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Phytochemical screening and investigation of the effect of *Alhagi maurorum* (Camel thorn) on carbon tetrachloride, acetaminophen and adriamycin induced toxicity in experimental animals

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Abstract

This study aims to evaluate the total phenolic and flavonoids contents of Camel thorn that reveals the higher concentration of flavonoids and phenolic especially in the ethanolic extract. In this study the administration of 660 mg/kg of the ethanolic extract of the *Alhagi maurorum* to mice showed a significant hepatoprotective activity against carbon tetrachloride and acetaminophen, by a significant decrease in the activity of serum transaminases. On the other hand the cardiac toxicity produced by adriamycine was significantly increased in the presence of the ethanolic extract of Camel thorn (*Alhagi maurorum*). Histopathological investigation confirmed that, Camel thorn extract protects liver against damage induced either by carbon tetrachloride or acetaminophen. Our study also suggested that Camel thorn can protect the liver against the injury produced by carbon tetrachloride or acetaminophen, with unexpected behavior for enhancing the cardiac toxicity of Adriamycin in mice.

Keywords: *Alhagi maurorum*, Hepatoprotective activity, Carbon tetrachloride, Acetaminophen.

Introduction

Hepatotoxicity implies chemical-driven liver damage. Liver plays central role in transformation and clearance of most chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometime even when introduced within therapeutic ranges, may injure the organ. Chemicals that cause liver injury are called hepatotoxins.[1] Chemicals produce a wide variety of clinical and pathological hepatic injury. Biochemical markers (i.e. alanine transferase, alkaline phosphatase and bilirubin) are often used to indicate liver damage.[2, 3] Liver damage is further characterized into hepatocellular (predominantly initial Alanine transferase elevation) and cholestatic type (initial alkaline phosphatase rise). However they are not mutually exclusive and mixed type of injuries are often encountered. Chemicals and drugs such as Carbon tetrachloride (CCl_4) and acetaminophen catabolised radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, causing the swelling and necrosis of hepatocytes and result to the release of cytosolic enzymes in to the blood.[4] A large number of medicinal plants have been shown

hepatoprotective effects.[5-9] Flavonoids and other phenolics of plant origin have been reported to have roles as scavengers and inhibitors of lipid peroxidation.[10-12]

Flavonoids may have existed in nature for over 500 million years and thus have interacted with evolving organisms over the eons. Clearly, flavonoids serve important purposes in nature, having survived in vascular plants throughout evolution.[13] Flavonoids not only equip the plants themselves with unique properties (such as colours), but also exert an influence on animals living with plants. The long association of plant flavonoids with various animal species and other organisms throughout evolution may account for the extraordinary range of biochemical and pharmacological activities of these chemicals in mammalian and other biological systems.[14]

In humans and higher animals, flavonoids have long been recognized to possess anti-inflammatory, antioxidant, ant-allergic and hepato-protective properties. They are also believed to be antithrombotic, antibacterial, antifungal, antiviral, and cancer protective, and also to protect against cardiovascular disease.[15-17]

Alhagi maurorum (Camel thorn) is a very common woody perennial shrub, rich in phenolic and flavonoid compounds with more than twelve different isolated flavonoids have been reported.[18] Antioxidants, anti-diarrheal and anti-ulcerogenic activity of Camel thorn with these contents have been reported.[19] Camel thorn used in folk medicine as a remedy for rheumatic pains, bilharziasis, liver disorders, urinary tract infection and for various types of gastrointestinal discomfort with both peripheral and central anti-nociceptive activity on the dose of 400 mg/kg through its work as antioxidant.[20]

The aim of this study is to evaluate the phenolic and flavonoid contents as well as hepatoprotective activity of Camel thorn as one of flavonoids rich plant against carbon tetrachloride and acetaminophen induced hepatotoxicity and against adriamycine produced cardiac toxicity.

Materials and methods

Plant collection and preparation of extracts

Fresh herb of *Alhagi maurorum* (Camel thorn) was collected from Al Kofra desert- Libya. The plant was identified by comparison with authentic sample in the herbarium of the Botany department, faculty of science, Benghazi University. The plants was washed with tap water and left for drying in the open air. 300 grams of the plants dried powders was gradually extracted by continuous soxhlation with petroleum ether, chloroform, ethyl acetate and ethanol (500 ml), respectively. All fractions were evaporated to dryness using rotavapor (IKA-WERKE, GMBH& Co.Kg, D-79219 Staufen, Germany) and the extractive value for each fraction was calculated as 8.1, 3.9, 1.5 and 6.0 grams, respectively. The different fractions were reconstituted in their extraction solvent to give the required concentration needed in this study.

