



Study the effect of ethanolic extraction of *Eucalyptus globulus* against aflatoxin which causes liver damage in males rats

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Abstract

This study has been designed to evaluate the beneficial effects of *Eucalyptus globulus* extract upon Aflatoxin-causes hepatic toxicity, This study has been conducted on adult male rats at biology department in the college of the science\ University of AL-Qadisiya during the period extended from November, 2015 to February, 2016 (4 months). 40 mature male Wister rats, (age 90 days, weight 190 ± 10 g) were separated in to four randomly equal groups Blood serum samples were separated for assessment of GOT, GPT, concentration. Under our experimental conditions, Aflatoxin poisoning resulted evidenced by statistically significant increase ($p > 0.05$) in the activities of GOT, GPT in group 2 of just Aflatoxin compared with the control group, and in the third and fourth groups showed statistically significant decrease ($p < 0.05$) in GOT, GPT level in Blood serum of Aflatoxin-treated group compared with the control group, After histological analysis of the group Aflatoxin proved change in the liver, after the gavage of (EUCE) The results showed that there is an improvement in liver tissues after treatment with EUCE .In present study we concluded to EUCE alcoholic dose 400mg/kg b.w. is better than dose 250mg/kg b.w. depending on results in recently study.

We concluded that the administration of (EUCE) act as antioxidant for Aflatoxin which induced toxicity of liver and antifungal against mycotoxins.

Keywords: Aflatoxin, Eucalyptus, Males rats, GOT, GPT.

Introduction

Aflatoxins are secreted by both *Aspergillus flavus* and *Aspergillus parasiticus*, which are common types of molds widespread in nature. Those molds present does not always indicate that harmful levels of aflatoxin are present, but does indicate a significant risk. The molds can colonize and pollute food

before fruitage or during storage, specially following continued exposure to a high-humidity environment, or to worrying disorders such as drought [1].

The innate habitat of *Aspergillus* is in soil, decomposing vegetation, hayd, and scraps suffering microbiological deterioration, but it attacks all types of organic substrates at any time disorders

are favorable for its growth. Favorable disorders include high moistness content (at least 7%) and high temperature. Aflatoxin conversion products are sometimes originate in eggs, milk foods, and meat when animals are nourished contaminated scraps [2].

A study showed in Kenya and Mali found that the main practices for dehydrating and putting away of maize were laughable in minimizing revelation to aflatoxins [3].

Eucalyptus globulus was proved fruitful in defensive different organs against oxidative in various experimental models [4]. Eucalyptus, a native plant of Australia, includes less than 800 species. Some of them, including *Eucalyptus globulus*, were introduced in Europe and North Africa where they are well acclimated to the Mediterranean shores

Material and method

Plant material collection:

The leaves of *Euclayptus globulus* plant were collected during August 2015, from Al-Dewanyia city from park.

Preparation of Ethanolic Extracts (*Eucalyptus globulus*):

20gm of *Eucalyptus globulus* powdered leaves were taken and extracted with soxhlet apparatus ethanol (70%) Within 24 hours, and then taking the extract and place it in a ptry dish and put in the oven at a temperature of (40°C) within 48 hours, The result of extract was stored at (4°C) until use [8].

The treatments:

40 mature male Wister rats, (age 90 days, weight 190±10g) were separated in to four randomly equal groups, the

[5]. Eucalyptus is mainly cultivated for the paper and cosmetic industries, while some of them are used in traditional medicine, certain species of Eucalyptus are even used in modern medicine [10]. Many researches were conducted on the medicinal properties of *Eucalyptus globulus*, The leaf extract or essential oil from the leaves of *Eucalyptus globulus* were reported to possess antifungal, antibacterial, anti-inflammatory and anthelmintic properties [6]. In addition, the beneficial effect of Eucalyptus was demonstrated in rats given toxic doses of aflatoxins. However, the therapeutic effect of *Eucalyptus globulus* against aflatoxins induced antifungal status of blood serum was assessed by measuring level and activities of enzymes GOT and GPT [7].

