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Effect of *Hibiscus sabdariffa* calyx Extract on Some Biochemical and Histological Parameters of Wistar Rats with Carbon Tetrachloride Induced Liver DamageSadi, N.S.¹, Sunusi, U.*¹, Kamal, R.M.², Ibrahim, S.³, Bello, A.S.¹, Muhammad, M.N.⁴

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Abstract

Hibiscus sabdariffa (Malvaceae) calyx extract is one of such important herbal hepatoprotective drug; it has a wide range of pharmacological properties, such as antioxidant and free radical scavenging capacity. The present study was carried out to determine the hepatoprotective effects of *Hibiscus sabdariffa* calyx extract on carbon tetrachloride (CCl₄) induced liver damage. Experimental rats were divided into seven groups. Group I: served as the control rats, group II: was administered with CCl₄ only. Group III and IV: were administered with 250 and 500mg/kg extract respectively. Group V: was administered with CCl₄ + standard drug. Group VI and VII: were administered with 250 and 500mg/kg extract only. Serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities, total cholesterol (TC) and triacylglycerol (TG) were measured. The liver tissues of different groups were examined. Oral administration of the extract following administration of CCl₄ significantly reduced the damage as determined by the activities of serum AST, ALT and ALP. Also, treatment with the extract shows a significant decrease in the level of TC and TG. Histopathological analysis of the liver tissues of CCl₄ group shows the presence of severe vacuolation and inflammation. Treatment with the extract promotes healing of the damage in the liver. *Hibiscus sabdariffa* calyx extract was effective in the treatment of liver damage induced by CCl₄.

Keywords: *Hibiscus sabdariffa* calyx extract, carbon tetrachloride, hepatoprotective

Introduction

Hibiscus sabdariffa Linn (Roselle) belongs to the family Malvaceae, and is widely grown in Africa, South East Asia, and some tropical countries of America). The two botanical types of roselle are *Hibiscus Sabdariffa* var. *sabdariffa*, grown for its fleshy, shiny-red calyx, and *Hibiscus Sabdariffa* var. *altissima* grown for its phloem fiber (Purseglove, 1974). In Hausa, the plant is locally called 'Yakuwa' while the fresh calyx is referred to as 'Zobo'. The Yoruba called the leaves 'Amukan' and the flowers 'Ishapa' (William, 1980). *H. sabdariffa* sepals (calyx and epicalyx) are the most important economic parts of the plant which is used in food (jam and jelly) and cosmetic industries as a source of natural colouring agent. The plant finds various uses in traditional medicine. The seeds contain 17.8–21% non-edible oil (Ahmed, 1980) and 20% protein, and are sometimes used for animal feed (Ahmed, and Nour, 1981). Truswell (1992), reported that *Hibiscus sabdariffa* have antiseptic, digestive, diuretic, emollient and purgative. It is a folk remedy for abscess and other numerous conditions; cancer, cough, debility, dyspepsia, dysuria, fever, hangover, heart ailments, hypertension, scurvy, neurosis and strangury (Duke, 1985). Liver disorders are one of the world problems. It is frequent in occurrence and it is associated with a high morbidity and high mortality (Vinoth *et al.*, 2009). Liver damage can be caused by certain xenobiotics and microbial infiltration from ingestion or infection (Hai *et al.*, 2011). Carbon tetrachloride (CCl₄) is a well-established xenobiotic. Previous studies showed that both liver and kidneys are the target organs of CCl₄.



Extensive evidence demonstrated that CCl_4 is activated in the liver to a highly reactive trichloromethyl radical which initiates free radical-mediated lipid peroxidation of the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane, which leads to an accumulation of lipid derived oxidants causing liver injury (Singh *et al.*, 2008).

Hibiscus sabdariffa extract (HPEEE) is one of such important herbal hepatoprotective drug. *Hibiscus sabdariffa* is a natural phenolic rich plant that has been reported to have a wide range of pharmacological properties, such as antioxidant activity and free radical scavenging capacity (Ali *et al.*, 2005). *Hibiscus sabdariffa* extract which is a group of phenolic compounds was isolated from the dried flowers of *H. Sabdariffa*. Ramirez *et al.*, (2011) reported that the constituents of the extract of *H. Sabdariffa* have strong antioxidant properties, while Tsai *et al.*, (2009) postulated that the effectiveness of plant extracts against many diseases are due to their antioxidant, antigenotoxic and biomodulatory effects on various cells and animals exposed to different agents. However, it is uncertain if HPEEE also has protective effects against CCl_4 toxicity. The aim of this research was to investigate the effect of HPEEE in-vitro as well as examining its protective effect on the CCl_4 -induced liver damage in rats.

Materials and Method

Chemicals

All chemicals and solvents used for this research work were of the analytical grade and were obtained from Merck (India). Cholesterol, triglyceride and liver function kits were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other reagents were obtained from recognized manufacturers.

Plant Materials

Fresh plant material (*Hibiscus sabdariffa* L. calyx) was obtained from a farm at Kofar Kabuga Gwale L.G.A Kano state, Nigeria. The plant was authenticated at the Biological Sciences Department, Bayero University Kano. It was then identified and given an accession number of BUKHAN0040. The plant material

was dried under shed prior to grinding into a fine powder using a laboratory mortar.

Preparation of Extract

One hundred and thirty eight-gram (138g) portion of the dried sample was weighed and packed in a big laboratory reagent's bottle and continuously extracted with 95% ethanol for 24 hours. The solvent was removed from the extract by filtration. The percent yield of HSEE was 16.5% (w/w). The extract was weighed and stored at 4°C until required.