Preliminary phytochemical screening of the different plants extracts

Preliminary screening of the different Camel thorn extracts were performed to investigate the presence or absence of the different phytochemical constituents such as phenolics, flavonoids, tannins, saponins and alkaloids using standard procedures described by Alex *et al*.[21] The result showed in table (1).

Quantitative estimation of total phenolics

Total phenol contents of different extracts were determined by the modified Folin-ciocalteu method according to Omoruyi *et al*.[22] An aliquot of 0.5 ml of each extract (1 mg/ml) was mixed with 2.5 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 2 ml (7.5 % w/v) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for colour development. Absorbance was then measured at 765 nm using spectrophotometer (Spectro UV-VIS double, 110 V, 60 Hz, Serial No. Double 001158, Labomed, Inc. U.S.A.). Total phenolic contents of different extracts were expressed as mg/g tannic acid equivalent using the following equation from the calibration curve: Y = 0.4879×, R2= 0.9064, where × is the absorbance and Y is the tannic acid equivalent in mg/g. The experiment was conducted in triplicate and the results were expressed as mean ± SD values.

Quantitative estimation of total flavonoids

Total flavonoid contents of different extracts were determined by method described by Ordonez *et al*.[23] Based on the formation of a flavonoid-aluminum complex. 0.5 ml of various solvent extracts (1 mg/ml) was mixed with 0.5 ml of aluminium chloride (2% in ethanol). The resultant mixture was incubated for 30 min at room temperature for yellow colour development which indicated the presence of flavonoid. Absorbance was measured at 420 nm using UV–VIS spectrophotometer. Total flavonoid content was

calculated as quercetin equivalent (mg/g) using the following equation based on the calibration curve: Y = 0.217x, R2 = 0.9582, where × is the absorbance and Y is the quercetin equivalent.

Quantitative estimation of total flavonols

Total flavonoid contents of different extracts were determined by method described by Omoruyi *et al*.[22] The reaction mixture consisting of 2 ml of various solvent extracts (1 mg/ml), 2 ml of aluminum chloride prepared in (2% in ethanol) and 3 ml of sodium acetate solution (50 g/l) was allowed to incubate for 2.5 h at 20°C. Absorbance at 440 nm was measured. Total flavonol content was calculated as mg/g of quercetin equivalent from the calibration curve using the equation: Y= 0.217×, R2 = 0.9582 where × is the absorbance and Y is the quercetin equivalent.

Experimental animals

Male Albino mice weighing 25-30 g, and Wister rats weighing 180-200g were maintained in the animal house of Faculty of Medicine- Benghazi University. They were healthy animals and housed in spacious polypropylene cages. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 25±2ºC and 12 hours light/dark cycle throughout the experimental period. All the animals were feed with standard pellet diet and water ad libitum.

Effect of CTE on carbon tetrachloride and acetaminophen-induced hepatotoxicity:

Male albino mice weighing 25-30g were divided into 6 different groups each of 10 mice. The 1st group (control) received normal saline i.p. The 2nd and 3rd groups received daily i.p dose (660 mg/kg) of Camel thorn ethanolic extract (CTE) for 3 days. Immediately after this treatment, the 3rd and 4th groups were given a single oral dose of carbon tetrachloride (1 ml/kg in maize oil). The 5th and 6th groups were pretreated with CTE as 3rd and 4th groups, and then given a single dose (500mg/kg i.p) of acetaminophen respectively. 24 hours after this treatment the animals will be sacrificed, the blood will be collected and used for the determination of liver transaminases, serum Alanine aminotransferase (ALT) and serum Aspartate aminotransferase (AST) according to Bergmeyer *et al*.[24] The liver will be removed, fixed in 10% formalin and used for histopathological investigation.

Effect of CTE on adriamycin-induced cardiac toxicity:

Male Wistar rats weighting 180-200 g were divided into four groups each consisting of 6 rats. The first group (control) received normal saline. The second group received 660 mg/kg of CTE i.p. daily for 4 days. The third group received a single dose 20 mg/kg of Adriamycin i.p. in the first day. The fourth group received a single dose 20mg/kg of Adriamycin i.p in the first day, followed by 660 mg/kg of CTE i.p daily for 4 days. All animal were fasted for 18 hrs before sacrifice. The collected blood was used to measure cardiac enzymes creatine phosphate (CPK) and lactate dehydrogenase (LDH).[25, 26]

Statistical Analysis

The experimental results were expressed as the mean ±S.E.M. and are accompanied by the number of observations. Data were assessed by one way analysis of variance (ANOVA). If this analysis indicated significant differences among the group means, then each group was compared by rather the LSD, or paired sample test. A P value of 0.05 was considered statistically significant, P value of 0.01 was considered statistically highly significant.