first served as negative control received only distilled water (C) , the second as positive control received with Aflatoxin in diet (2.5 g/kg b.w.) as a single dose (T1). third and fourth groups received Aflatoxin in diet (2.5 g/kg b.w.) as a single dose then treated after 2 days with *Eucalyptus* Extraction (EUCE) (250,400 mg/kg b.w.) (T2), (T3) respectively orally through stomach tube during 42 days. males were anaesthetized (by injection ip of 0.3ml ketamine+0.1ml of xylazine/kg b. w. ip), blood samples were obtained from heart in non-heparinized tubes, Blood serum samples were separated for assessment of GOT, GPT, concentration. Under our experimental conditions, Aflatoxin poisoning resulted evidenced by statistically significant

increase ($p > 0.05$) in the activities of GOT, GPT in group 2 of just Aflatoxin compared with the control group, and in the third and fourth groups showed statistically significant decrease ($p < 0.05$) in GOT, GPT level in Blood serum of Aflatoxin-treated group compared with the control group.

Serum Preparation:

Blood was collected in test tubes with cap and allowed to clot (for 20 minutes), then serum was separated by centrifugation at 4000rpm for 10 minutes [9]. The separated serum of each animal was subdivided nearly into 6 samples using appendroff tubes 0.5 ml and kept at deep freezer until using for assessment of the biochemical parameters.

Results:

Serum oxidant-antioxidant concentrations:

Serum GOT concentration

The results illustrated in figure (1) showed significant differences between all experimental groups and control group. In group A with gavage Aflatoxin showed significant increase ($p < 0.05$) in serum concentration of GOT compared with control group, and in

Biochemical Assay oxidant-antioxidant:

Oxidant-antioxidant are determinate by kits of spectrophotometer to GOT and GPT this kits it's from US bio USA [10].

Microscopic examination:

Liver were quickly excised and fixed in 10% formalin neutral buffer solution. The trimmed tissues were first washed with tap water followed by dehydration through a graded alcohol series and then passed through xylol and paraffin series before being embedded in paraffin. The paraffin blocks were cut into 5-6 μ m sections stained with Hematoxylin and Eosin and examined under a light microscope [11].

groups gavage with single dose of Aflatoxin and continuous gavage Eucalyptus daily (AE250, AE400), showed significant decrease ($p > 0.05$) in serum concentration of GOT compared with Aflatoxin group.

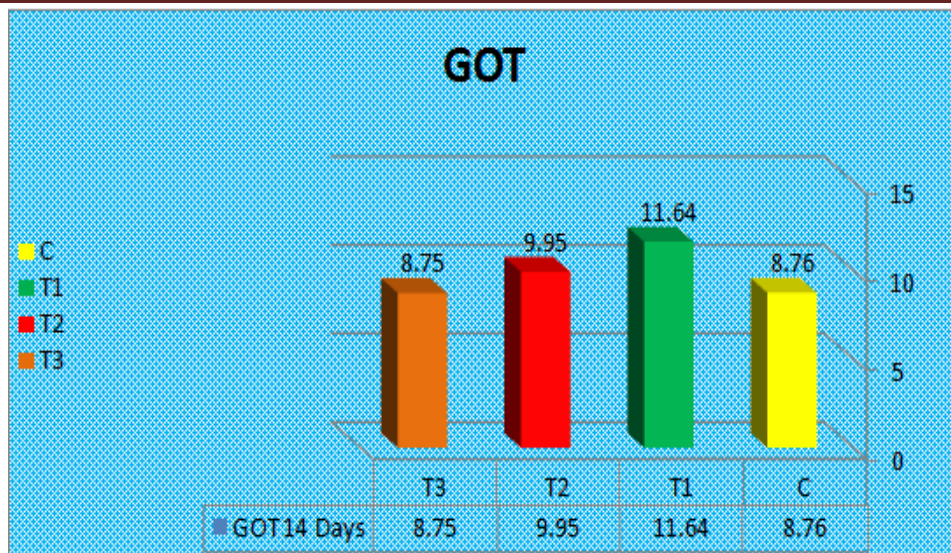


Figure (1): Effect of *Eucalyptus globulus* treatment on serum GOT concentration (µMole/ml) in mature male rats gavaged Aflatoxin