Phytochemical Screening

The extract were subjected to preliminary phytochemical tests using standard methods as previously described (; to detect the presence of anthraquinones, tannins, cardiac, terpenoids, glycosides, alkaloids, saponin, phenols, glycosides, steroidal nucleus.

Experimental Animals

An apparently healthy thirty-five either gender adult albino rats of Wistar strain weighing about 120 - 150g were purchased from the animal house, Department of Biological Sciences, Bayero University, Kano. The Animals were housed in well ventilated cages at a room temperature of 27 ± 1 °C, kept on 12±1h day/night regular cycle schedules (Reeves *et al.*, 1993) and were fed with water *ad libitum*. Carbon tetrachloride (CCl_4) (1.0mg/kg) and extracts were administered to the animals orally by gastric intubation. CCl_4 used was of best quality available (Merck, Darmstadt, Germany). All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

Experimental Design

The animals were kept for one week to adapt to the environment after which they were divided into seven groups (each of 5 rats) as follows: Group I (control): Rats were administered distilled water (Group II): Rats were orally administered a single daily dose of CCl_4 in mineral oil (1:1 v/v). (Group III): Rats in addition to the CCl_4 administered 48hr later, a single daily dose of a standard drug, 360.01mg/kg for 14 days. (Group IV and V): Rats were treated with CCl_4 as in III, but instead given 250 and 500

mg/kg extracts respectively. (Group VI and VII): Rats received only 250mg/kg and 500mg/kg extracts respectively, for 14 days.

Sample Collection and Preparation

Rats from the various groups were sacrificed by decapitation treatment 24h after respective treatment period. The blood was collected into a plain container and allowed to stand for 30 min to clot before being centrifuged at 2000rpm for 10 min to separate the serum. Immediately, the serum was used to estimate the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triacylglycerol (TAG) and total cholesterol or kept at - 20°C if the analysis was not immediate. Immediately after blood sampling, animals were sacrificed and the liver of each animal was dissected out, a part of liver was fixed in 10% formalin for histopathological studies and the other part was washed with ice-cold saline to remove as much blood as possible and stored at - 20°C until examined.

Determination of Liver Enzymes Activities

Serum samples were used for the determination of ALT and AST activities (Reitman and Frankel, 1957) and ALP (Belfied and Goldberg, 1971).

Determination of Lipid Profile Parameters

Serum samples were used for determination of TC (Allain *et al.*, 1974) and TAG (Fossati and Prencipe, 1982).

Histopathological Analysis

The biopsies of the liver of albino rats were fixed with 10% formal saline, dehydrated with ascending grade of alcohol cleared with toluene, infiltrated with molten paraffin wax. The microtome sections were stained with

Haematoxylin and Eosin staining technique and examined with Leica DM 750 microscope and photographed with Leica ICC 50HD camera (Auwioro, 2010).

Statistical Analysis

All experimental values were expressed as mean \pm standard deviation. The data were subjected to one-way analysis of variance (ANOVA) and comparison by Dunnett's post hoc test for multiple comparisons among the groups. A p-value < 0.05 were considered to be statistically significant.

Results

The results of hepatoprotective effects of *Hibiscus sabdariffa* calyx extract on rats treated with carbon tetrachloride CCl_4 are shown below (Table 1 and 2). Table 1 showed the effect of different doses of *Hibiscus sabdariffa* calyx extract on liver enzymes activities in CCl_4 induced hepatotoxicity in rats. There was a significant difference ($p < 0.001$) in the levels of ALT, AST and ALP in CCl_4 group compared to the control group. Treatment with extract at different doses (250 and 500mg/kg) significantly reduced the level of liver enzymes activities ($p < 0.001$) in treated groups compared with untreated group. Administration at the dose of 500mg/kg body wt. showed marked reduction as compared with the group treated with 250mg/kg body wt. At the same time, there were non-significant difference when compared to the group treated with 250mg/kg body weight and the group treated with standard 3 group. The extract at both 250 and 500mg/kg administered alone did not show any significant effect on levels of ALT, AST and ALP compared to control.

Table 1: Effect of *Hibiscus sabdariffa* calyx extract on serum ALT, AST and ALP levels of CCl₄ treated rats.

Group	Treatment	ALT	AST	ALP
I	Control	32.66±7.10	37.66±4.73	43.33±4.51
II	CCl ₄	73±6.93 ^{a***}	82.33±6.51 ^{a***}	79.66±2.52 ^{a***}
III	CCl ₄ + 250mg/kg extract	43.33±4.51 ^{b***}	59.66±7.51 ^{a**b**}	47.66±2.52 ^{b***}
IV	CCl ₄ + 500mg/kg extract	40±2.00 ^{b***}	56.66±6.51 ^{a*b***}	44.66±2.52 ^{b***}
V	CCl ₄ + standard drug	44.01±5.13 ^{b***}	60.66±5.13 ^{a**b**}	46±2.00 ^{b***}
VI	Extract 250mg/kg only	34.66±4.04	41±5.30	45.33±4.51
VII	Extract 500mg/kg only	38.33±4.04	43.33±1.53	47.33±4.51

Key

Values are mean ± S.D. n=5

^aAs compared with control group. ^bAs compared to CCl₄ group (p<0.05*, 0.01** and 0.001***)

Table 2 showed the effect of different doses of *Hibiscus sabdariffa* calyx extract on serum lipid profile parameters in CCl₄ induced hepatotoxicity in treated and untreated groups. The results indicated that levels of TC, TG in CCl₄ group were significantly elevated (p<0.001) with respect to control group. Administration of *Hibiscus sabdariffa* calyx extract resulted in the reduction of TC and TG in comparison to CCl₄ untreated group, there was a very highly significant difference in TC and TG treated groups at all level doses compared to CCl₄ untreated group (p<0.001). Administration at the dose of 500mg/kg body wt. showed a significant difference (p<0.001) compared with CCl₄ untreated group. The extract at both 250 and 500mg/kg administered alone did not show any significant effect on the levels of TC and TG compared to control.

