lipid peroxidation leads to the progressive accumulation of lipid hydroperoxides in plasma membranes, which then decomposes to form MDA under stress or toxic conditions **(Kandeil and Abu El-Saad, 2005).** AFs may decrease vitamin absorption and reduce their levels in the body, and hence weaken the antioxidant defense mechanism **(Decoudu et al., 1992).** This may be one of the reasons for the increase in MDA level.

However, the decline in superoxide dismutase (SOD) activity which was observed in case of administration of AFs may be related to the consumption of highly active components during conversion into H_2O_2 due to the effect of AFs **(Gokhan,et al; 2005 and Abdel-Wahhab, & Aly, 2003)**.Catalase (CAT) activity was significantly decreased in rabbits received AFB1 alone compared to the other groups, this result was in agreement with many researches carried out on this subject in different animal species **Verma and Nair (2001)** and **Rastogi et al. (2001)** who suggested that AFs decreased catalase in testicles and liver. The significant reduction in activities of CAT and SOD could be responsible for increased lipid peroxidation observed during aflatoxicosis.

CAT and SOD are the main antioxidant enzymes in the body, which scavenge unwanted nacent oxygen, H_2O_2, and OH produced by free radical. The decreased enzyme activities and increased MAD levels produced by AFB1 (Table, 4) can be attributed to lower ability of the tissue to scavenge free radicals and prevent the action of lipid peroxidation (**Amresh et al., 2007)**.

The antioxidants have been definitively linked to anti-inflammatory and immunosuppressive properties and they may include increased superoxide dismutase, glutathione peroxidase, catalase and glutathione reductase activity **(Lee et al., 1999).**

Reactive oxygen species (ROS) and lipid peroxidation (LPO) have been considered to be the main mechanisms in AFB1 toxicity **(Sohn et al., 2004).**

The significantly reduced activities of SOD and CAT (Table, 4) in this work point out to the hepatic damage in the rabbits received AFB1 alone. Otherwise, treatment with vitamin E + selenium alone or with charcoal showed significant increase in the level of these enzymes which indicates their antioxidant activity.

Vitamin E had been reported to be an excellent biological chain breaking antioxidant that protects cells and tissues from lipoperoxidative damage induced by free radicals **(Koinarski et al., 2005).** Selenium is an essential trace element in animal known as a highly effective antioxidant. A very important metabolic role of selenium in animals is the presence in the active site of the selenoenzyme (GSH-PX). This enzyme, together with superoxide dismutase and catalase, protects cell against damage caused by free radicals and lipoperoxides **(Wang et al., 2013).** In addition, it was reported that selenium is required for normal functions of pancreas and vitamin E plays a role in selenium metabolism **(Combs and Combs 1986 and Ibrahim et al., 2011).**

Microelements and vitamin E protect cells and subcellular structures from oxidative damage by inhibiting oxygen formation, and by decreasing MDA levels. In addition the increased levels of the antioxidant enzyme GSH-Px and CAT accompanied with vitamin E supplementation might thus normalize that lipid peroxidation reaction and related biochemical changes which in turn protect the cells from increased risk of peroxidation damage as a result of administration of cytotoxic drugs **(Ibrahim et al., 2011).**

Rabbits received AFB1 alone provoked significant increase in the total chromosomal aberrations and total aberrant cells compared with the other groups. Concerning the different types of chromosomal aberrations, it was clearly indicated that centromeric attenuation, deletions and pulverized chromosomes are the most sensitive types of aberrations to be induced. Ring, gap and end to end association were also recorded. Numerical chromosomal aberrations in the form of hypoploidy were observed (Table, 5 and figures 11 through14). The findings in present study are in corroboration with those reported by **Yoshiaki and Mitsuo (2001)** who recorded that AFB1 induced chromosome aberrations (CA) in rat bone marrow cells. The exposure to AFB1 led to gene mutations, chromosomal aberrations, formation of micronuclei, sister chromatid exchanges in bone marrow cells and dominant lethal mutations **(Eaton and Gallagher, 1994).**

The activated charcoal did not affect adsorption of AF (5 and 20 ppm) in vitro **(Maryamman et al., 1991).** Similarly**, Edrington et al. (1996)** reported that activated charcoal did not reduce urinary excretion of AF in turkey poults orally dosed with 0.75 mg

AF B1/kg body weight, also it did not have any protective effects on AF toxicity.In our opinion, the clear differences between the ability of charcoal in alleviating the toxic effects of AFB1 may be due to failure of charcoal to adsorb all the amount of aflatoxin leaving free parts which caused these alterations.