Results

Result of phytochemical screening and estimation of total phenolic and flavonoids

The result of the preliminary phytochemical screening give a clear evidence for the presence of phenolics, flavonoids, tannins and alkaloids in addition to carbohydrates and sterols. The tests also revealed that the absence of anthraquinons and saponins. The table (1) showed these resulted in a qualitative manner. Quantitative analysis of the total phenolics and flavonoids showed in table (2) indicated that Camel thorn extracts containing high percentage of phenolic and flavonoids with especial concern to the ethanol extract.

Table 1: Results of phytochemical screening for the Camel thorn different extracts

Phytochemicals	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
Phenolics	-ve	+	++	+++
Flavonoids	-ve	+	+++	+++
Tannins	-ve	-ve	+	+++
Anthraquinon	-ve	-ve	-ve	-ve
Alkaloids	-ve	-ve	++	++
Saponins	-ve	-ve	-ve	-ve
Carbohydrates	-ve	-ve	+	+++
Sterols	+++	++	-ve	-ve

Key: −ve (Absent), + (Low in abundance), ++ (Moderate in abundance), +++ (High in abundance)

Table 2: results of quantitative estimation of the total phenoilc, flavonoids and flavonols in mg/grams of the dried extracts

Phytochemicals	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
Phenolics (TE/gm)	Nd	156.12±3.51	218.57±0.55	537.29±4.23
Flavonoids (QE/gm)	Nd	142.13±5.65	153.20±13.24	224.92±1.25
Flavonols (QE/gm)	Nd	58.15±0.33	60.43±0.22	79.31±2.09

Values are expressed in mean ± standard deviation of the mean (n = 3). Nd: not detected; (TE) tannic acid equivalent; (QE) quercetin equivalent.

Result of the hepatoprotective activities:

Data in figure (1) indicated that the level of both ALT & AST, were significantly reduced ($P < 0.001$) in groups received CTE+CCl_4 as compared to CCl_4 treated animals.

During our study the percentage of death in the acetaminophen treated group was 30% (3 out of 10 mice) whereas in the group receiving both CTE+ acetaminophens it was 10% (i.e. one out of ten).