Table 2: Effect of *Hibiscus sabdariffa* calyx extract on serum total cholesterol (TC) and triacylglycerol (TG) in CCl₄ induced hepatotoxicity rats.

Group	Treatment	TC mg/dl	TG mg/dl
I	Control	96.20±9.96	86.47±9.73
II	CCl ₄	219.69±44.75 ^{a***}	127.21±4.42 ^{a**}
III	CCl ₄ + 250mg/kg extract	173.79±11.68 ^{a**}	75.22±10.46 ^{b***}
IV	CCl ₄ + 500mg/kg extract	129.91±4.28 ^{b***}	59.14±13.03 ^{b***}
V	CCl ₄ + standard drug	102.36±3.70 ^{b***}	86.31±12.49 ^{b**}
VI	Extract 250mg/kg only	98.88±10.26	89.32±9.28
VII	Extract 500mg/kg only	99.87±10.30	92.12±6.20

Key: Values are means ± S.D. n=5

^aAs compared with control group. ^bAs compared to CCl₄ group (p<0.05*, 0.01** and 0.001***).

Histopathological Results

The histological findings showed normal histological features in the liver hepatocytes of the control group, which consists of central vein and hepatocytes arranged in hepatic cords (Figure 1), while that of CCl₄ intoxicated rats shows areas of severe vacuolation and areas of inflammation (Figure 2). Liver section of treated

rat at dose level of 250 and 500mg/kg shows areas of mild vacuolation with no inflammation (Figure 3 and 4); also group treated with standard drug (Figure 5) shows areas of mild vacuolation. Administration at the dosage of 250 and 500mg/kg only (Figure 6 and 7) also shows areas of mild vacuolation almost similar to that of the control group.

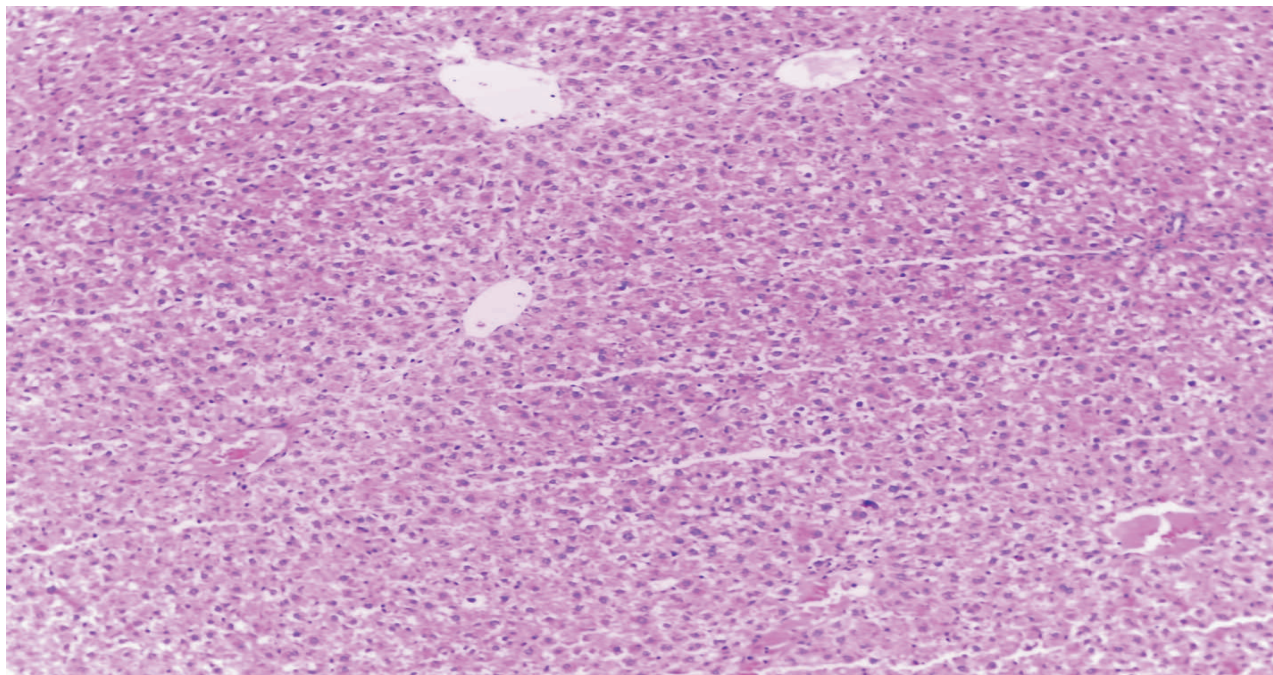


Figure 1. Liver of control group showing the normal histological structure of hepatic lobule (H&E stain, Mag x100).

