Genotoxic And Cytotoxic Biomarkers Of Aflatoxin B1 And The Use Of Curcumin As Antidotal Therapy

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ABSTRACT

This study was carried out to spot light on the different biomarkers caused by aflatoxin B1 oxidative damage, in order to get an early diagnosis, and study the effect of curcumin as a model used as an antidotal therapy to reduce or even ameliorate the oxidative damage induced by aflatoxin B1. Forty apparently healthy male adult albino rats weighing 120-150 gm were divided into four equal groups, the 1st group was orally administered 250 µg/kg Body weight aflatoxin B1 using stomach gavage, the 2nd group was orally administered 250 µg/kg body weight in combination with 200 mg/kg body weight Curcumin, the 3rd group was orally administered 200 mg/kg body weight Curcumin, while the fourth group was administered distilled water and kept as negative control group. Rats in all groups were administered 10 oral doses for two weeks (5 successive doses /week). Serum samples were obtained to estimate different biochemical parameters including 8-Hydroxy-2-deoxy guanisine (marker of DNA damage), and cytokines (IL1-IL5-TNF) level .while liver tissues were used for histopathological examination and Comet assay for detection of DNA degeneration. The results revealed that Aflatoxin B1 caused significant increase of 8-hydoxy guanisine, and increase of cytokines level. While comet assay for detection of DNA degeneration revealed that tail length, DNA tail %, and DNA moment were significantly increased indicating sever DNA degeneration in liver. Generally, Curcumin administeration in a dose of 200 mg /kg body weight orally for 2 weeks in combination with Aflatoxin B1 caused significant alteration on all parameters toward the normal state, although curcumin administration alone caused no significant changes in all parameters level when compared to the control group. The histopathological examination of liver tissue revealed sever hepatic damage induced by Aflatoxin B1; although curcumin treated rats in combination with aflatoxin B1 revealed less sever changes.

INTRODUCTION

Biomarkers that are diagnostic of liver histomorphologic change are being evaluated, developed, and improved largely by industrial efforts. Thus, novel biomarkers of liver injury are sought to lower the incidence of false negative results, thereby leading to more accurate prediction of liver injury in preclinical and ultimately clinical studies *(1)*. During recent years, active principles with diverse chemical structures have been isolated from plants reportedly possessing hepatoprotective effects. For instance, various triterpenes like, oleanolic acid, ursolic acid, *curcumin*, and celastrol are effective in protecting against liver *(2)*.

Aflatoxins are the most dangerous mycotoxin known, owing to their high toxicity to both animals and human. Aflatoxin B1 is a metabolite of *Aspergillus flavus*.It is a potent hepatotoxic and hepatocarcinogenic mycotoxin *(3)*.

Definitive ways for complete detoxification of mycotoxin-contaminated food do not exist, therefore new methods to eliminate and control mycotoxicosis are sought *(4)*.

Suplementation of mice (6-7 weeks, 30 gram body weight) by curcumin 200 mg/kg body weight with Phenytoin as hepatotoxic drug (300 mg/kg body weight) orally for 3 weeks, showed significant decrease in the increased level of 8-OH-dg in liver tissue (the marker of DNA oxidative damage) that induced by Phenytoin *(5)*.

Aflatoxin B1 treated Young male F344 rats by gavage with 250 µg/kg b.w for 3 weeks (5 days/week)revealed that urinary excretion of the oxidative DNA damage biomarker, 8-

hydroxydeoxyguanosine, was increased after treatment, reached a maximum level at day 9 and went down thereafter *(6)*.

Paracetamol (hepatotoxic drug) oral administration to rats (500 mg/kg b.w.) daily dose for 5 days caused significant increase in serum TNF-α than control rats, whereas paracetamol injected rats-treated with the *curcumin* (50mg/kg b.w.) orally for 10 days after or before paracetamol restored the altered values to the near normalcy *(7)*.

Oral administration of aflatoxin B1 at dose of 25 µg /rat (150-180gram)/day orally for 90 days produced in focal necrosis, interface hepatitis, dense portal infiltration and inflammation when compared with the control group. Rats treated with curcumin (200 mg/kg BW) orally for 90 days in concurrent with aflatoxin B1 showed minimal degree of liver necrosis and inflammation when compared with AFB1 treated group *(8)*.

Chicks fed ration containing 150 ppb or 300 ppb aflatoxin B1 for 21 days showed mild to severe vacuolar degeneration of liver, in which irregularly shaped vacuoles in hepatocytes were apparent in the livers of occasional necrotic cells of both doses. Most of the hepatocytes also contained sharply edged vacuoles that were sign of fatty accumulation. Proliferation of Kupffer cells and occasional bile duct hyperplasia were the other observed changes *(9)*.

Increased comet length and DNA percent in comet tail present in blood culture containing arsenic (1.4 lM) and Fluoride (34 lM) indicating the induction of DNA damage when added alone as well as in combination with curcumin, This DNA damage was escorted with the decreased percent DNA in comet head. Noteworthy decrease was observed in the comet length as well as DNA percent in comet tail in cultures supplemented with *curcumin* (1.7 lM) along with Arsenic and Fluoride. The above data was further supported by the convincing rise in percent DNA in comet head *(10)*.

MATERIAL AND METHODS

Material

Aflatoxin B1 pure powder from *Aspergillus Flavus* was purchased from Sigma-Aldrich (USA) the compound is soluble in water and polar organic solvents.

Curcumin was purchased from BIO BASIC INC. Company is a water soluble yellow colored polyphenol; it is the active principle of *Curcuma longa.*

Experimental animals

In this study a forty apparently healthy male adult albino rats weighing 120-150 gm were used. They were obtained from Lab. animal colonies –ministry of public health, Helwan, Animals were housed in metallic cages (10 rats/cage) and acclimatized for 2 weeks to the laboratory condition before starting the experiment. The hygienic condition was kept constant throughout the experimental period; Food and water were offered *ad libtium.* The normal day light was only used.

Grouping, dosing, and samples

Rats were divided into four groups each of 10 rats, the 1st group was orally administered 250 µg/kg Body weight Aflatoxin B1 using stomach gavage, the 2nd group was orally administered 250 µg/kg (1/4 LD50) body weight in combination with 200 mg/kg body weight Curcumin *(11)*, the 3rd group was orally administered 200 mg/kg body weight Curcumin administration with either curcumin , AFB1 or both was 5 successive doses /week for two weeks. while the fourth group was administered distilled water only and kept as a control group.

Blood samples were taken 24 hours after the last treatment from the orbital venous plexuses using a capillary tube the collected samples were left to clot at room temperature then centrifuged for about 20 minutes at 3000 r.p.m to obtain clear serum. The sera were labeled and stored in deep freezer at -20C until used for the biochemical analysis.

Liver samples for histopathological examination and comet assay

Liver from each rat in each group were immediately dissected out after 24 hours of the last dose then prepared for histopathological examination and Comet assay.

Methodology

1. Determination of serum 8-Hydroxy-2-deoxy guanisine (8-OH-dG)

Serum 8-Hydroxy-2-deoxyguanisine was determined spectrophotometerically using ready made kits of molecular probes company, Egypt *(12)*

2. Determination of serum cytokines (IL1-IL5-TNF)

Serum IL1 *(13)*, IL5 *(14)*, and TNF *(15)* were determined using ELISA kits of Vitro Scient Company, Egypt.

3.Protocol for the single cell gel electrophoresis / comet assay for rapid genotoxicity assessment

The single cell gel electrophoresis (SCGE)/ comet assay, developed by N.P. Singhl, combines the simplicity of biochemical techniques for detecting DNA single strand breaks (frank strand breaks and incomplete excision repair sites), alkali-labile sites and cross linking with the single cell approach typical of cytogenetic assays. The advantages of the SCGE technique include:

(1) The collection of data at the level of the individual cell, allowing for more robust types of statistical analyses.

(2) The need for small numbers of cells per sample (<10,000).

(3) Its sensitivity for detecting DNA damage.

(4) That virtually any eukaryotic cell population is amenable to analysis.

Working protocol that can be used to detect DNA damage is given below *(16)*.

The evaluation of DNA Damage was estimated as the following

Observations are made of stained DNA using a 40x Objective on a fluorescent microscope use a comet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, UK) Linked to a CCD camera to assess the quantitative and qualitative extent of DNA damage in the cells by measuring the length of DNA migration and the percentage of migrated DNA.