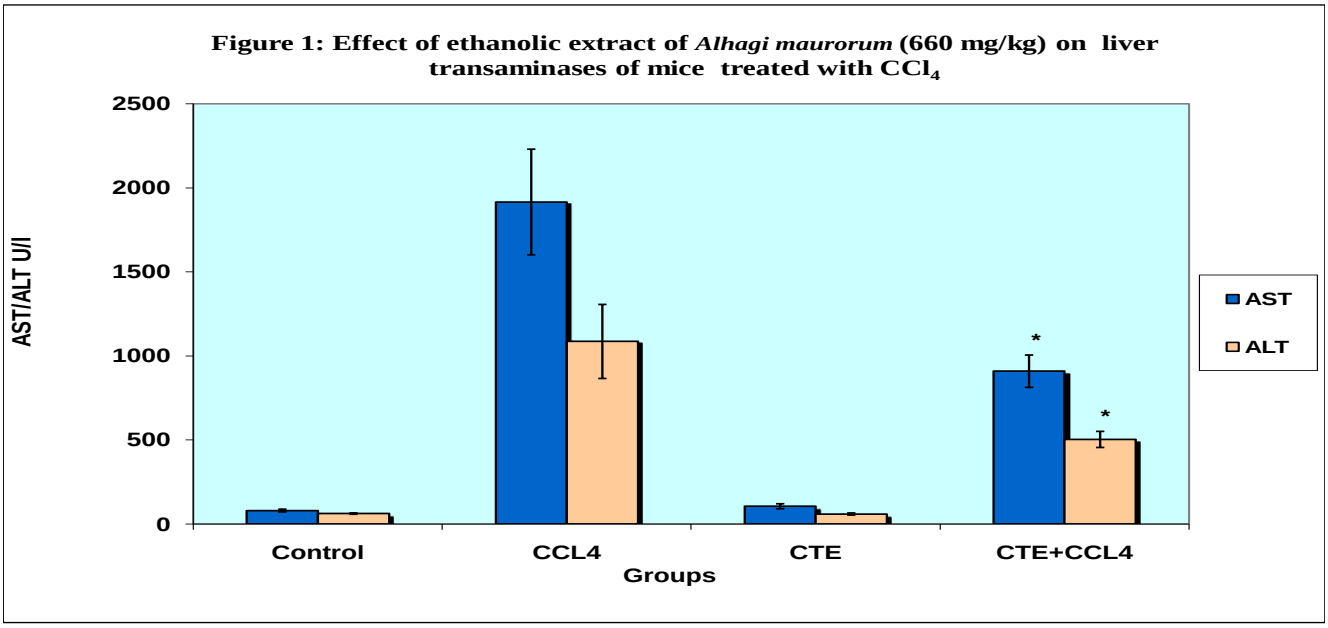
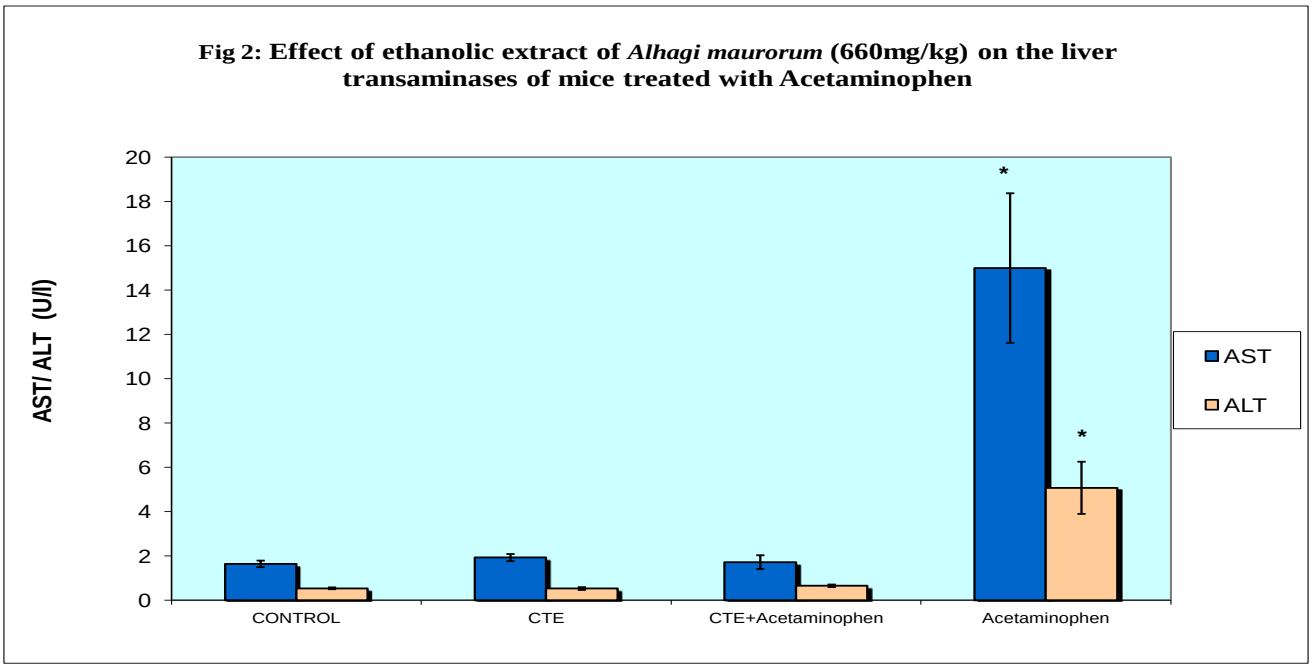


Figure 1: Effect of ethanolic extract of *Alhagi maurorum* (660 mg/kg) on liver transaminases of mice treated with CCl_4

*** Significantly decreased as compared to the CCl_4 treated group ($P<0.01$).**

Results in figure 2 showed that the level of AST and ALT in the group of animals treated with CTE+ Acetaminophen were significantly lower (P<0.01) as compared to acetaminophen- treated group.