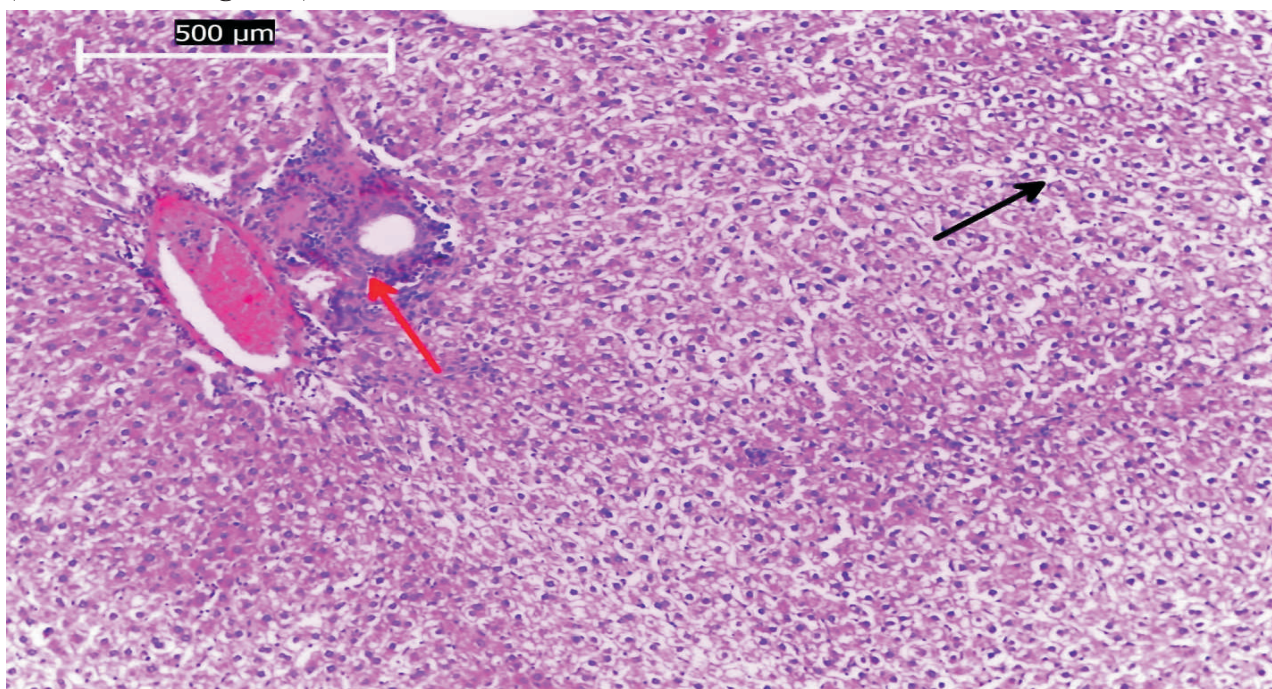


Figure 2. Liver section of CCl₄-intoxicated rats showing severe vacuolation and areas of inflammation (H&E, Mag X100)

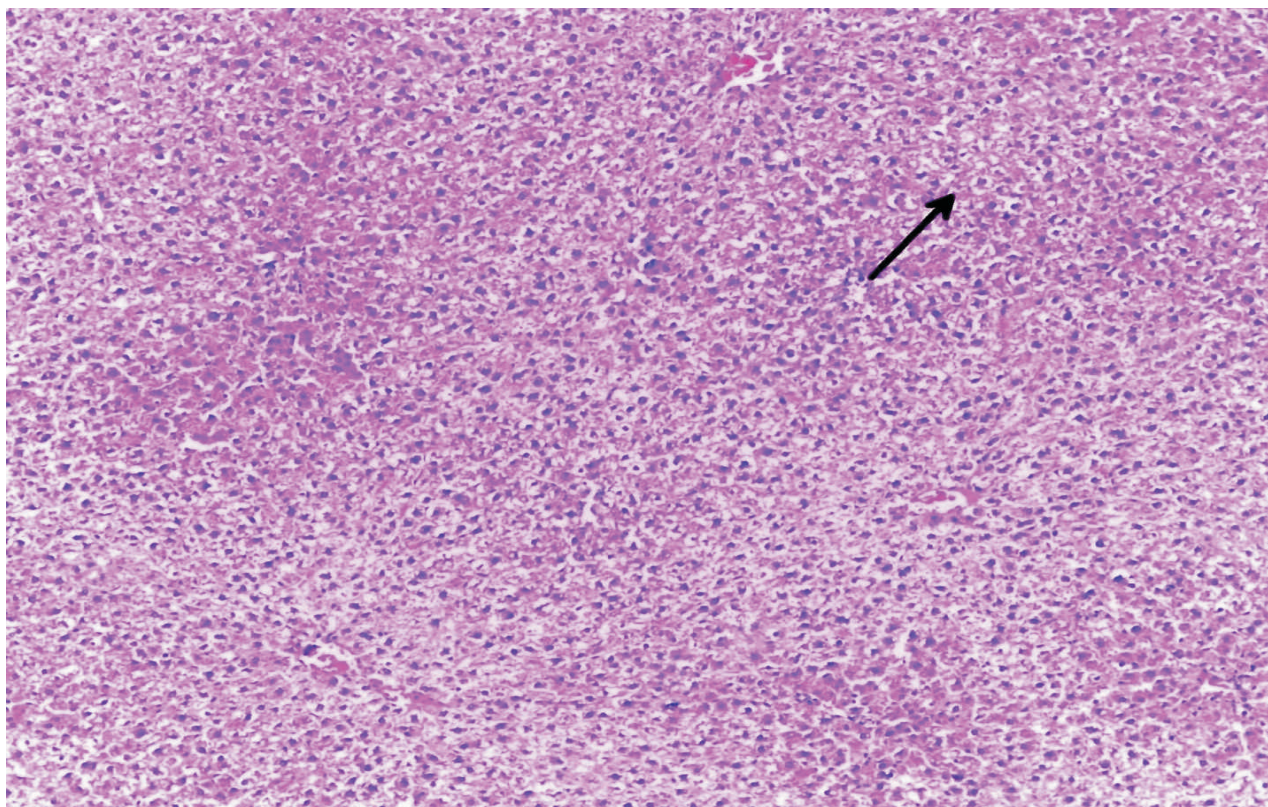


Figure 3. Liver section of CCl₄- intoxicated rats pretreated with HPEEE (250mg/kg b.wt.) showing kuffer cells activation (arrow) and mild vacuolation (H&E stain, Mag x400)