Finally, the program calculates tail moment. Generally, 50 to 100 randomly selected cells are analyzed per sample.

4.Histopathological studies

At the termination of the experiment Speicement of liver from both control and treated groups then dried by filter paper and fixed by formalin 10% solution till used in histopathologicalexamination.The isolated organs were processed for preparing paraffin sections of 3-5 microns thickness, the paraffin sections were stained with Mayer's heamatoxilline and eosin for microscopic examination according to *(17)*.

5.Statistical analysis

The results are expressed as mean±standard deviation (SD). Differences between groups were assessed by one-way ANOVA analysis using the SPSS software package for Windows. Significance at P-values ≤ 0.001, ≤ 0.01, ≤ 0.05 had been given respective symbols in the tables.

RESULTS

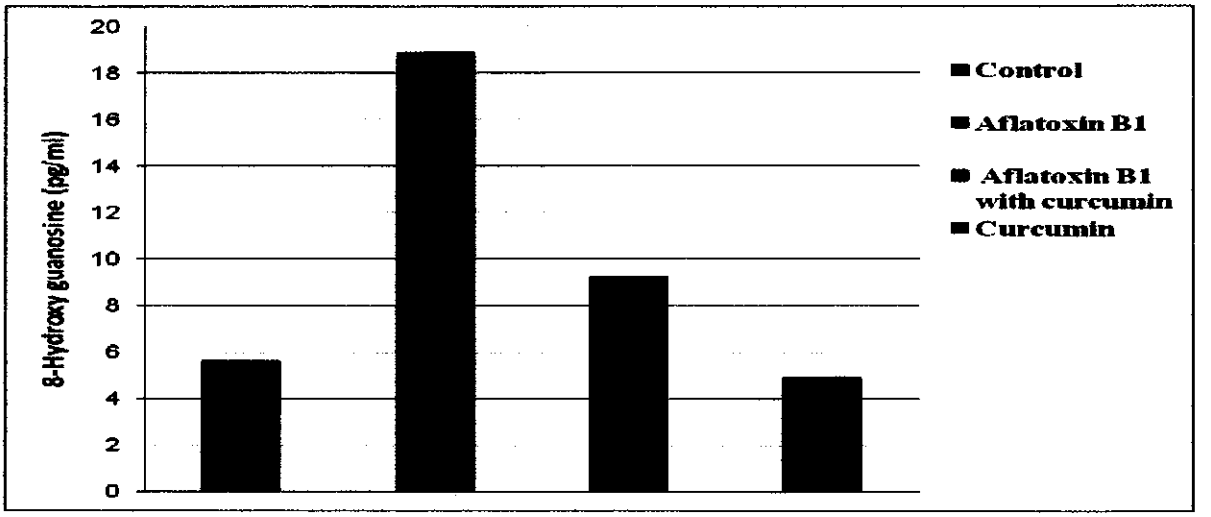
Oral administration of aflatoxin B1 (250µg/kg body weight) for 2 weeks to adult male albino rats caused significant elevation of 8-hydoxy guanisine. This elevation is decreased when curcumin was administered in a dose of 200 mg /kg body weight orally for 2 weeks in combination with aflatoxin B1 but the decrease of 8-hydoxy guanisine was not reach the level of the control group, although curcumin administration alone caused no significant changes in 8-hydoxy guanisine level when compared to the control group.

Table 1. Effect of AFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) on serum 8-hydoxy guanisine (pg/ml) of adult male albino rats.

Group / Parameter	Control group	1st group Aflatoxin B1	2nd group Aflatoxin B1 with Curcumin	3rd group Curcumin
8-Hydroxy guanosine (pg/ml)	5.60 ^{c}±0.35	18.87 ^{a}±0.57	9.23 ^{b}±0.22	4.88^{c}±0 .28

Means carrying different superscripts within the same row are highly significant from each others ($P \leq 0.001$).

Figure 1. Effect of AFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) on serum 8-hydoxy guanisine (pg/ml) of adult male albino rats.



Cytokines (Interleukin 1, Interleukin 5, and Tumor necrotic factor) level in serum was significantly increased after oral administration of aflatoxin B1 (250µg/kg body weight) for 2 weeks even when curcumin (200 mg /kg body weight orally for 2 weeks) was administered in combination with aflatoxin B1, Although curcumin administration alone caused no significant changes in Cytokines level when compared to the control group.

Table 2.Effect of AFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) on serum cytokines of adult male albino rats.

Group / Parameter	Control	1st group Aflatoxin B1	2nd group Aflatoxin B1 with Curcumin	3rd group Curcumin
IL1(pg/ml)	70.40 ^{d}±4.23	170.80 ^{b}±5.48	221.20 ^{a}±8.43	137.80 ^{c}±4.47
IL5(pg/ml)	42.40 ^{d}±3.41	135.40 ^{b}±3.70	169.00 ^{a}±4.98	61.80 ^{c}±3.90
TNF-α(pg/ml)	170.00 ^{c}±1.41	126.20 ^{b}±2.46	167.20 ^{a}±7.66	148.00^{c}±0.86

Means carrying different superscripts within the same row are highly significant from each others ($P \leq 0.001$).

Figure 2.Effect of AFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) on serum interleukin-1 type of cytokines (pg/ml) of adult male albino rats.

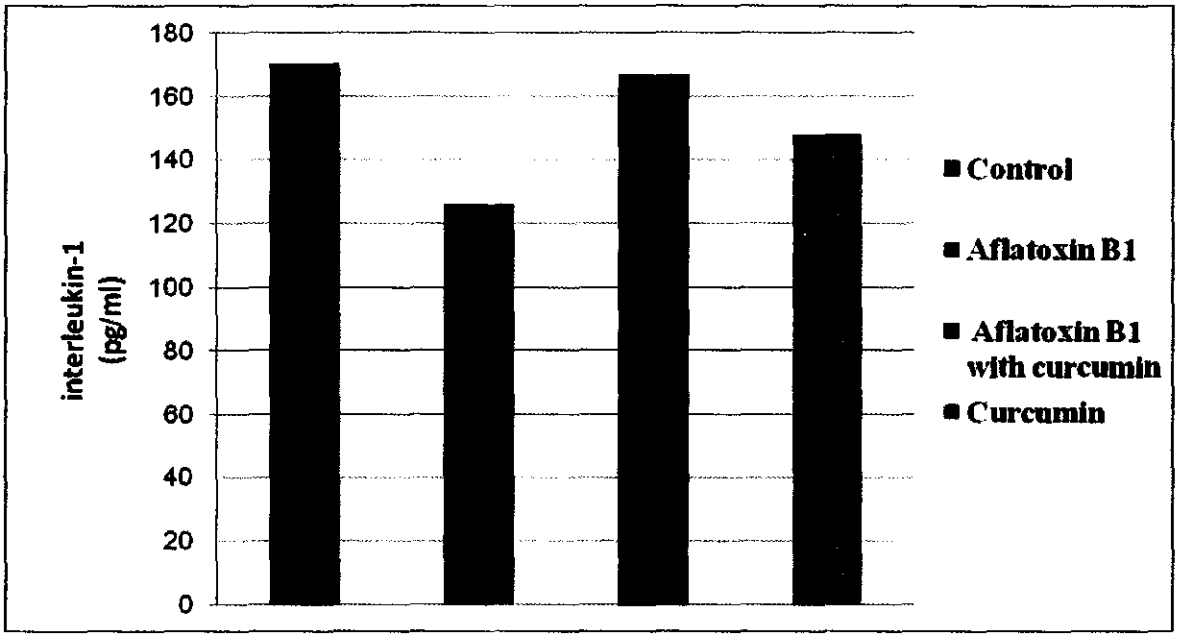


Figure 3.Effect of AFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) on serum interleukin-5 type of cytokines (pg/ml) of adult male albino rats.