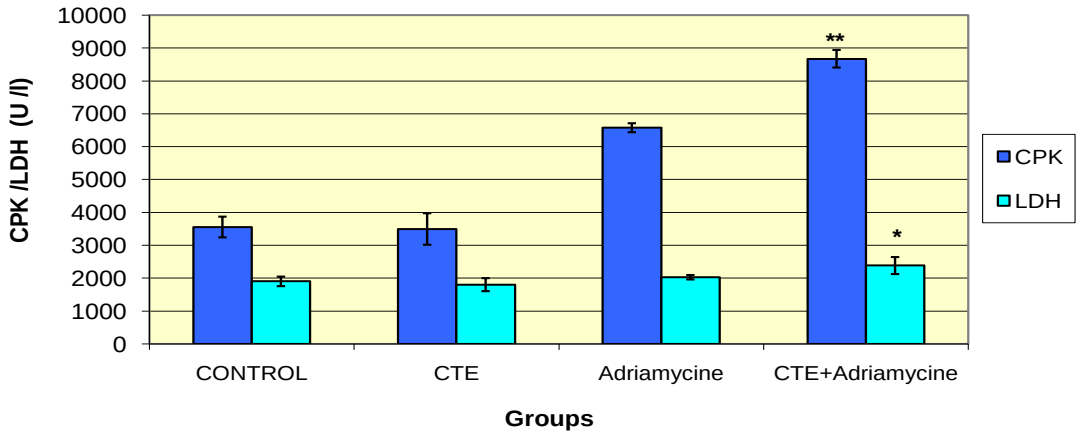
Fig 2: Effect of ethanolic extract of *Alhagi maurorum* (660mg/kg) on the liver transaminases of mice treated with Acetaminophen



***Significantly increased as compared to all groups (P < 0.001).**

With regard to the effect of CTE on the adriamycin-induced cardiac toxicity, our data presented in figure 3 indicated that the level of CPK and LDH were significantly increased respectively (P<0.01 or 0.05) in the group treated with the combination of both CTE+ adriamycin as compared to the control or other treated groups.

Figure 3:Effect of ethanolic extract of *Alhagi maurorum* on cardiac enzymes of mice treated with Adriamycine



****Significant increased as compared to the other groups (P < 0.001).**
***Significant increased as compared to CTE treated group (P <0.05).**

Histopathological studies also provided supportive evidence for the biochemical analysis. As presented in figure 4 and 5 the livers of control and CTE treated mice showed normal parenchymal architecture with cord of hepatocytes, portal tract and central veins without any alterations.

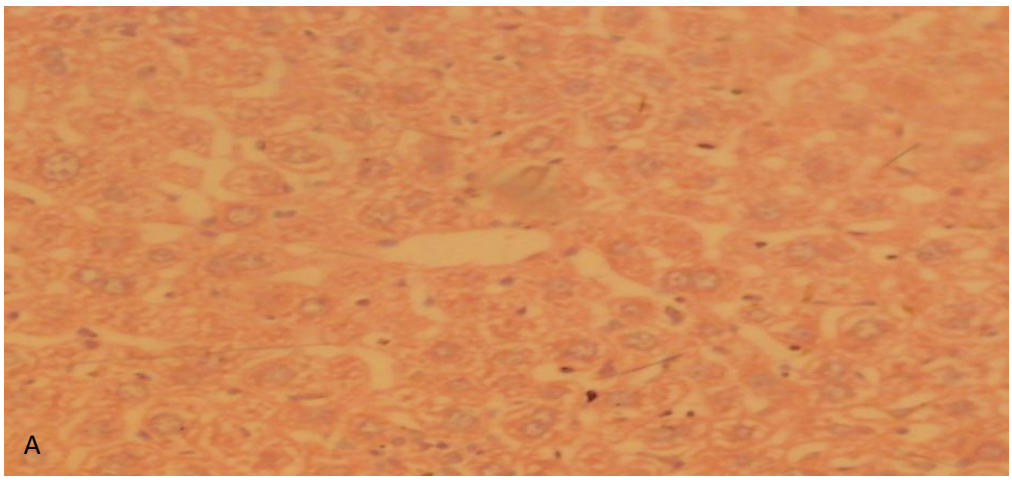


Figure 4: Light photomicrographs (10 x magnifications) of hematoxylin and eosin–stained sections of liver of normal mice, control (A) showed normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins without noticeable alterations