Serum GPT concentration:

The results illustrated in figure (2) showed significant difference between all experimental groups and control group. In group A with gavage Aflatoxin showed significant increase ($p > 0.05$) in the serum concentration of GPT compared with the

control group, and in groups gavage with single dose of Aflatoxin and continuous gavage *Eucalyptus* daily (AE250, AE400) is showed significant decrease ($p < 0.05$) in the serum concentration of GPT compared with the Aflatoxin group.

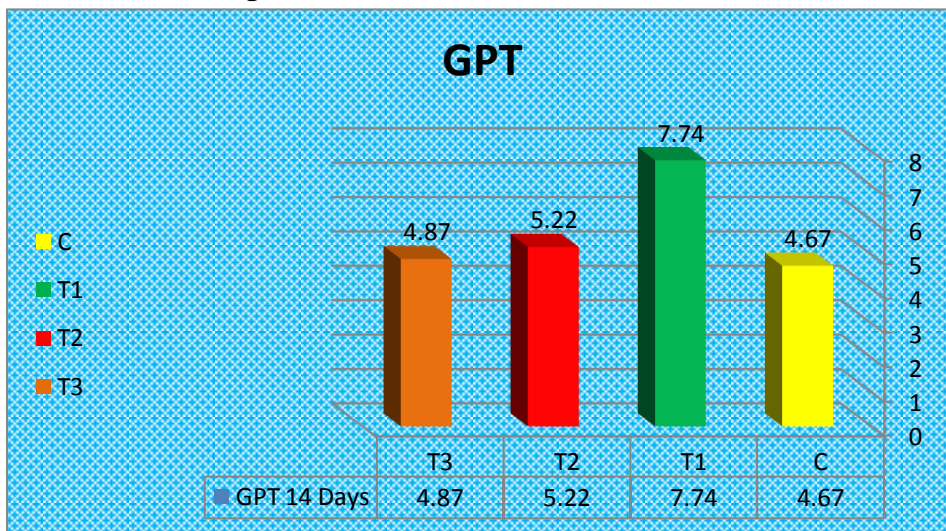


Figure (2): Effect of *Eucalyptus globulus* treatment on serum GPT concentration (µMole/ml) in mature male rats gavaged Aflatoxin.

Histopathology profile:

The sections obtained from liver of rat were explained of the many histological changes in tissue of liver in all groups of experiments, in control group was explain normal radial arrange around central vein and hepatocyte showed with hexagonal shape with acidophilic cytoplasmic and central permanent nuclei in liver tissue (FIG. (3) & FIG. (4)). Where as in Aflatoxin group was explained extensive necrosis in the hepatic tissue (FIG.(5) & FIG.(6)), loss of radially arrangement of hepatic cords

around the central vein in liver tissue, congestion and hyper aplasia of bile duct in liver tissue. As for the AE250 group was explained some of cell showed bionuclatied, degeneration of hepatocyte, normal control vein, present of radial arrangement of hepatocyte and proliferation in liver tissue. And finally in AE400 group was explained normal radial arrangement hepatocyte clear regeneration of hepatocyte which showed vacuolated and bionuclatid, mild dilation of cytoside in liver tissue.