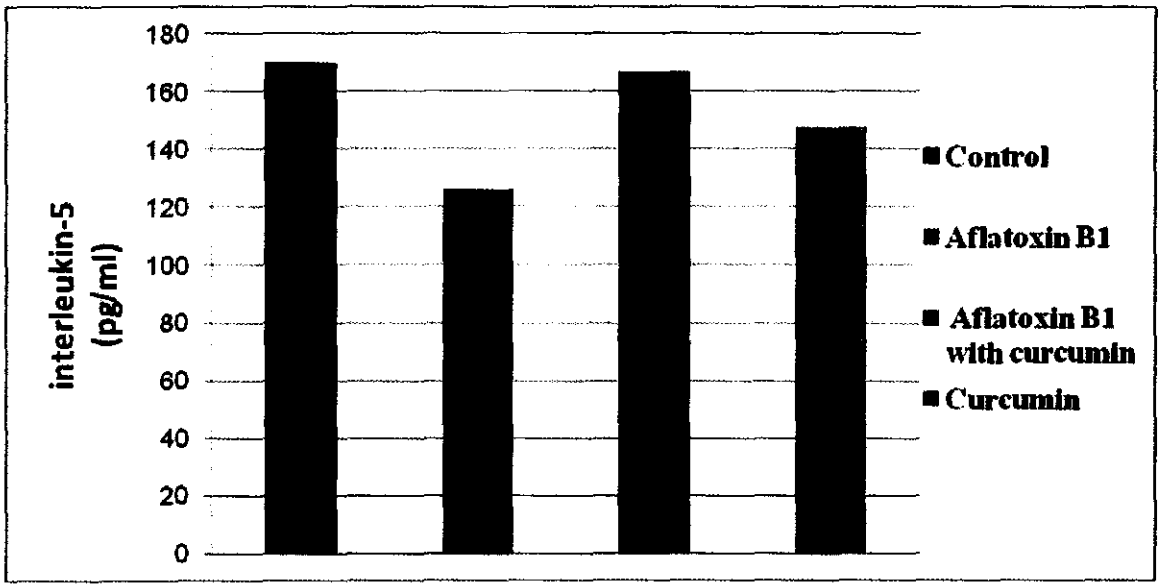
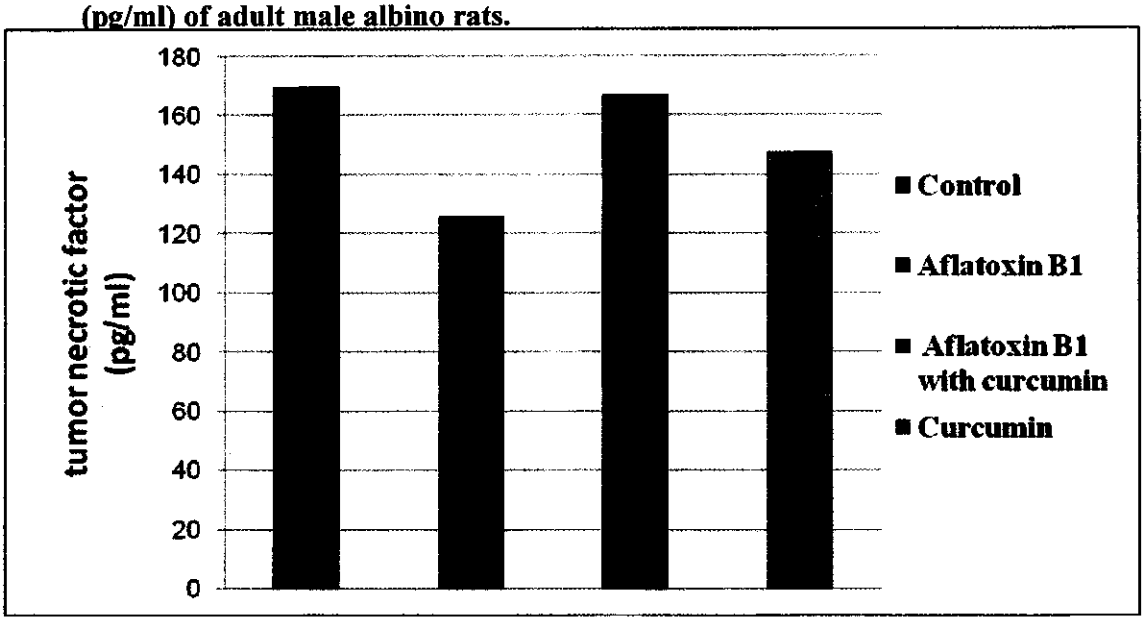


Figure 4.Effect of AFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) on serum tumor necrotic factor type of cytokines (pg/ml) of adult male albino rats.



Means carrying different superscripts within the same row are highly significant from each others ($P \leq 0.001$).

Comet assay for detection of liver DNA degeneration after oral administration of aflatoxin B1 (250µg/kg body weight) for 2 weeks revealed that tail length, DNA tail %, and DNA moment were significantly increased indicating sever DNA degeneration in liver , this elevation was significantly decreased when curcumin was administered in a dose of 200 mg /kg body weight orally for 2 weeks in combination with aflatoxin B1, Although curcumin administration alone caused no significant changes in level of tail length, DNA tail %, and DNA moment when compared to the control group.

Table 3.Effect of AFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) on liver DNA Tail length (µm), Tail DNA %, and Tail moment of adult male albino rats.

Group / Parameter	Control	1st group Aflatoxin B1	2nd group AFB1 with Curcumin	3rd group Curcumin
Tail length (µm)	1.30±0.11	11.70±1.21	7.86±0.43	1.34±0.12
Tail DNA %	1.07±0.07	11.05±1.04	7.42±0.38	1.13±0.12
Tail moment	1.41±0.20	133.04±26.05	58.77±6.19	1.55±0.29

Means carrying different superscripts within the same row are highly significant from each others ($P \leq 0.001$).

Figure 5.Effect of AFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) on liver DNA Tail length (µm) of adult male albino rats.

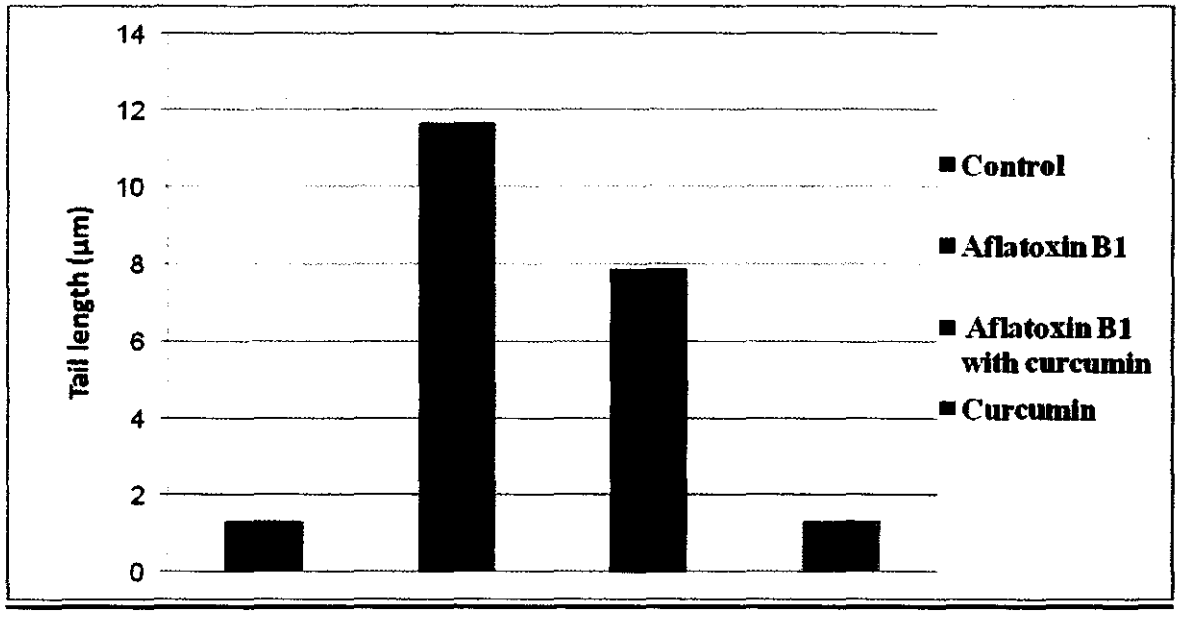


Figure 6.Effect of AFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) on liver Tail DNA %, of adult male albino rats.