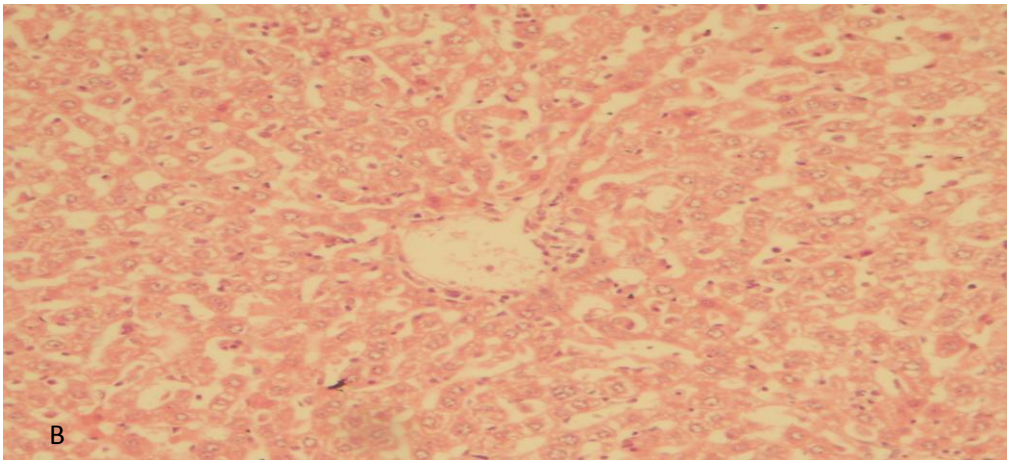


Figure 5: Light photomicrographs (10 x magnifications) of hematoxylin and eosin–stained sections of liver of mice treated with CTE (B) showed normal hepatocytes

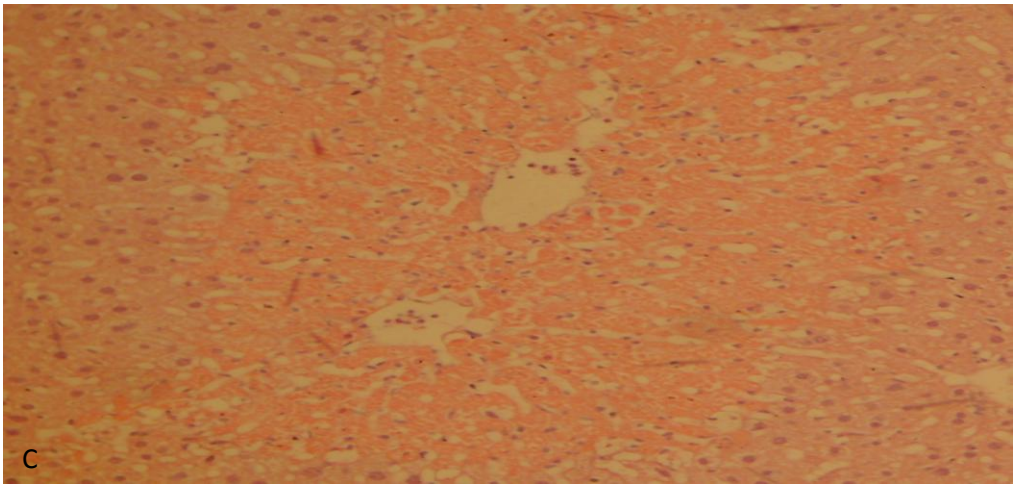


Figure 6: Light micrographs of hematoxylin and eosin–stained sections of liver of mice treated with CCl_4. (C) showed severe Centrizonal necrosis accompanied by fatty changes were observed in the hepatocytes of the livers

Whereas figure 6 indicated a centrizonal necrosis accompanied by fatty changes in the hepatocytes of the livers of the CCl_4 treated mice. As shown in figure 7 the extent of cellular necrosis was lesser in the animal treated with CTE+CCl_4. As indicated in figure 8, a centrizonal necrosis accompanied by fatty changes were observed in the hepatocytes in the livers of mice of the acetaminophen treated groups, however as presented in figure 9, the cellular necrosis was almost completely disappearing in the group receiving CTE+ acetaminophen.