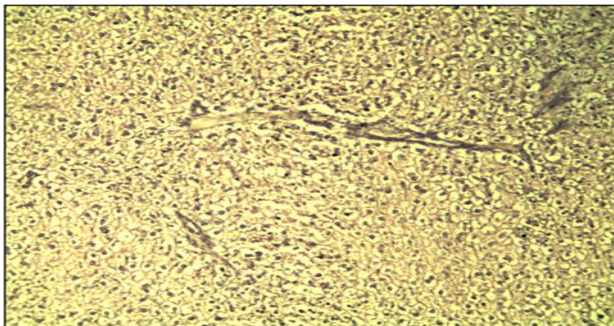


Figure (3): section of Liver of rat, Gavage of D.W control group (C). (H&E x10)

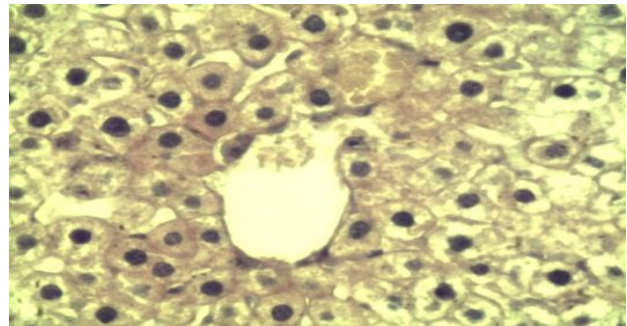


Figure (4): section of Liver of rat, Gavage of D.W control group (C). (H&E x40)

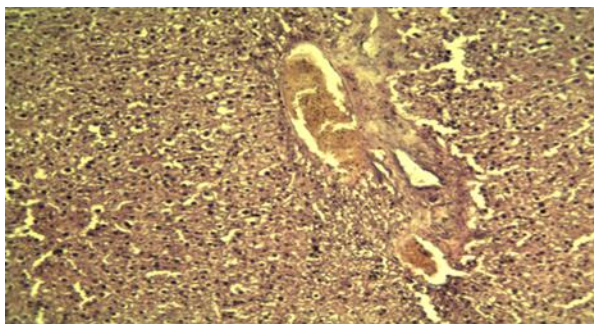


Figure (5): section of Liver of rat, Gavage of Aflatoxin (2.5g/ kg b.w) (T1) once for 42 days. (H&E 10)

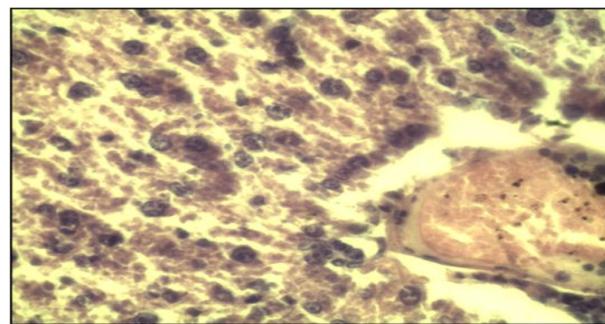


Figure (6): section of Liver of rat, Gavage of Aflatoxin (2.5g/ kg b.w) (T1) once for 42 days. (H&E 40)



Figure (7): liver of rat, Gavage of Aflatoxin (2.5g/ kg b.w.) one time and Gavage of EUE. 250mg/ kg b.w.) (T2) for 42 days. (H&E x10)

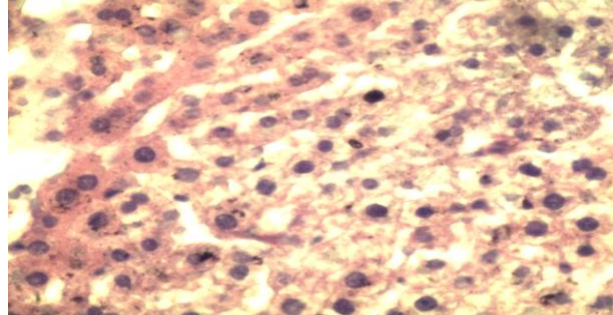


FIG.(8) liver of rat, Gavage of Aflatoxin (2.5g/ kg b.w.) one time and Gavage of EUE. 250mg/kg b.w.) (T2)for 42 days. (H&E x40)