Results of the present study revealed many hematological, biochemical, chromosomal and histopathological changes in experimentally induced aflatoxicosis in rabbits. Liver was the most affected organ according to the biochemical and histopathological findings. It could be said that administrations of vitamin E + selenium alone or with charcoal to rabbits with aflatoxicosis reduced the toxic effects of AF.

Table 1. Erythrogram (Mean values ± S.E) of rabbits received AFB1 and treated with vitamin E+ selenium, charcoal and vitamin E+ selenium with charcoal compared with control group on the 1st and 21th day post treatment.

(n= 5)

Parameters	RBC_s $x10^6$/ul		Hb gm/dl		PCV %		MCV fl		MCH pg		MCHC g/dl	
Sampling	1st	21th	1st	21th	1st	21th	1st	21th	1st	21th	1st	21th
Healthy control group	5.686 ± 0.245a	5.742 ± 0.322a	11.92 ± 0.287a	11.77 ± 0.33a	38.08 ± 0.75a	38.33 ± 0.98 a	59.77 ± 1.18 b	66.94 ± 2.52 a	21.04 ± 0.49a	20.76 ± 0.71 a	31.30 ± 0.19ab	30.72 ± 0.59 a
Aflatoxin group	3.518 ± 0.233c	4.21 ± 0.094bc	8.0 ± 0.219c	8.19 ± 0.22c	27.07 ± 1.02b	29.30 ± 0.81c	77.48 ± 2.06a	69.63 ± 1.65 a	23.14 ± 0.94a	19.45 ± 0.22 a	29.64 ± 0.85b	28.01 ± 0.80 a
Aflatoxin + Vit. E &Selenium group	4.676 ± 0.202b	4.512 ± 0.214c	9.4 ± 0.201b	9.17 ± 0.23c	30.15 ± 0.65c	29.91 ± 0.92c	64.478 ± 3.35b	66.27 ± 3.96 a	20.10 ± 0.88b	20.32 ± 0.21 a	31.18 ± 1.25a	30.66 ± 1.57 a
Aflatoxin + Charcoal group	4.004 ± 0.103c	4.226 ± 0.24bc	8.15 ± 0.237c	8.29 ± 0.29c	27.15 ± 0.92b	27.76 ± 0.65c	68.19 ± 3.96b	66.65 ± 4.51 a	20.41 ± 0.19b	19.74 ± 0.71 a	30.30 ± 1.6ab	29.98 ± 1.55 a
Aflatoxin+ Vit. E &Selenium + Charcoal group	4.742 ± 0.226 b	4.812 ± 0.213b	9.650 ± 0.344b	10.01 ± 0.24b	29.95 ± 0.62ab	31.94 ± 0.58b	63.16 ± 2.91b	66.37 ± 2.09 a	20.35 ± 1.0b	20.80 ± 1.1 a	32.22 ± 1.36a	30.39 ± 1.6 a

Different letters in the same column means significant difference at ($p \leq 0.05$)

Table 2. Leukogram (Mean values ± S.E) of rabbits received AFB1 and treated with vitamin E+ selenium, charcoal and vitamin E+ selenium with charcoal compared with control group on the 1st and 21th day post treatment. (n= 5)