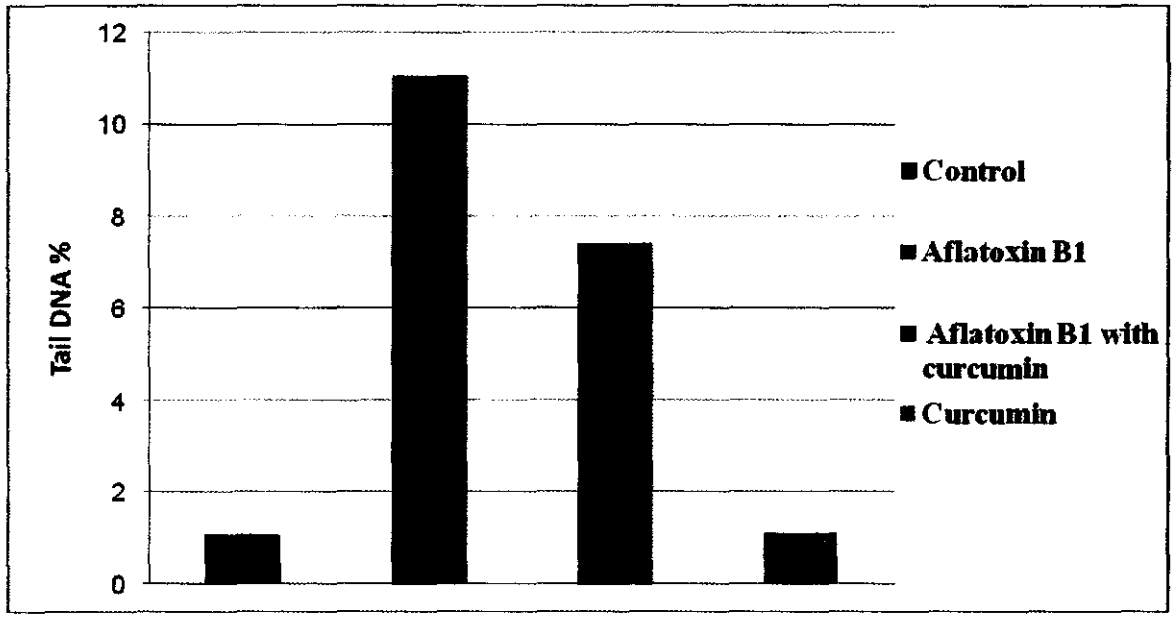


Figure 7.Effect of AFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) on liver Tail moment of adult male albino rats.

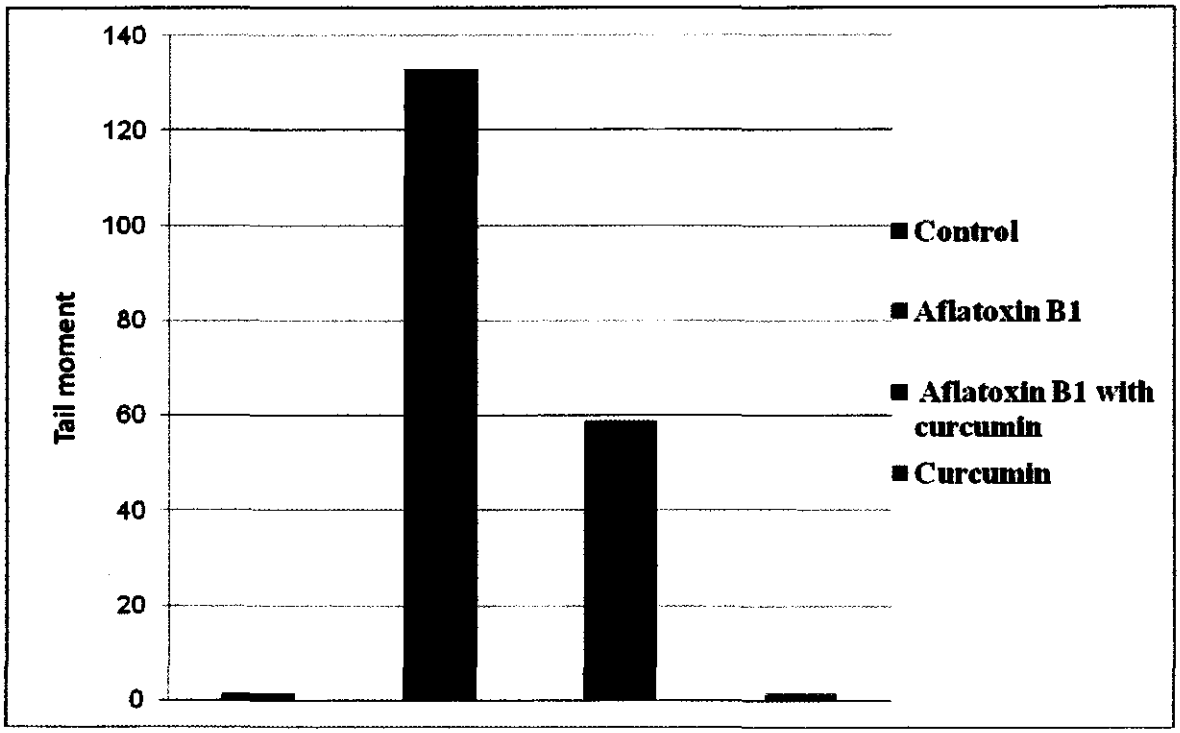
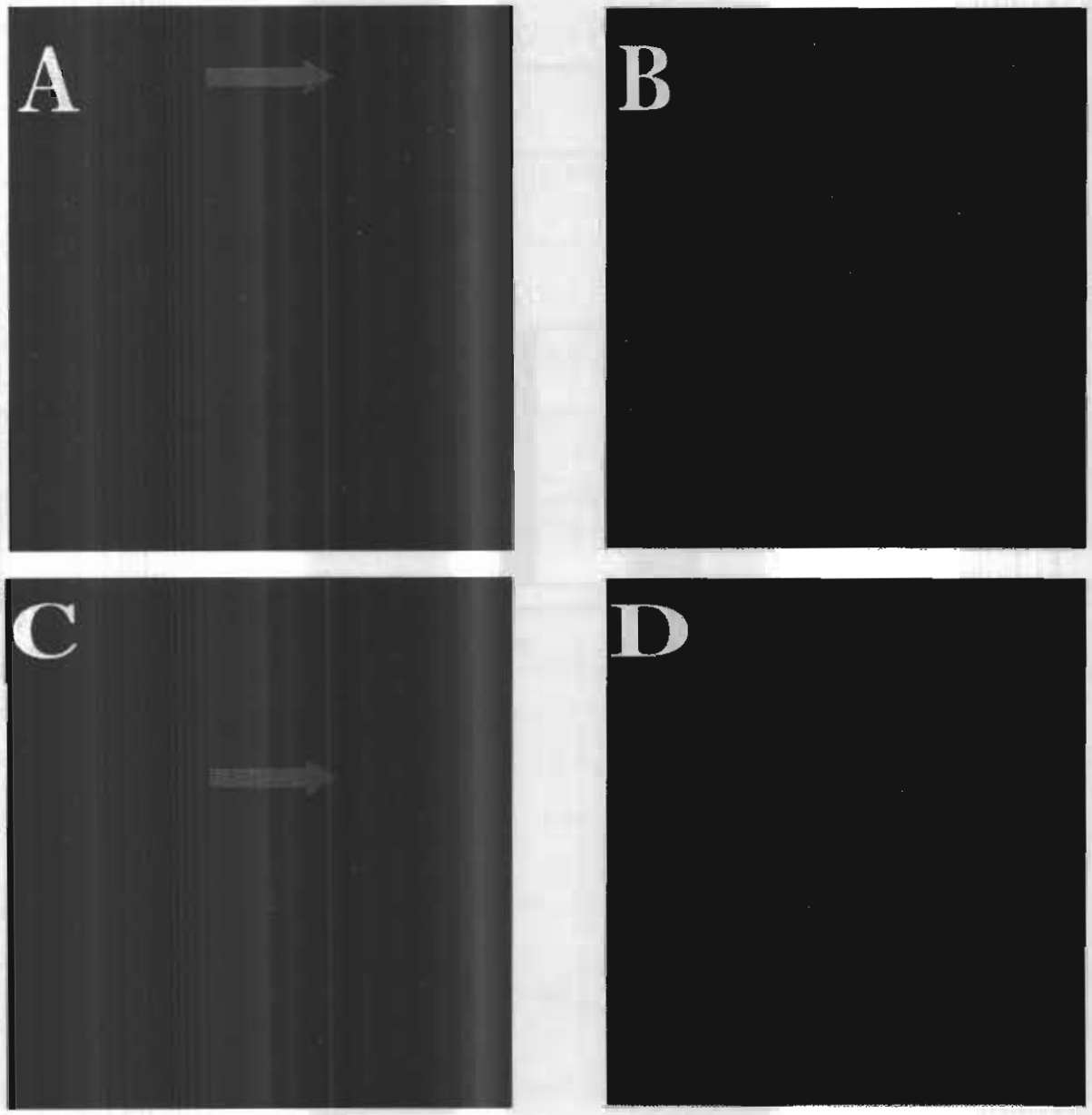


Figure 8.Comet assay of liveraffectedbyAFB1 (250 µg /kg body weight), AFB1 with Curcumin (250 µg /kg and 200 mg /kg body weight), and Curcumin alone (200 mg /kg body weight) for 2 weeks (5 successive doses /week) of adult male albino rats.