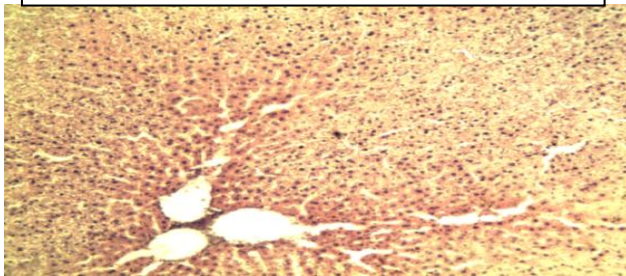


FIG.(9) Liver of rat, Gavage of Aflatoxin (2.5g/ kg b.w.) one time and Gavage of EU. 400mg/kg b.w.) (T3) for 42 days. H&Ex 10)

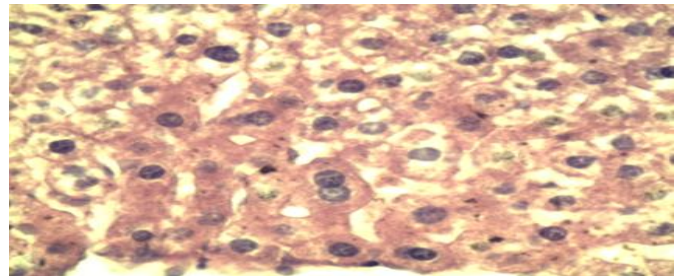


Figure (10) Liver of rat, Gavage of Aflatoxin (2.5g/ kg b.w.) one time and Gavage of EU. 400mg/kg b.w.) (T3) for 42 days. (H&E x40)

FIG.(7) and FIG.(8) showed histological section, obtained from AE250 male rat with gavage single dose of Aflatoxin (2.5 g/kg b. w.) and with gavage (250 mg/kg b. w.) of alcoholic extracted of *Eucalyptus globulus* for 42 days revealed the presence of was explained normal radial arrangement hepatocyte clear regeneration of hepatocyte which showed vacuolated and

Discussion:

The aim of this study was to investigate the protective effect of *Eucalyptus globulus* on the Aflatoxin-induced liver toxicity evidenced by biochemical measurements of GOT and GPT levels in blood serum and histopathological changes. In fact, our

bionuclatid, mild dilation of cytoside in liver tissue (Regeneration), in the hepatic tissue.

Histological section, obtained from AE400 male rat with gavage single dose of Aflatoxin (2.5g/kg b. w.) and with gavage (400 mg/kg b. w.) of alcoholic extracted of *Eucalyptus camaludlensis* for 42 days revealed the presence of (Regeneration) hepatic tissue.

study clearly demonstrates that acute Aflatoxin is now important consideration in the etiology of human hepatocellular carcinoma. In the present study, we found also that Aflatoxin-induced Liver damage by histological changes in liver of rat were explained of the many histological changes in tissue of liver in

all groups of experiments, in control group was explain normal radial arrange around central vein and hepatocyte showed with hexagonal shape with acidophilic cytoplasmic and central permanent nuclei in liver tissue. Where as in Aflatoxin group was explained extensive necrosis in the hepatic tissue, loss of radially arrangement of hepatic cords around the central vein in liver tissue, congestion and hyper aplasia of bile duct in liver tissue. As for the AE250 group was explained some of cell showed bionuclatied, degeneration of hepatocyte, normal control vein , present of radial arrangement of hepatocyte and proliferation in liver tissue. And finally in AE400 group was explained normal radial arrangement hepatocyte clear regeneration of hepatocyte which showed vacuolated and bionuclatid, mild dilation of cytoside in liver tissue, and we when discussed of my results in this study the effect of EUE on regeneration of hepatocytes is very good by as antioxidants activity and the EUE is richen with the flavonoids, phenols and alkaloid this compound is very active to repair of tissues of liver and regeneration, also exerts antifungals properties and may participate to the defence against an oxidative stress by scavenging ROS [12, 13]. Histopathological observations also substantiated the biochemical findings with improvements in Liver histology after administration of plant extract [14]. Other studies attribute the fall in the rate of plasma proteins caused