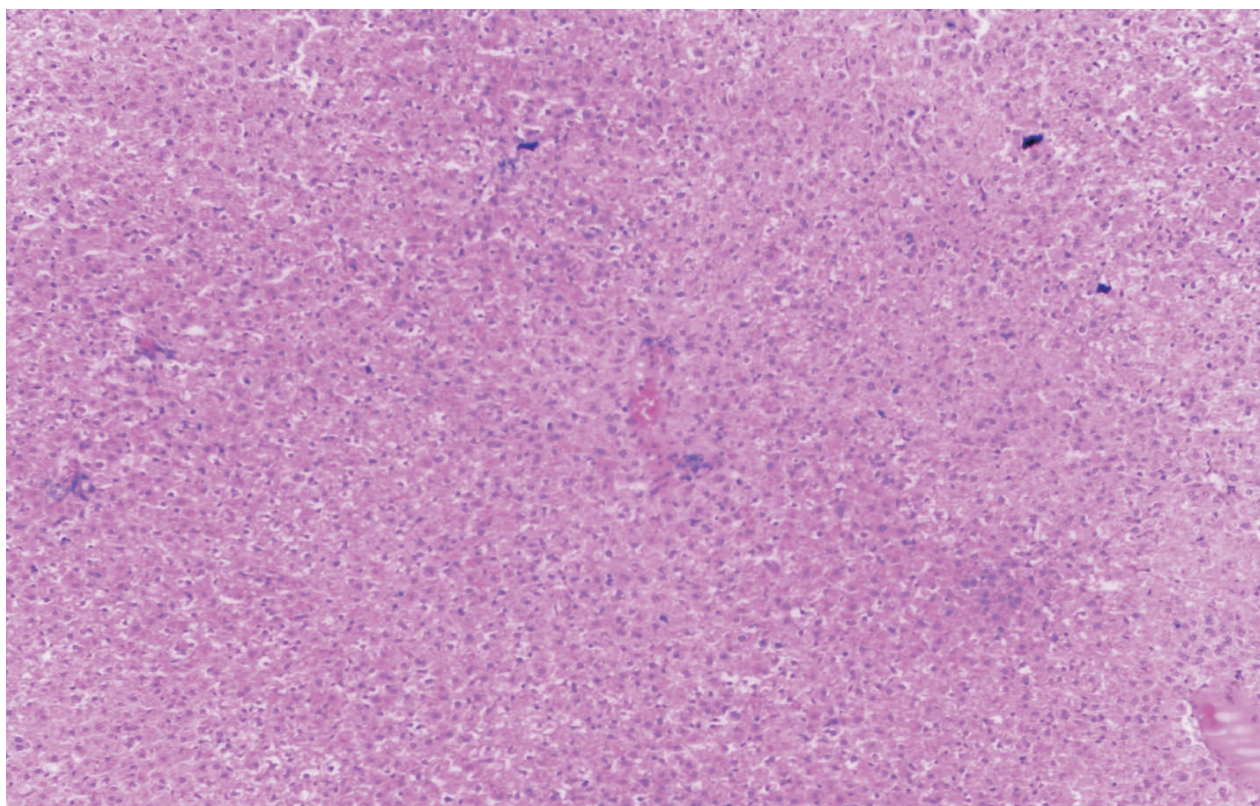


Figure 4. Liver section of CCl₄- intoxicated rats pretreated with HPEEE (500mg/kg b.wt.) showing kuffer cells activation (small arrow) and little mild vacuolation (H&E stain, Mag X100)