A =Control group B= Aflatoxin B1 C=Curcumin group D=Aflatoxin B with Curcumin

Histopathological findings
Aflatoxin B1 group

Diffusely, the hepatic architecture is disrupted by fragmentation of hepatic cords resulting in small aggregates and single hepatocytes (Fig.9). Massively the hepatic cells are suffered from vacuolar degeneration at different degrees ranging from sever type at the periphery of hepatic lobules to moderate around

the central vein (Fig.10). The sever type characterized by large clear cytoplasmic lipid vacuole which peripheries the nucleus (Fig.11).The milder one showed clear areas within the cytoplasm surrounding a centrally located nucleus. Multifocally, there are randomly distributed foci of hepatocellular necrosis characterized by hyperesinophilia, loss of cellular details, and pyknosis as well as karyorrhectic nuclei (coogulative necrosis) (Fig.12). Some hepatocytes are enlarged up to 2-3 times normal , with abundant esinophilic cytoplasm and large vesicular nuclei with marginated chromatin and prominent nucleus (megalocytosis) (Fig.13).Very few number of mitotic figures were seen . the hepatic sinusoids were engorged with blood (Fig.14) .

Curcumin group in combination with aflatoxin B1

In comparison to the aflatoxin B1 group , the histopathological changes were less sever and this was evident by , no necrotic hepatocytes were seen in the hepatic tissue, the hepatic vacuolation was mostly seen as variable sized cytoplasmic vacuoles around centrally located nucleus (Fig.15) and the sever type of the vacuolar degeneration was very minimal.

Most of areas of hepatic lobules especially around the central vein maintained normal hepatic architecture (hepatic cords) .no mitotic figures or megalocytosis was noted (Fig.16).

Curcumin group and the control group showed no histopathological lesions in liver (Fig.17).

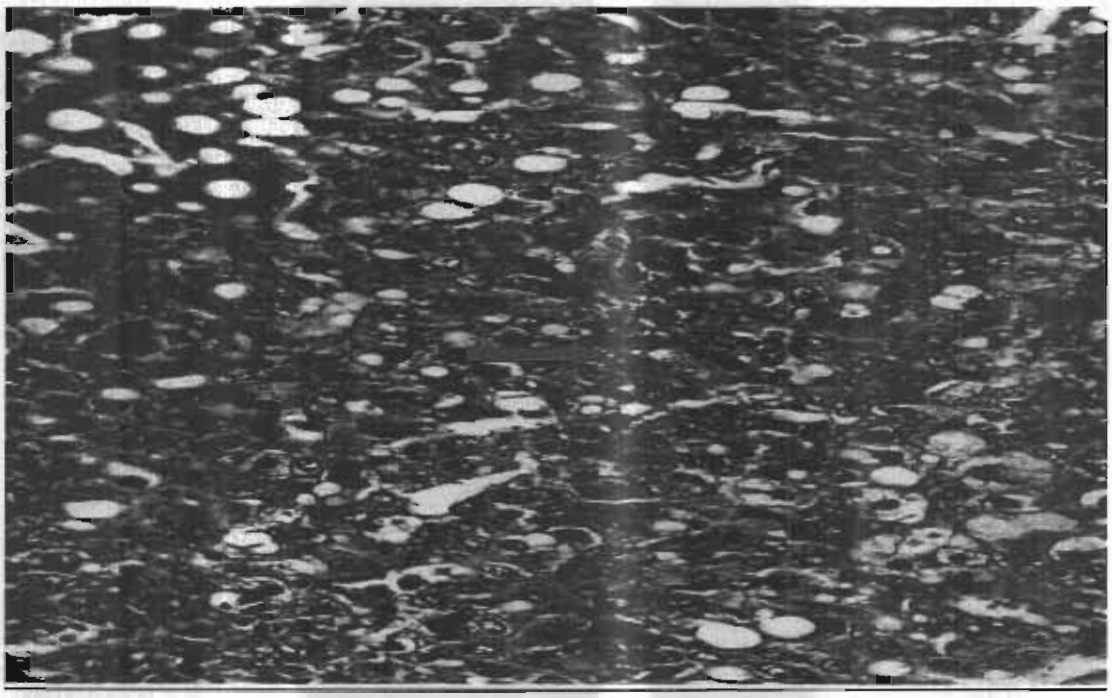


Figure 9.Liver of adult male albino rat treated with AFB1 (250 µg /kg body weight) for 2 weeks (5 doses /week), showed disruption of hepatic cords.H&E.X400.

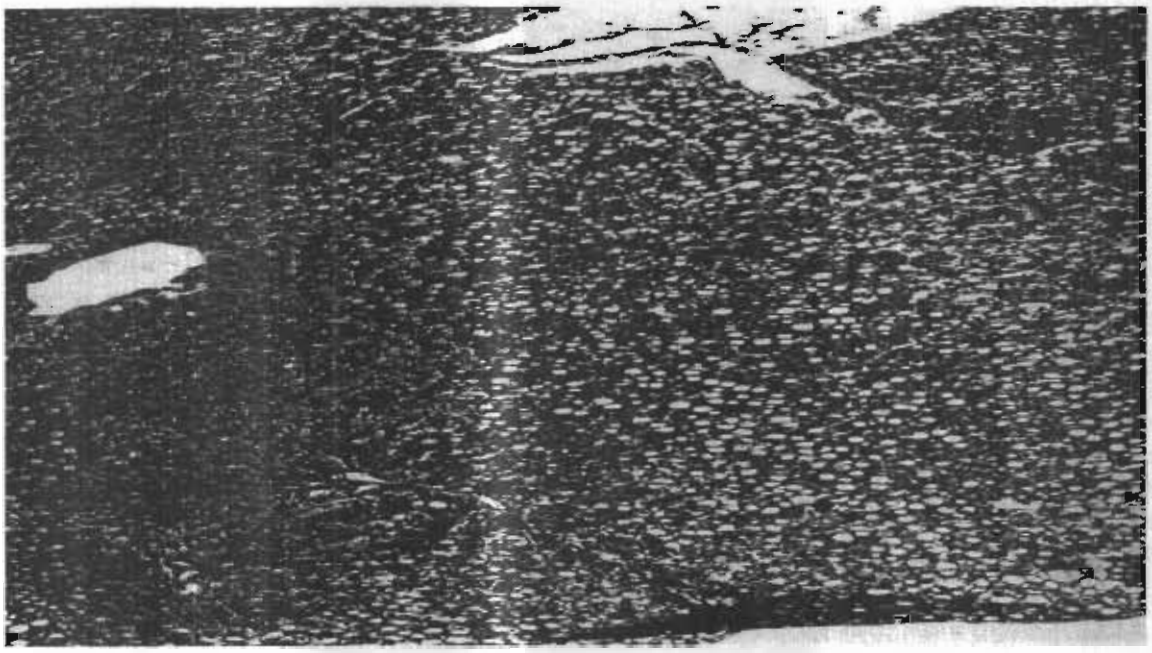


Figure 10.Liver of adult male albino rat treated with AFB1 (250 µg /kg body weight) for 2 weeks (5 doses /week), showed diffuse vacuolar degeneration of the hepatocyte . H&E X40.