Parameters / Groups	TLC X10³/UL		Lymphocyte X10³/ UL		Neutrophil X10³/ UL		Monocytes X10³/ UL		Basophil X10³/ UL		Eosinophil X10³/ UL	
	1st	21th	1st	21th	1st	21th	1st	21th	1st	21th	1st	21th
Healthy control group	6.352 ±0.17a	6.432 ±0.16a	3.114 ±0.23a	3.087 ±0.29a	2.41 ±0.16a	2.508 ±0.15a	0.508 ±0.02a	0.515 ±0.03a	0.127 ±0.08a	0.064 ±0.02a	0.193 ±0.02a	0.258 ±0.08a
AFB1 group	4.699 ±0.09d	4.826 ±0.17d	2.255 ±0.14c	2.258 ±0.12b	1.833 ±0.08c	1.931 ±0.09b	0.329 ±0.05c	0.337 ±0.2b	0.114 ±0.03a	0.058 ±0.04a	0.168 ±0.01a	0.241 ±0.03a
AFB1 + Vit. E &Selenium group	5.628 ±0.13b	5.666 ±0.18bc	2.87 ±0.06b	2.835 ±0.07a	2.08 ±0.15ab	2.094 ±0.14ab	0.391 ±0.02b	0.453 ±0.04a	0.116 ±0.02a	0.056 ±0.03a	0.169 ±0.03a	0.227 ±0.04a
Aflatoxin + Charcoal Group	5.135 ±0.1c	5.332 ±0.23cd	2.536 ±0.18bc	2.709 ±0.11a	1.95 ±0.11bc	1.919 ±0.12b	0.360 ±0.04	0.401 ±0.07	0. 115 ±0.07a	0.068 ±0.05a	0.174 ±0.02a	0.233 ±0.05a
AFB1+ Vit. E &Selenium + Charcoal group	6.021 ±0.14ab	6.16 ±0.18ab	3.07 ±0.18ab	3.04 ±0.13a	2.17 ±0.11ab	2.32 ±0.09a	0.472 ±0.03ab	0.493 ±0.06a	0.120 ±0.05a	0.062 ±0.05a	0.189 ±0.05a	0.246 ±0.04a

Different letters in the same column means significant difference at ($p \leq 0.05$)

Table 3. Biochemical parameters of liver and kidney function in serum of rabbits received AFB1 and treated with vitamin E+ selenium, charcoal and vitamin E+ selenium with charcoal compared with control group on the 1st and 21th day post treatment.

Mean± S.E (n= 5)

Parameters / Groups	AST u/l		ALT u/l		Alp u/l		LDH u/l		Urea mg/dl		Creatinin mg/dl	
	1st	21th	1st	21th	1st	21th	1st	21th	1st	21th	1st	21th
Healthy control group	23. 0 ±02.4 c	20.4 ±2.1c	30.4 ±3.6bc	26.6 ±1.5c	187.4 ±17.3c	177.8 ±17.1c	187.45 ±6.0c	189.60 ±7.3d	24.2 ±3.0c	27.4 ±1.9b	0.99 ±0.14a	1.08 ±0.2a
AFB1 group	144.0 ±12.9a	99.4 ±2.8a	210.0 ±13.8a	149.4 ±11.1a	912.8 ±34.9a	710 ±64.1a	403.34 ±24.2a	434.47 ±18.1a	55.2 ±6.8a	48.0 ±4.8a	1.02 ±0.13a	1.07 ±0.08a
AFB1 + Vit. E &Seleniu m group	98.2 ±8.94b	70.0 ±6.7b	121.0 ±11.4b	89.2 ±10.4b	519.8 ±53.5b	239.8 ±41.8b	303.47 ±13.5b	297.35 ±13.6c	40.4 ±6.3ab	33.0 ±3.4b	1.02 ±0.1a	0.95 ±0.11a
AFB1 + Charcoal Group	138.6 ±13.2a	75.8 ±8.4b	182.4 ±20.1a	132.6 ±12.5a	780.6 ±56.5a	634 ±53.2a	362.6 ±23.5a	352.41 ±15.3b	47.2 ±2.9ab	42.8 ±1.9a	1.44 ±0.13a	1.318 ±0.2a
AFB1+ Vit. E &Seleniu m+ Charcoal group	89.0 ±9.27b	68.0 ±5.61b	116.4 ±10.4b	84.8 ±11.4b	515.4 ±52.0b	235.2 ±44.2b	190.43 ±4.5c	191.35 ±7.8d	37.4 ±4.8cb	28.6 ±1.6b	1.21 ±0.17a	1.196 ±0.08a

Different letters in the same column means significant difference at ($p \leq 0.05$)

Table 4. Oxidative parameters in liver tissues homogenate of rabbits received AFB1 and treated with vitamin E+ selenium, charcoal and vitamin E+ selenium with charcoal compared with control group on the 1st and 21th day post treatment.