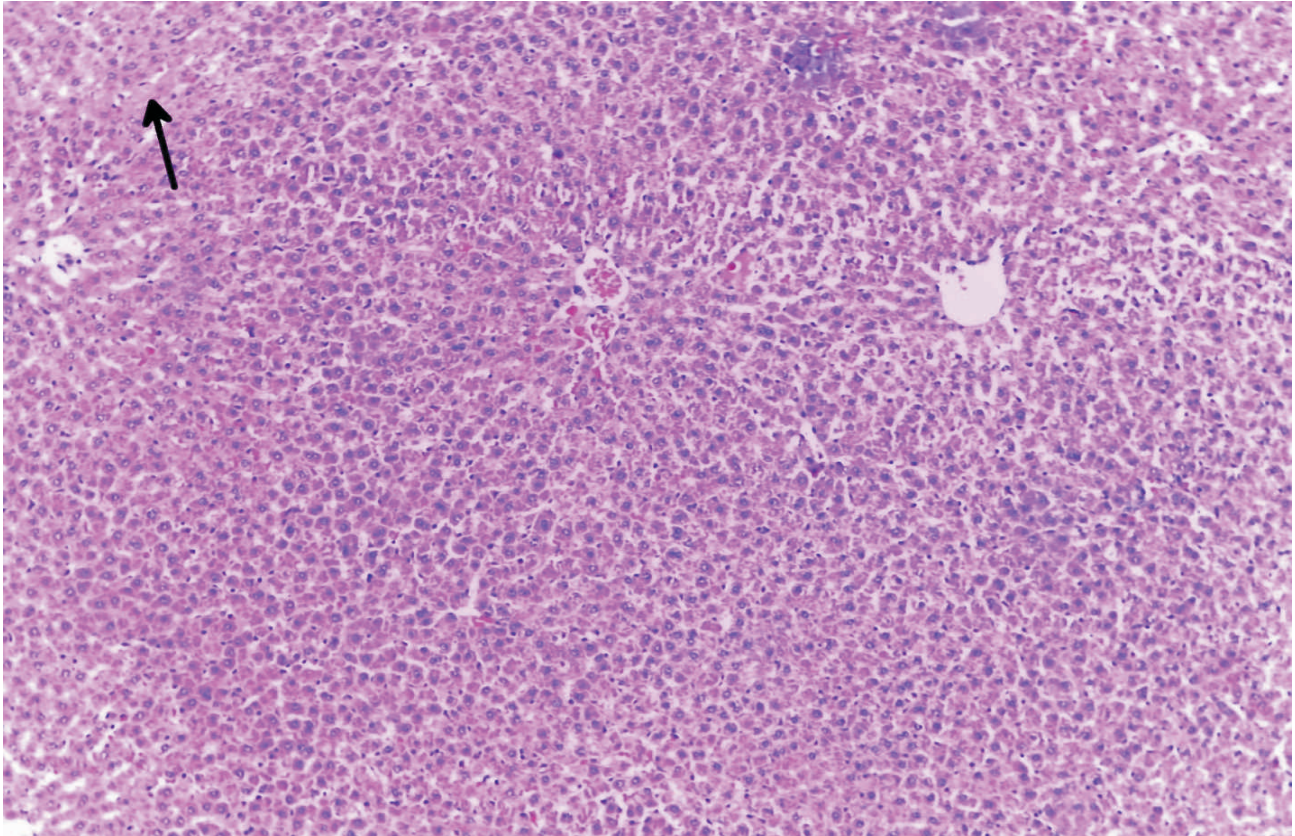


Figure 5. Liver section of CCl₄- intoxicated rats pretreated with standard drug showing kuffer cells activation, and mild vacuolation (H&E stain, Mag X100)

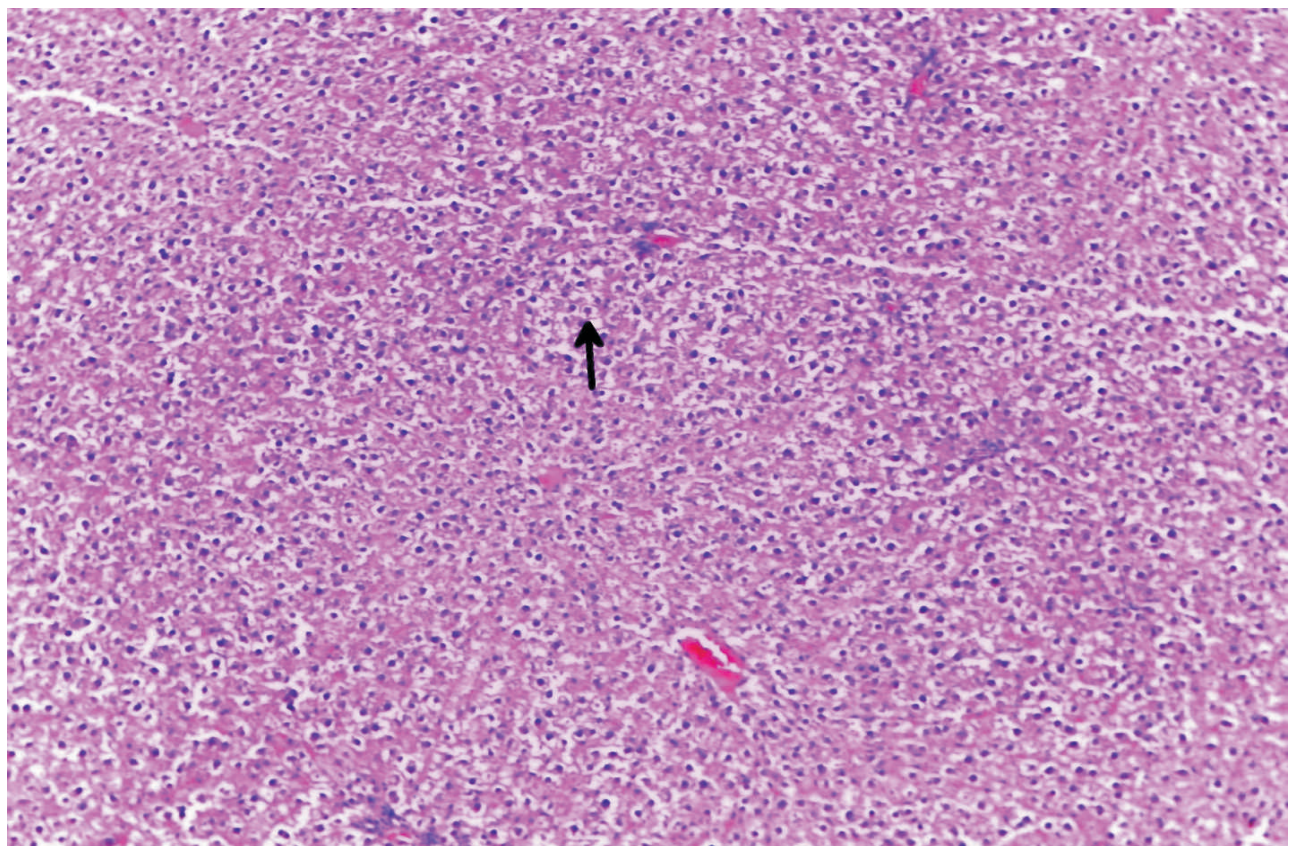


Figure 6. Liver section after administering HPEEE (250mg/kg b.wt.) (H&E stain, Mag X100)