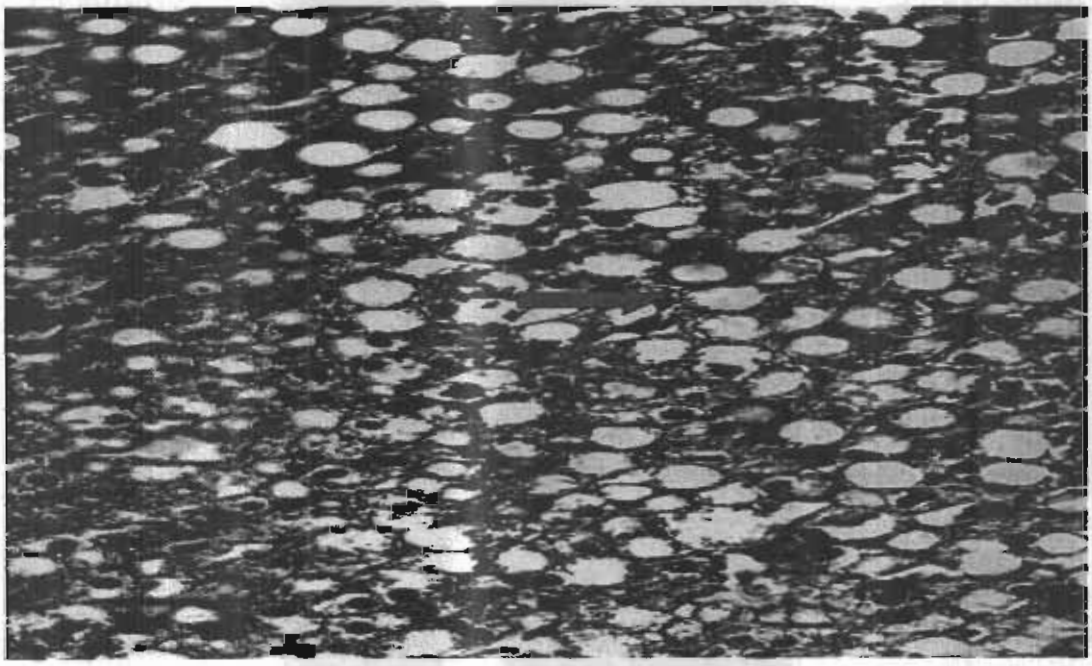


Figure 11. Higher magnification of the previous image showed sever vacuolar degeneration of the hepatocyte with peripheral nucleus. H&E X400.

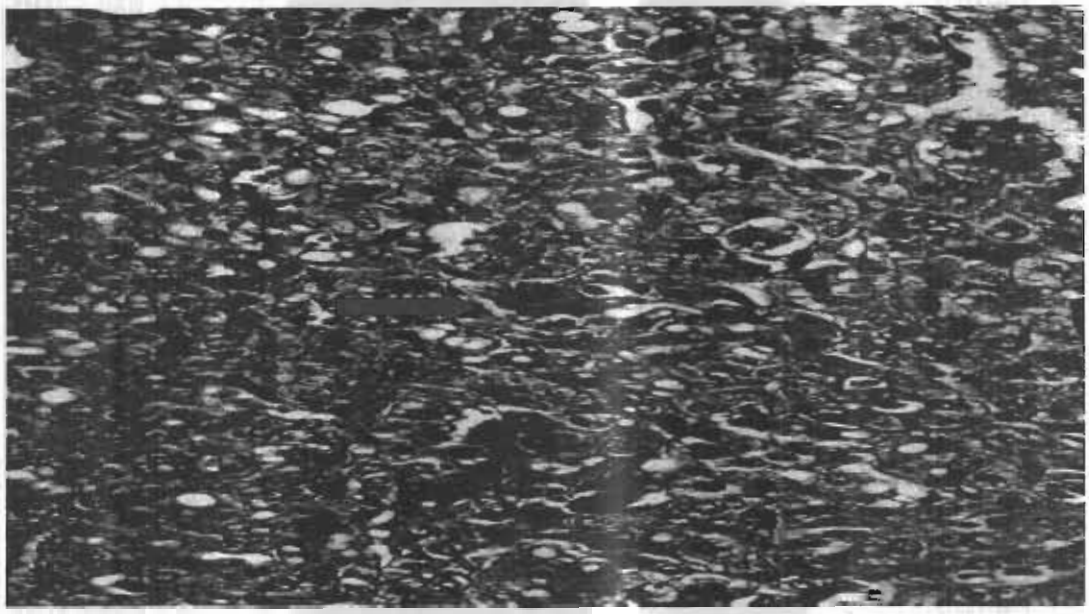


Figure 12.Liver of adult male albino rat treated with AFB1 (250 µg /kg body weight) for 2 weeks (5 doses /week), showed multifocal necrotic cells. H&E X400.

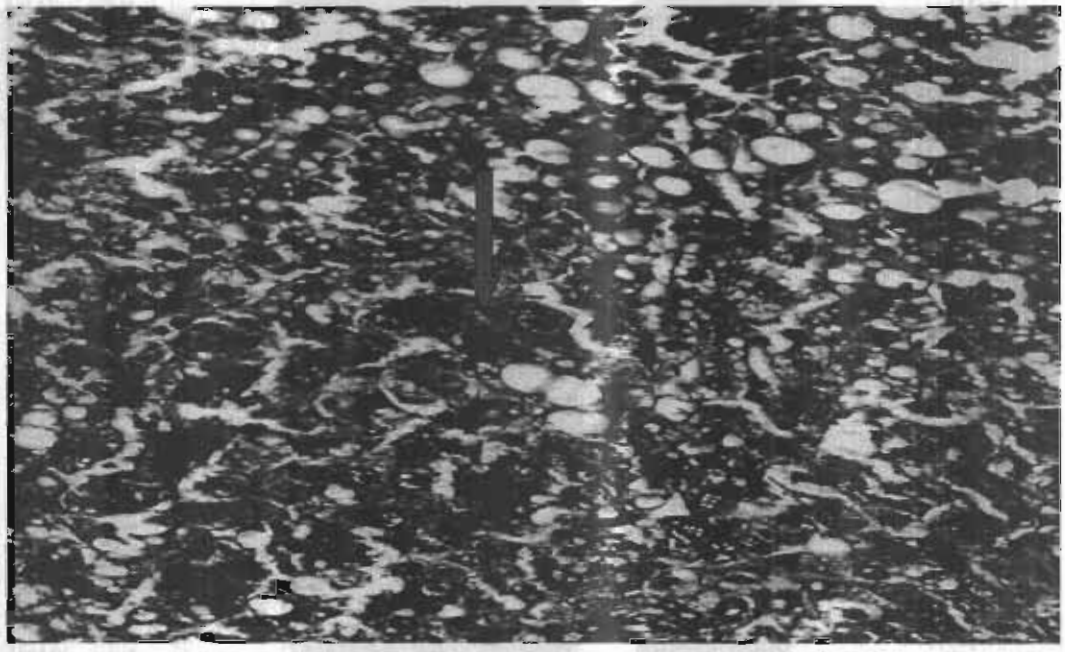


Figure 13.Liver of adult male albino rat treated with AFB1 (250 µg /kg body weight) for 2 weeks (5 doses /week), showed large hepatocyte (megalocytosis) . H&E X400.

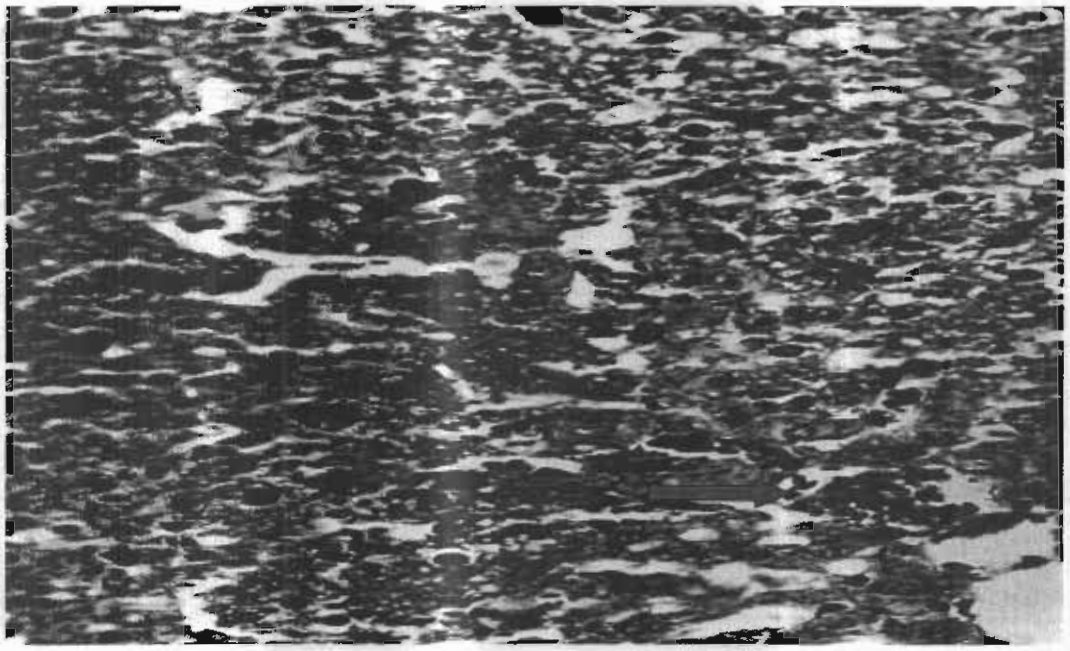


Figure 14.Liver of adult male albino rat treated with AFB1 (250 µg /kg body weight) for 2 weeks (5 doses /week), showed hepatic sinusoids filled with blood (congestion). H&E X400.