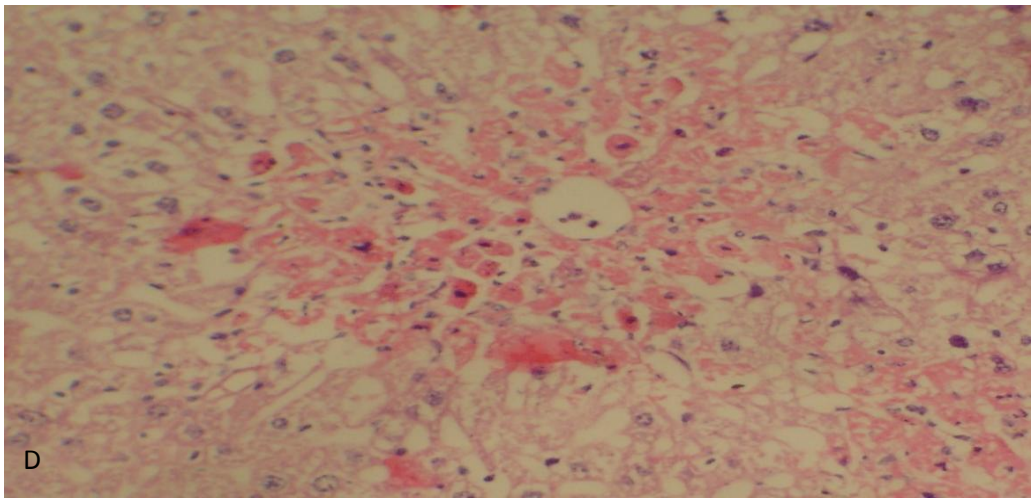


Figure 7: Light micrographs of hematoxylin and eosin–stained sections of liver of Mice treated with CCl_4 + 660 mg/kg of CTE (D) showed less Centrizonal necrosis

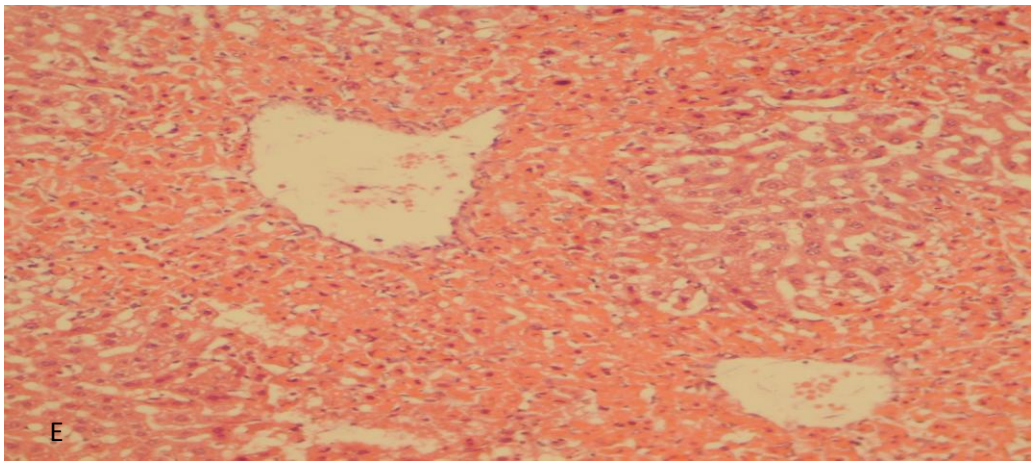


Figure 8: Light photomicrographs (10 x magnifications) of hematoxylin and eosin–stained sections of liver of from treated with acetaminophen (E). Centrizonal necrosis accompanied by fatty changes

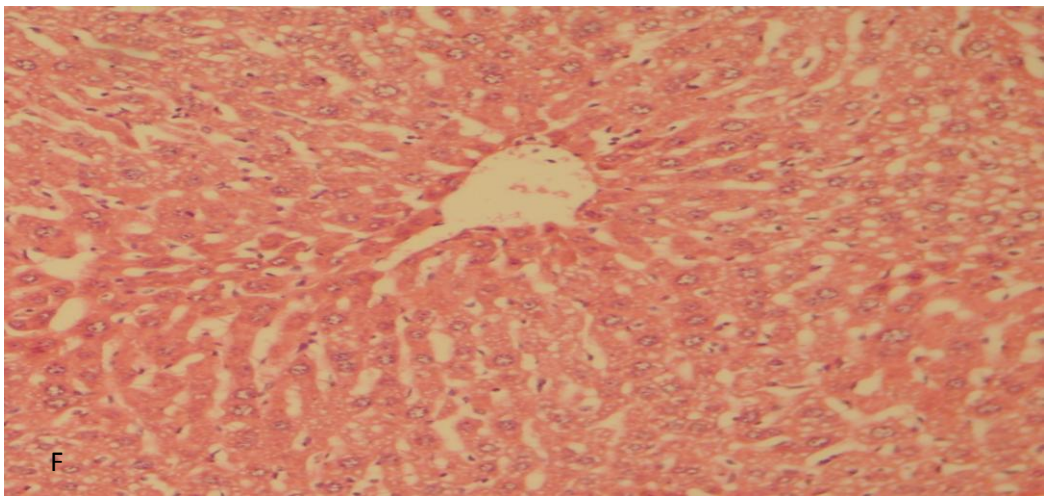


Figure 9: Light photomicrographs (10 x magnifications) of hematoxylin and eosin–stained sections of liver of mice treated with acetaminophen plus CTE (F) showed less centralized necrosis