Mean± S.E (n= 5)

Parameters / Groups	MDA nmol/g tissue		SOD u/g tissue		CAT u/g tissue	
	1st	21th	1st	21th	1st	21th
Healthy control group	16.2 ±0.7d	15.6 ±0.7c	6.4 ±0.2a	6.5 ±0.1a	0.68 ±0.08a	0.69 ±0.04a
AFB1 group	37.4 ±4.01a	30.4 ±2.7a	3.2 ±0.3c	4.8 ±0.1b	0.38 ±0.05b	0.46 ± 0.02b
AFB1 + Vit. E &Selenium group	23.8 ±1.3c	17.4 ±1.1c	4.7 ±0.1b	6.5 ±0.1a	0.68 ±0.3a	0.65 ±0.02a
AFB1+ Charcoal group	30.8 ±1.2b	21.8 ±1.2b	3.6 ±0.2c	4.8 ±0.2b	0.57 ±0.02a	0.65 ±0.04a
AFB1+ Vit. E &Selenium+ Charcoal group	19.5 ±0.7dc	15.8 ±0.7c	4.8 ±0.2b	6.1 ±0.2a	0.71 ±0.03a	0.70 ±0.02a

Different letters in the same column means significant difference at ($p \leq 0.05$)

Table 5. Chromosomal aberrations induced in bone marrow of rabbits received AFB1 and treated with vitamin E+ selenium, charcoal and vitamin E+ selenium with charcoal compared with control group on the 21th day post treatment.

Mean± S.E (n= 5)

Parameters / Groups	No. of examined metaphase cells	Total aberrant cells			Types of structural aberration						Types of numerical aberration		Total chromosomal aberration
		No.	%	Mean± S.E	Ring	Gap	Centromeric attenuation	Deletion	Pulverization	End to end association	hypoploidy	hyperploidy	
Healthy control	250	5	2	1.0± 0.4d	-	-	6	-	-	-	2	-	1.6±0.7 d
AFB1 group	250	133	53.2	26.6 ± 1.7a	6	2	54	34	20	4	26	-	29.2±2.0 a
AFB1 + Vit. E &Selenium group	250	35	14	7.0± 0.6c	-	-	26	6	4	-	4	-	8.0±0.4 c
AFB1 + Charcoal group	250	77	30.8	15.4 ± 1.7b	8	2	32	20	14	2	10	-	17.6±1.6 b
AFB1+ Vit. E &Selenium+ Charcoal group	250	17	6.8	3.4± 0.5d	2	-	9	4	2	-	3	-	4.0±0.3 d

Different letters in the same column means significant difference at ($p \leq 0.05$)