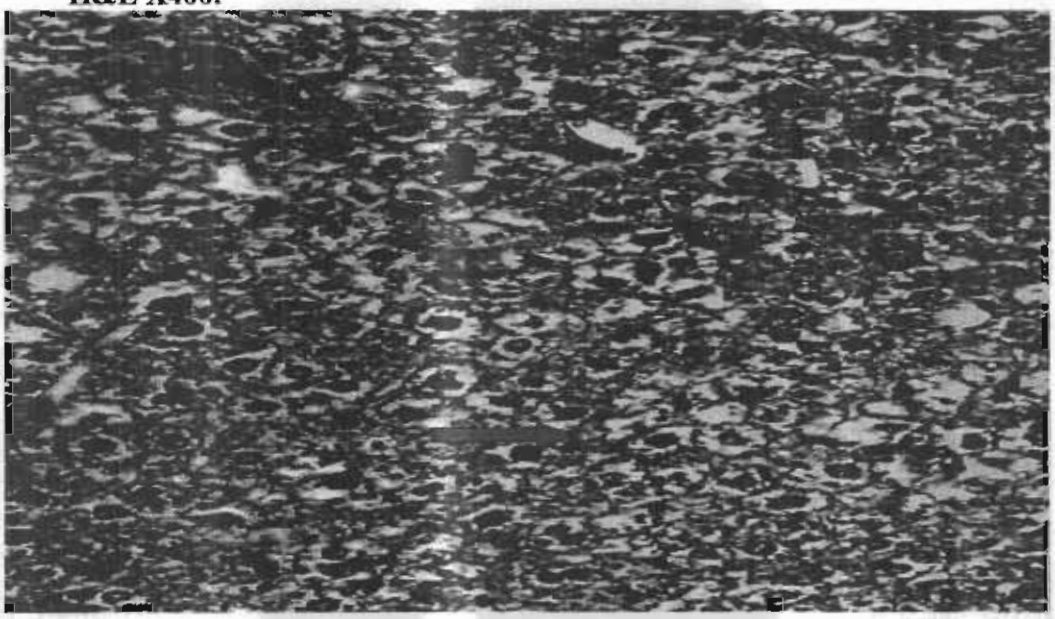


Figure 15.Liver of adult male albino rat treated with AFB1 (250 µg /kg body weight) in combination with curcumin (200 mg/kg body weight) for 2 weeks (5 doses /week), showed mild vacuolar degeneration in hepatocytes with centrally located nucleus .

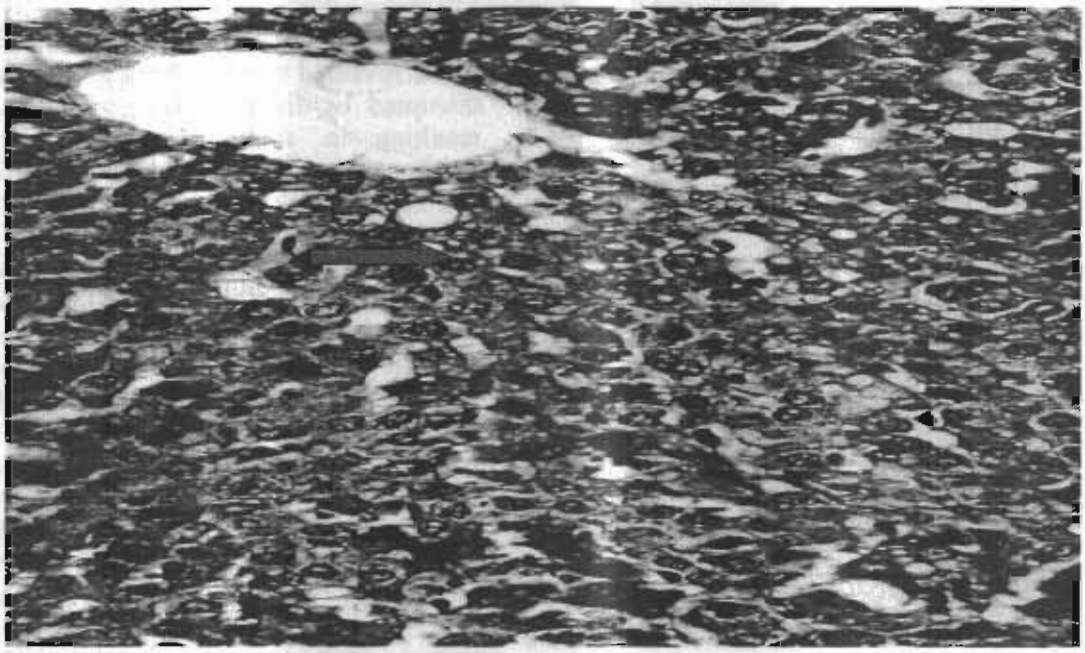


Figure 16.Liver of adult male albino rat treated with AFB1 (250 µg /kg body weight) in combination with curcumin (200 mg/kg body weight) for 2 weeks (5 doses / week), showed maintaining of the hepatic cord aroud the central vein. H&E X100.

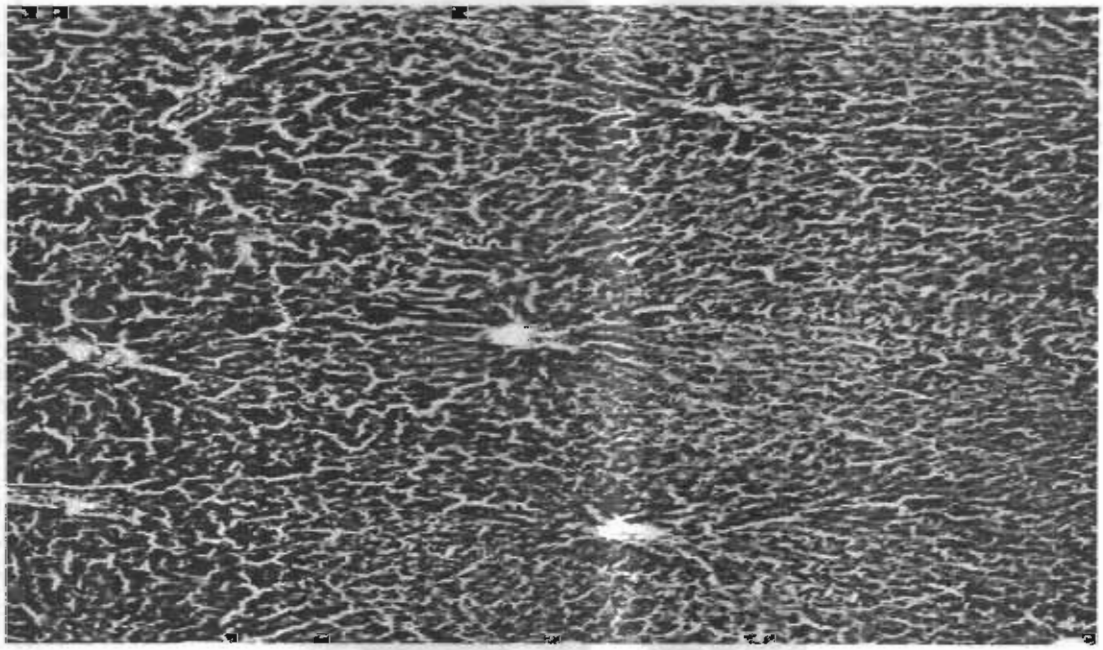


Figure 17.Normal liver. H&E X40.