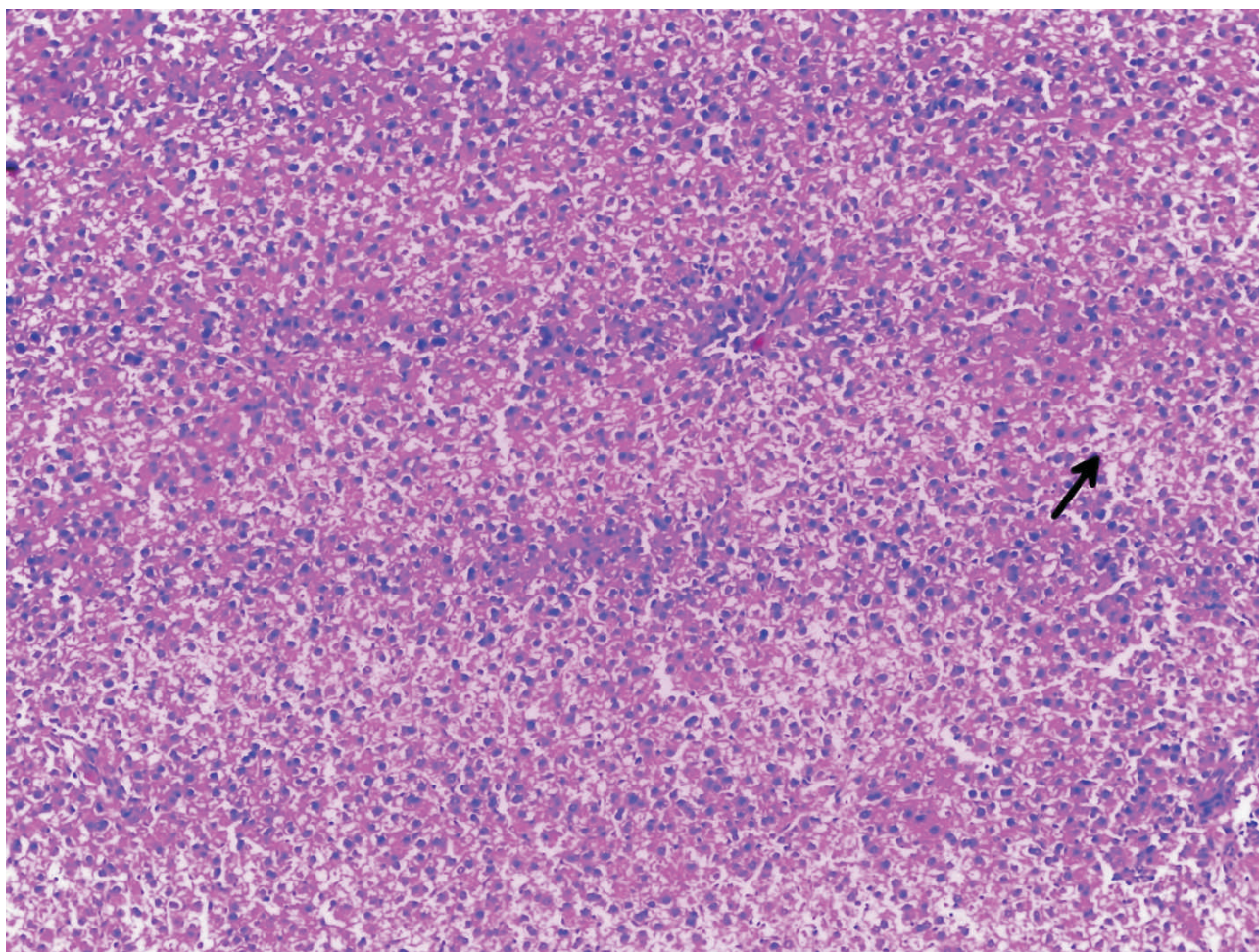


Figure 7. Liver section of after administering HP EEE (500mg/kg b. wt.) (H&E stain, Mag X100)

Phytochemical Screening Result

Table 4.1: Qualitative Phytochemical Analysis of Ethanolic Extract of Calyces of Red and Green Varieties of *H. sabdariffa*

Test	Ethanolic extract of red Variety	Ethanolic extract of green Variety
Flavonoid	+	+
Saponin	-	-
Glycoside	-	-
Alkaloid	+	+
Tannin	+	+
Terpenoid	+	+

Key: (+) =present (-) = absent

The result for the qualitative phytochemical analysis of ethanolic extract of calyces of red and green varieties of *H. sabdariffa* is presented in table 4.1. It indicates the presence of alkaloid, terpenoid, tannin and flavonoid in both varieties while saponin and glycoside are absent in both varieties.

Table 4.2: Total Flavonoid, % Alkaloid, Tannin and Vitamin C Contents of Ethanolic Extract of Calyces of Red and Green Varieties of *H. sabdariffa*.

Compounds (n=3)	Red variety contents	Green variety contents
Total flavonoid (mg/g)	2.21 ± 0.00 ^a	1.22 ± 0.10 ^a
Percentage alkaloid (%)	15.19 ± 0.20 ^a	24.99 ± 0.10 ^a
Tannin content (mg/g)	8.11 ± 0.30 ^a	1.22 ± 0.10 ^a
Vitamin C content (mg/g)	0.16± 0.00 ^a	0.042± 0.00 ^a

The values are expressed as Mean ± Standard Deviation of triplicate determination. Values in the same rows with the same superscript are significantly different ($p < 0.05$).

The results for the concentration of flavonoid, % alkaloid, tannin, and vitamin C of ethanolic extract of calyces of the red and green varieties of *H. sabdariffa* were presented in Table 4.2. There was a significant increase ($p < 0.05$) when the alkaloids content of the green variety ($24.99 \pm 0.10\%$) was compared to that of the red variety ($15.19 \pm 0.20\%$). The flavonoids and tannin contents of the red variety was found to be significantly higher ($p < 0.05$) than that of the green variety, with the red variety having 2.21 ± 0.00 mg/g and 8.11 ± 0.30 mg/g while the green variety having 1.22 ± 0.10 mg/g and 1.22 ± 0.10 mg/g respectively. Also, the concentration of vitamin C of the green variety (0.04 ± 0.00 mg/g) significantly lower ($p < 0.05$) when compared to that of the red variety (0.16 ± 0.00 mg/g).