by the administration of Aflatoxin to the poor efficiency of damaged Livers to filter and reabsorb the protein [15]. In our work, Eucalyptus treatment effectively protected against Aflatoxin-induced hepatotoxicity by restoring almost normal activities of GOT and GPT. The attenuation of Liver damage was confirmed by microscopic examination. These findings are in agreement with those reported earlier by others [16]. Previous studies showed that nutraceutical benefit of the extract of our plant have been attributed to the flavonoids, flavonols, and phenolics compounds [17, 18]. The localization of flavonoids in the membrane interiors and their resulting restrictions on the fluidity of membrane components could strictly hinder the diffusion of free radicals generated during Aflatoxin oxidation, and there by decrease resulting damage effects [19, 20]. Therefore, Eucalyptus extract might play a key role in protection against Aflatoxin intoxication by modulating the cellular GSH pool [21]. In this regard, we suggest that Eucalyptus globulus exerts an in vivo antioxidant activity against harmful reactive oxygen species generated following Aflatoxin oxidation, and could therefore prevent Liver damage.

Conclusion: we concluded that the administration of (EUCE) act as antioxidant for Aflatoxin which induced toxicity of liver and antifungal against mycotoxins.

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دراسة تأثير مستخلص الكالبتوس الايثانولي ضد سم الأفلاتوكسين الذي يسبب تلف الكبد في ذكور الفئران

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الخلاصة:

تم تصميم هذه الدراسة لتقييم التأثيرات النافعة لمستخلص الكافور الكالبيوس على السمية الكبدية للأفلاتوكسين، وقد أجريت هذه الدراسة على ذكور الفئران البالغة في قسم علوم الحياة- كلية العلوم جامعة القادسية خلال الفترة الممتدة من نوفمبر، 2015 إلى فبراير، 2016 (4 شهور). 40 من ذكور الفئران الناضجة، (90 يوماً من العمر، وزن 190 ± 10 غم) تم فصلها إلى أربع مجموعات متساوية عشوائياً، تم فصل عينات الدم، المصل، لتقييم GOT، GPT. في ظروف التجربة، نتج عن التسمم بالأفلاتوكسين زيادة معنوية إحصائياً ($p > 0.05$) في أنشطة GOT، GPT في المجموعة الثانية المعاملة بالأفلاتوكسين فقط مقارنة مع مجموعة السيطرة، وفي المجموعتين الثالثة والرابعة أظهرت انخفاضاً معنوياً إحصائياً (0.05) في GOT، GPT في مصل الدم لمجموعة معاملة الأفلاتوكسين مقارنة مع مجموعة السيطرة، بعد التحليل النسيجي للمجموعة المعاملة بالأفلاتوكسين أثبتت تغير في الكبد، بعد التجريع من (مستخلص الكالبيوس) أظهرت النتائج أن هناك تحسناً في أنسجة الكبد بعد العلاج بالمستخلص. في الدراسة الحالية وجد ان جرعة 400ملغم/كغم من وزن الجسم هو أفضل من الجرعة 250ملغم /كغم اعتماداً على النتائج في الدراسة الحالية.

وتبين ان مستخلص اليوكالبيوس الكحولي يعمل كمضاد للأكسدة للأفلاتوكسين التي تسبب سمية الكبد ومضاد للفطريات ضد السموم الفطرية.

كلمات مفتاحية: الأفلاتوكسين، الكالبتوز، ذكور الفئران، GOT ، GPT.