Discussion

Results of the phytochemial screening and quantitative analysis of Camel thorn extracts raveled that, high percentage of phenolics and flavonoids present in the ethyle acetate and ethanol extracts, with especial concern to the later one (Table 2). The percentage of phenolics and flavonoids including flavonols in ethanol extract (537.29, 224.92, 79.31 mg/g) respectively, make point of interest for the antioxidant activity that expected from this extract. The hepatoprotictive activity of flavonoids has been reported16. In addition to the antioxidant activity may be enable Camel thorn extracts especially ethanol extract to protect all sensitive tissues, particularly liver against all kind of oxidative damage. It is known that CCl4 and acetaminophen (at high doses) is hepatotoxic.[26] It is well established that CCl_4 is metabolized in the liver to highly reactive trichloromethyl radical and this free radical leads to auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and cause functional and morphological changes in the cell membrane.[27, 28] This is evidenced by an elevation of the serum AST, ALT in CCl_4 treated rats.[29, 30]

In the liver, CCl_4 is metabolized by the cytochrome P450-dependent monooxygenase systems[31], followed by its conversion to more chemically active radical, This radical initiates the chain reaction of lipid peroxidation, which attacks and destroys polyunsaturated fatty acids.[32-34] This resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage.

It was reported that the antioxidant activity or inhibition of the generation of free radicals is important in the protection against the CCl_4 induced liver lesion.[31]

In cases of acetaminophen toxicity, the sulfate and glucuronide pathways become saturated, and more acetaminophen is shunted to the cytochrome P-450 system to produce N-acetyl-p-benzo-quinone imine.[35, 36] Resulting in widespread hepatocyte (liver cell) damage and death, clinically leading to acute hepatic necrosis.[37-39]

Little is known about the mechanism of hepatoprotective activity of CTE against CCl_4 or acetaminophen hepatotoxicity. Flavonoids group of naturally occurring benzo-g-derivatives, have been shown to possess several biological properties, many of which may related, partially at least, to their antioxidant and free radical- scavenging ability.[40, 41] In some studies the authors described the antioxidant activity of flavonoids from some plants like *Solanum melongena*, or *Licania licaniaeflora*.[42, 43]

Our results suggest that the CTE has hepatoprotective activity against both CCl_4. and acetaminophen induced hepatotoxicity. The mechanism of such hepatoprotective activity may be related to the phenolic and flavenoids of CTE or the CTE may alter the activity of cyp450. Adriamycin has been postulated to induce cardiotoxicity through redox cycling and reactive oxygen species.[44] This reactive oxygen species (ROS) hypothesis, however, has been tempered by a series of studies in which treatment with a ROS scavenger failed to prevent cardiac toxicity caused by doxorubicin.[45, 46] Thus, an alternative hypothesis is needed to explain doxorubicin-induced cardiotoxicity.

Sui Z *et al* suggested that doxorubicin-induced cardiotoxicity is not solely due to redox cycling of doxorubicin.[47] But maybe also Topoisomerase 2β (Top2β) as an essential driver of doxorubicin-induced cardiotoxicity. In the presence of Top2β, doxorubicin activates the DNA response and apoptosis pathways and triggers a marked alteration in the transcriptome that selectively affects oxidative phosphorylation and mitochondrial biogenesis in cardiomyocytes.

Maria Oliveira *et al* reported that flavonoid extract from *Camellia sinensis* did not protect the cardiac toxicity of adriamycin.[48] Our results indicated that CTE extract has failed to inhibit the cardiac toxicity of adriamycin and this may be due to interference with the metabolic pathway of adriamycin plus the other mechanism of toxicity of adriamycin that previously mentioned about the role Top2ß in adriamycin toxicity.

From this study we may conclude that CTE protect the liver from the injury produced by oxidative damage of carbon tetrachloride or acetaminophen due to its high contents of phenolic and flavonoids. This study also reveled that significant increase in cardiac toxicity produced by Adriamycin in presence of CTE extract and further future study is need to explain the mechanism behind this action.

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