Discussion

The present study revealed the ameliorative effect of *Hibiscus sabdariffa* calyx extract on CCl_4 induced liver toxicity. Liver diseases reduce people's quality of life and frequently lead them to death. Jaeschke (2008) reported that occupational exposure to chemical compounds like aliphatic hydrocarbons alters the liver structure and functions. CCl_4 is used as a hepatotoxic agent in animal research work to study the hepato-curative agent in plants and other compounds (Aliyu *et al.*, 1995). *Hibiscus sabdariffa* Linn. (HPEEE) is a valuable source of traditional medicine (Ubani *et al.*, 2010). In the present study, serum hepatic biomarkers, AST and ALT activities were greatly increased ($p < 0.001$) in rats with the CCl_4 treatment compared

to control. Similarly, the elevation of serum ALT and AST is a known effect of CCl_4 toxicity which specifically affects the liver (Anand *et al.*, 1992). The increased serum levels of hepatic markers have been attributed to the liver injury, because these enzymes are placed in the cytoplasmic area of the cell and are released into circulation in the case of cellular damage (Brent and Rumack, 1993). Reports indicated that these enzyme activities were significantly elevated after CCl_4 treatment (Arici and Cetin, 2011; Yin *et al.*, 2011; Mehmetcik *et al.*, 2008). On the other hand, decrease in the serum levels of AST, ALT and ALP enzymes with HPEEE is an indication of the stabilization of plasma membrane as well as repair of liver damage caused by CCl_4 . Dahiru *et al.* (2003) stated that the levels of liver enzymes ALT, AST, in the serum were lower when CCl_4 treated rats are given *Hibiscus sabdariffa* calyx extract than in untreated CCl_4 control which is evidence that the extract promotes healing of peroxidised liver. This observation is in agreement with the view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Jaishree and Badami, 2010). Administration of 500mg/kg extract was more effective in lowering the enzymes level compared to 250mg/kg in line with a previous report (Hashemi, 2014). Our finding is however at variance with a previous report by Dahiru *et al.* (2003). It was earlier suggested that the extract at higher doses, and when given for a long period could be toxic (Akindahunsi and Olaleye, 2002). Akanya *et al.*, (1997) reported that the protective effect of the extract could be due to the rich Vitamin C content of the extract which serves as an antioxidant and a reductant, especially in the conversion of any α -tocopheroxyl radicals formed, to α -tocopherol. The presence of

Hibiscus protocatechuric acid (phenol) and Hibiscus anthocyanins both isolated from the flower were reported to have protective effect against tart butyl hydroperoxide induced hepatic toxicity in rats (Wang *et al.*, 2000; Tseng *et al.*, 1996). The present results revealed significant elevation in serum TC and TG levels in CCl₄ intoxicated rats, as compared to control rats. These results are in agreement with the previous reports (Al-Dosari, 2010; El-Habibi *et al.*, 2009; Hashemi, 2014). Treatment of rats with hibiscus sabdariffa calyx extract resulted in significant improvement in the tested lipid profile parameters.

Liver biopsy indicated damage in the CCl₄ treated group. Previous findings revealed that CCl₄ causes necrosis (Sun *et al.*, 2001), fibrosis (Natsume *et al.*, 1999), mononuclear cell infiltration (Natsume *et al.*, 1999), steatosis and degeneration of hepatocytes, increase in mitotic activity (Teocharis *et al.*, 2001) and cirrhosis (Zalatnai *et al.*, 1991) in the liver. Sun *et al.* (2001) reported that CCl₄ causes apoptosis in liver. Therefore, liver damage as a result of administration of CCl₄ are in agreement with previous studies. Treatment with *Hibiscus sabdariffa* calyx extract improved the structure of the hepatic cells. Liu *et al.* (2006) reported that this protective effect appears due to *Hibiscus sabdariffa* calyx extract antioxidant properties.

The qualitative analysis on the red and green varieties of *Hibiscus sabdariffa* calyces revealed the presence of tannins, alkaloids, flavonoids and terpenoids in the ethanolic extract of both varieties. Both calyces do not show the presence of Saponins and glycosides (Table 4.1). The various phytochemicals detected are known to have beneficial importance in industries and medicinal sciences. Phytochemical screening test is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth (Mungole and Chaturvedi, 2011).

Flavonoids are well known for their anti-viral, anti-inflammatory, antioxidant activity, cytotoxic and also used in the treatment of

hypertension, diabetes and rheumatic fever (Akambi *et al.*, 2009). *H. sabdariffa* shows the presence of flavonoids in the calyces, it may be of use to cure the above- mentioned disorders and as antioxidant agent.

Alkaloids are basic natural products occurring primarily in plants. They occur as one or more heterocyclic nitrogen atoms and are generally found in the form of salts with organic acids. Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents (Ocho-Anin *et al.*, 2010).

Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism. Tannins play important role such as potent antioxidant (Tom *et al.*, 2013). Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). It is clear that calyces of *H. sabdariffa* possess good phytoconstituent that would be helpful in future for the cure of different types of diseases.

Conclusion

It was found that CCl₄ caused toxicity and *Hibiscus sabdariffa* calyx extract was effective in the treatment of CCl₄ induced hepatotoxicity in rats. Histopathological analysis shows the level of damage caused by administration of CCl₄ as well as the effectiveness of *Hibiscus sabdariffa* calyx extract in clearing the damage.

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