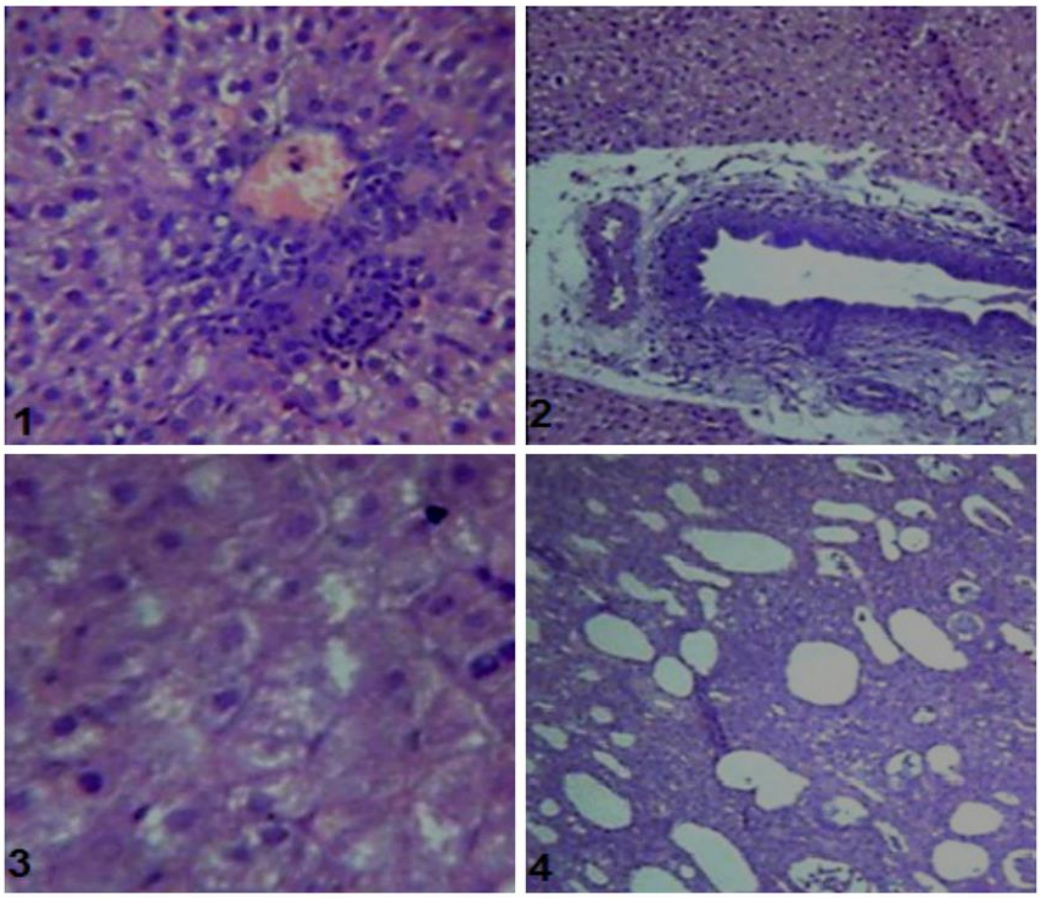

Plate 1: Fig. (1-4): Liver and kidney sections, on the 1st day post treatment, of rabbits received AFB1 alone showing:

Fig.1: Congestion of hepatic B.VS. with perivascular leucocytic cells infiltration. (H&E X 100)

Fig.2: Hyperplasia of epithelial lining of the bile duct with periductal fibrosis and edema (H&E X 40)

Fig.3: Focal area of coagulative necrosis of hepatic cells in the hepatic parenchyma. (H &E X 200)

Fig.4: Cystic dilatation of some renal tubules. (H

& E X 200)