DISCUSSION

Oral administration of aflatoxin B1 (250µg/kg body weight) for 2 weeks to adult male albino rats caused significant elevation of 8-hydoxy guanisine. This elevation is decreased when curcumin was administered in a dose of 200 mg /kg body weight orally for 2 weeks in combination with aflatoxin B1. these results are due to contribution of aflatoxin B1 to increase oxidative stress due to induction of large amount of ROS causing oxidative DNA damage and release of 8-hydoxy guanisine as marker in DNA damage. and that curcumin can act as a non-enzymic antioxidant by direct interaction with ROS or act as scavenger to ROS and reduce the oxidative damage to DNA reducing the level of 8-hydoxy guanisine *(6,7)*.

Cytokines (Interleukin 1, Interleukin 5, and Tumor necrotic factor) level in serum was significantly increased after oral administration of aflatoxin B1 as hepatocytes treated with Aflatoxin B1 release factors, which activate of proinflammatory cytokines as TNF-α and interlukins. The hepatoprotective effect on curcumin was demonstrated through correcting the value of serum α-glutathione S transferase (α-GST) that were significant raised by Aflatoxin B1 administration. So Curcumin also increased the level of cytokines in presence of Aflatoxin B1 *(7)*.

Comet assay in liver tissue for detection of liver DNA degeneration after oral administration of aflatoxin B1 revealed that tail length, DNA tail %, and DNA moment were significantly increased indicating sever DNA degeneration in liver, this elevation was significantly decreased when curcumin was administered in combination with aflatoxin B1. AFB1 contributes to increased oxidative stress due to induction of large amount of ROS causing oxidative DNA damage. Curcumin can act as a non-enzymic antioxidant by direct interaction with ROS or it act as scavenger to ROS and reduce the oxidative damage to DNA .these results are in agreement with toxicity in cases of arsenic and Fluoride *(10)*.

The histopathological examination of liver tissue revealed that, the hepatic architecture is disrupted by fragmentation of hepatic cords resulting in small aggregates and single hepatocytes. Massively the hepatic cells are suffered from vacuolar degeneration at different degrees ranging from sever type at the periphery of hepatic lobules to moderate around the central vein . The sever type characterized by large clear cytoplasmic lipid vacuole which peripheries the nucleus .the milder one showed clear areas within the cytoplasm surrounding a centrally located nucleus. Multifocally, there are randomly distributed foci of hepatocellular necrosis characterized by hyperesinophilia, loss of cellular details, and pyknosis as well as karyorrhectic nuclei (coogulative necrosis). Some hepatocytes are enlarged up to 2-3 times normal , with abundant esinophilic cytoplasm and large vesicular nuclei with marginated chromatin and prominent nucleus (megalocytosis) very few number of mitotic figures were seen. The hepatic sinusoids were engorged with blood.

The histopathological changes in liver of Curcumin treated rats of in combination with aflatoxin B1 in comparison to the aflatoxin B1 group revealed less sever changes and this was evident by , no necrotic hepatocytes were seen in the hepatic tissue , the hepatic vacuolation was mostly seen as variable sized cytoplasmic vacuoles around centrally located nucleus.and the sever type of the vacuolar degeneration was very minimal. Most of areas of hepatic lobules especially around the central vein maintained normal hepatic architecture (hepatic cords) .no mitotic figures or megalocytosis was noted. These results are due to the damages caused by Aflatoxin B1 in liver tissue by the action of ROS that leads to hepatocytes damage . The antioxidant effect of Curcumin and ROS scavenger caused repair to liver tissue to be near the normal state *(8,9)*.

Conclusion

Novel biomarkers of genotoxicty and cytotoxicity should be used to make an early and accurate diagnosis and lower the incidence of false negative results of diagnosis.

Curcumin is a potent antioxidantagent that could be used in prevention and control of genotoxicty and cytotoxicity caused by aflatoxin B1, so we should use it at the recommended dose 200 mg/kg body weight to decrease the hepatotoxic hazards induced by aflatoxin B1 in human and animal .

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الملخص العربى

الدلالات الجينية والخلوية للتسمم بالأفلاتوكسين ب١ ودور الكوركيومين كترياق علاجى

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أجرى هذا البحث لإلقاء الضوء على الدلالات المختلفة للأعراض الجانبية الناتجة عن الأفلاتوكسين ب١ وهو من أهم السموم الفطرية المؤثرة على الكبد وكذلك دراسة دراسة مادة الكوركيومين واستخدامها كترياق مضاد لتلك الآثار السمية لهذا السم الفطرى على الكبد .

وقد تم اجراء هذا البحث على اربعين من ذكور الفئران البالغة والتى قسمت الى اربعة مجاميع كل منها عشرة فئران .

جرعت المجموعة الاولى عن طريق الفم بجرعة تعادل ٢٥٠ ميكروجرام لكل كيلوجرام من وزن الجسم لمدة اسبوعان (خمس جرعات متتالية فى الاسبوع) وجرعت المجموعة الثانية ايضا عن طريق الفم بنفس الجرعة السابقة من اللأفلاتوكسين بالاضافة الى ٢٠٠ مجم لكل كيلو جرام من وزن الجسم من الكوركيومين لمدة اسبوعان (خمس جرعات متتالية فى الاسبوع) ايضا .

اما المجموعة الثالثة فقد جرعت عن طريق الفم بجرعة الكوركيومين ٢٠٠ مجم لكل كيلو جرام من وزن الجسم من الكوركيومين لمدة اسبوعان (خمس جرعات متتالية فى الاسبوع

وكانت المجموعة الرابعة هى المجموعة الضابطة والتى جرعت بالماء المقطر فقط لا غير.

بعد اربع وعشرون ساعة من آخر جرعة فى نهاية الأسبوعان الخاصة بالتجربة تم أخذ عينات من الدم من كل فأر على حدى فى كل مجموعة وذلك لإجراء بعض التحاليل الكيميائية فى مصل هذه المجاميع من الفئران كما تم اخذ عينات من الكبد لفحص انسجتها بالاضافة الى اجراء ال Comet assay المستخدم لإيضاح الخلل الطارئ على DNA وقد اسفرت نتائج هذا البحث على الآتى:-

المجموعة الاولى :- وهى التى تجرعت بالأفلاتوكسين ٢٥٠ ميكروجرام لكل كيلوجرام من وزن الجسم على مدار المده الزمنية التى اجرى فيها البحث عن زيادة فى نسبة guanisine 8-hydroxy فى مصل الفئران وهذا دليل على زيادة نواتج تكسير DNA

واتضح ايضاً من نتائج هذا البحث زيادة نسب السيتوكينز Interleukin 1, Interleukin 5, and Tumor Necrotic Factor .

وقد أظهر فحص النسيج الخلوى للكبد مدى تأثير تجريع جرعة ٢٥٠ ميكروجرام لكل كيلوجرام من وزن الجسم من الأفلاتوكسين لمدة اسبوعان (خمس جرعات متتالية فى الاسبوع) الى تغيرات جوهرية بالمقارنة بالمجموعة الضابطة كما اظهر اختبار الكوميت وجود زيادة معنوية فى كل من DNA tail %, DNA moment, and Tail length وهذا دليل على التغير جوهرى فى التركيب الوراثى للDNA.

اما المجموعة الثانية والتى تجرعت بكل من الأفلاتوكسين بذات الجرعة السابقة مضافاً اليها الكوركيومين ٢٠٠ مجم لكل كيلوجرام من وزن الجسم لمدة اسبوعان (خمس جرعات متتالية فى الاسبوع) الى تحسين نسب كل النتائج السابقة بنسب متفاوته .

وقد أثبتت نتائج المجموعة الثالثة التى تجرعت بالكوركيومين فقط بنفس ذات الجرعة ٢٠٠ مجم لكل كيلوجرام من وزن الجسم بعدم وجود أى تأثيرات جوهرية على النسب الطبيعية وذلك بالمقارنة بالنتائج التى حصلنا عليها مع المجموعة الضابطة .