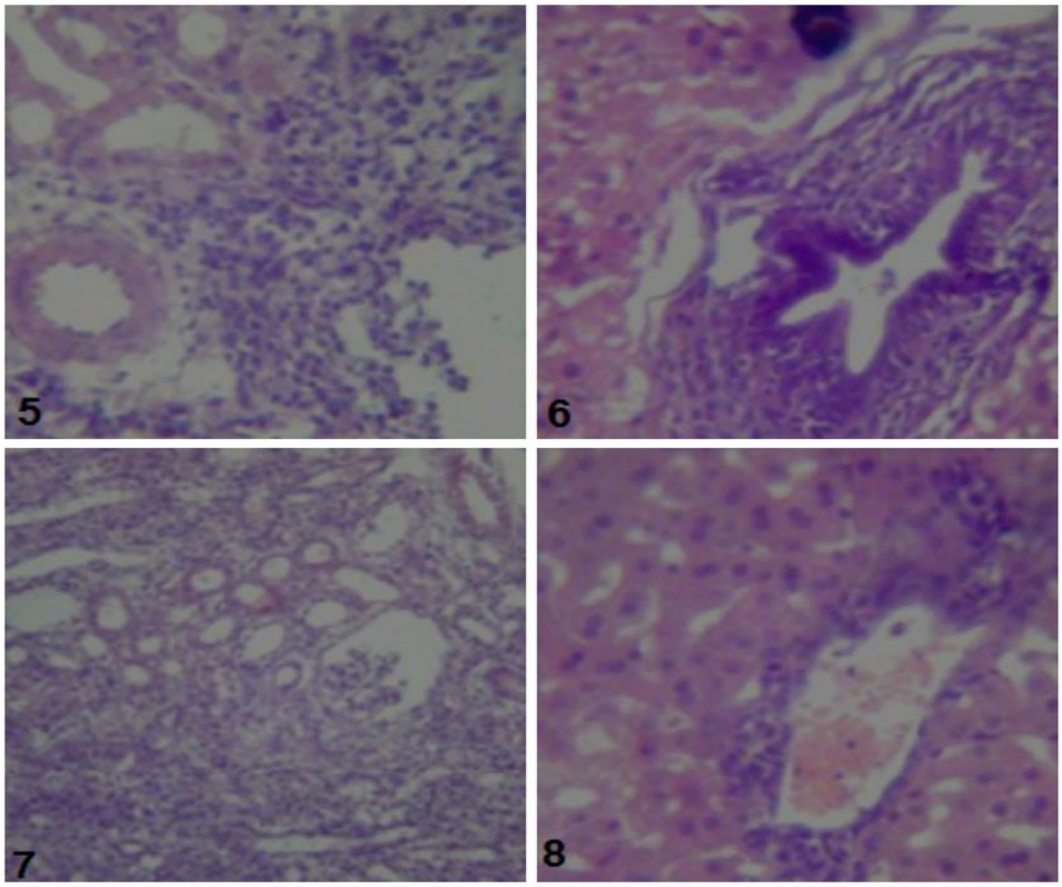

Plate 2: Fig. (5-8): The examined specimens showed

Fig.5: kidney Section on the 1st day post treatment from rabbits received AFB1 alone showing peritubular leucocytic cells infiltration. (H& E X 100)

Fig.6: liver Section on the 21th day post treatment from rabbits received AFB1 alone showing hyperplasia of epithelial lining of bile ducts with peri ductal fibrosis (H & E X 40)

Fig.7: kidney Section on the 21th day post treatment from rabbits received AFB1 alone showing hypocellularity of glomeruli (H & E X 40)

Fig.8: liver Section on the 21th day post treatment from rabbits received AFB1 and treated with charcoal showing mild congestion of the hepatic B.Vs with perivascular leucocytic infiltration (H&E X 100)

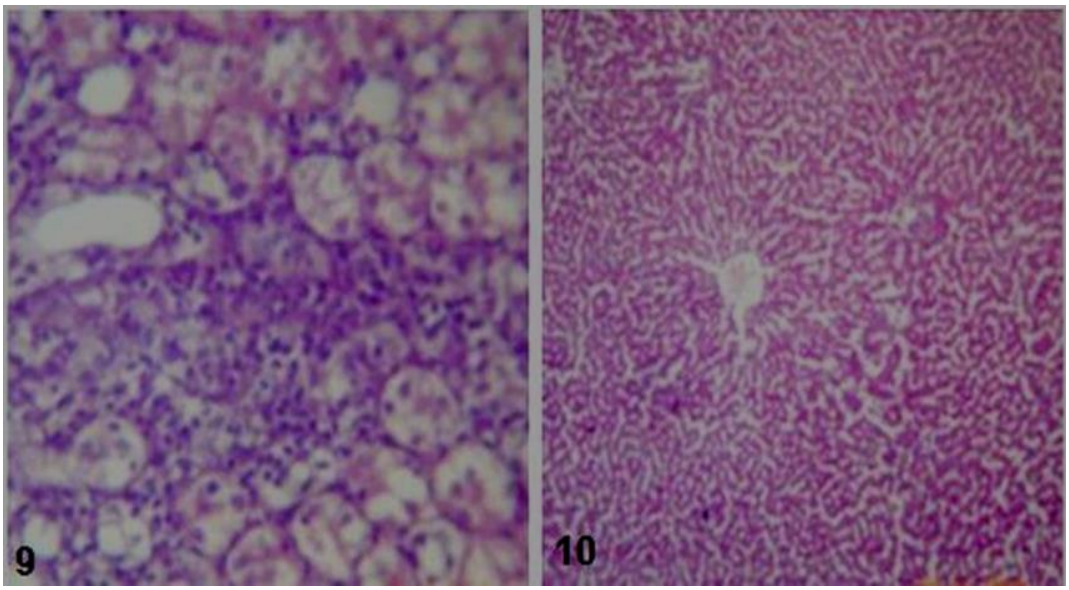

Plate 3: Fig. (9-10): The examined specimens showed

Fig.9: Kidney section on the 21th day post treatment from rabbits received AFB1 alone showing cloudy swelling of some renal tubules with focal infiltration of leucocytic cell to renal tubules (H &E X 100)

Fig.10: liver Section on the 21th day post treatment from rabbits received AFB1 and treated with E + selenium showing normal hepatic architecture with minimal cellular details abnormalities (H &E X 40)

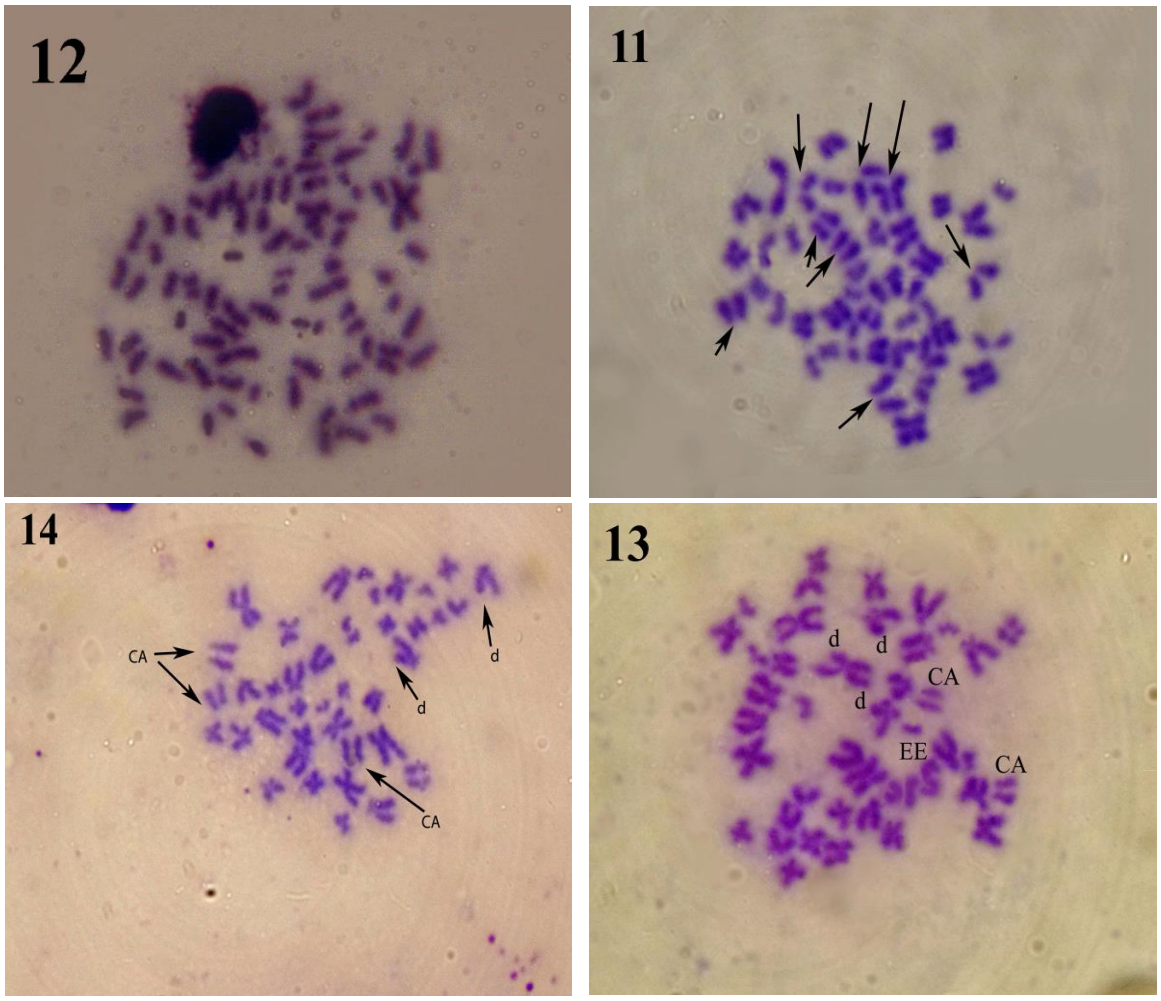

Plate 4: Fig. (11-14): The chromosomal study revealed

Fig.11: Metaphase spread obtained from femur bone marrow culture of rabbits received AFB1 alone displaying centromeric attenuation (CA).

Fig.12: Metaphase spread obtained from femur bone marrow culture of rabbits received AFB1 alone displaying pulverized chromosome.

Fig.13: Metaphase spread obtained from femur bone marrow culture of rabbits received AFB1 and treated with charcoal displaying centromeric attenuation (CA), deletion (d) and end to end association (EE).

Fig.14: Metaphase spread obtained from femur bone marrow culture of rabbits received AFB1 and treated with E + selenium displaying centromeric attenuation (CA) deletion (d) and hypoploid

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