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Effects of methanolic calyx extract of *Hibiscus sabdariffa* on body weight, blood cholesterol and liver marker enzymes in Wistar rats

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Eighty male albino rats were used to investigate the effects of methanolic calyx extract of *Hibiscus sabdariffa* on body weight, blood cholesterol and liver enzymes markers. Twenty-eight days oral administration of 100, 200, 400 and 800 mg/kg body weight of the extract showed a significant ($p<0.05$) time-dependent decrease in the body weights of all the treated groups when compared with the control, with 100 mg/kg causing significant ($p<0.05$) decrease at weeks 2 and 4, respectively when compared with other treatment doses. The extract significantly ($p>0.05$) decreased the serum cholesterol and increased the liver marker enzymes (ALP, ALT and AST) in dose-dependent and time-dependent manner, when compared with the control. However, on day 21, the group treated with 400 mg/kg showed a significant ($p<0.05$) increase the serum cholesterol, and decrease in liver marker enzymes when compared with the rest of the treatment groups. Histopathology from all the treatment groups revealed graded degrees of vacuolar degeneration of the hepatocytes and peri-portal infiltration of mononuclear leucocytes. The results of this present study suggests that the methanolic calyx extract of *H. sabdariffa* possesses anti-obesity and hypocholesterolemic potentials which should be harnessed with caution due to its tendency to adversely affect the liver.

Key words: *Hibiscus sabdariffa*, hypocholesterolemia, anti-obesity, liver maker enzymes, Wistar rats.

INTRODUCTION

Hibiscus sabdariffa (family Malvaceae) is an herb cultivated for its leaf, fleshy calyx, seed or fibre (Dalziel, 1973). The dried flowers of *H. sabdariffa* are used as a local juice by Nigerians (Usoh et al., 2005) while its floral parts serve as colourant in food industries (El-Meleigy, 1989). The broad usefulness of this plant as a food agents and herb has attracted the interest of researchers in the last two decades.

In most body tissues such as blood, bile and brain tissues, cholesterol is the main lipid found which according to research is the key lipid linked with arteriosclerotic vascular diseases. It is also required for steroids and cellular membranes formation. Although the liver metabolizes, the cholesterol increased levels are

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found in hypercholesterolaemia, hyperlipidaemia, hypothyroidism, uncontrolled diabetes, nephritic syndrome, and cirrhosis. Whereas decreased levels are found in malabsorption, malnutrition, hyperthyroidism, anaemia and liver diseases (Sood, 2006).

Research has revealed that aqueous extract of red and green *H. sabdariffa* petals caused a significant decrease in the LDL-cholesterol levels while no significant effect was observed on HDL-cholesterol and triglycerides (Olatunji et al., 2005). Carvaja-Zarrabal et al. (2009) and Nnamonu et al. (2013) research findings showed that *H. sabdariffa* calyx aqueous extract at intermediate and greater concentrations could be considered possible anti-obesity agents. Chen et al. (2003) reported of its anti-atherosclerotic property.

As reported by Wang et al. (2000), anthocyanin performs more activities than other antioxidants like ascorbate. In related researches, Olaleye (2007), Powers (1999) and Jonadet et al. (1990) reported that anthocynin were cardioprotective, while Olaleye (2007) Chen et al. (2003), Powers (1999) and Nnamonu et al. (2013) opined that *H. sabdariffa* possesses hypocholesterolemic property. The results of Amin and Hamza (2005) and Wang et al. (2000) revealed that *H. sabdariffa* possesses hepatoprotective and anti-oxidative effects on experimental animals. It has also been reported that, a *Hibiscus* anthocyanin induced apoptosis in human leukemia cells through oxygen reactive species-mediated mitochondrial pathway. Hou et al. (2005) reported apoptosis was induced in human leukemia cells by one anthocynin in Hibiscus (Delphinidin-3-sambubioside), Brunold et al. (2004) discovered differentiation and proliferation of human keratinocytes was initiated by polysaccharides taken from the flowers *H. sabdariffa.*

The aim of this present study was to evaluate the effects of methanolic calyx extract of *H. sabdariffa* on body weight blood cholesterol and liver marker enzymes of albino rats.

MATERIALS AND METHODS

Collection and preparation of *H. sabdariffa* calyx extract

Fresh calyces of *H. sabdariffa* were bought directly from Kalaah farm at Mubi, Adamawa State, Nigeria. The calyces were identified and validated by a botanist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, using the identification key of Morton (1987).

Preparation of *H. sabdariffa* calyx extract

The plant materials were air-dried at room temperature. The dried plant materials were subsequently pulverized into a fine powder. One hundred and thirty-five grams of the powdered plant material was extracted with 500 ml of 80% methanol for 72 h. The set up was agitated every 2 h in order to ensure thorough and homogeneous extraction. The filtrate was dried in a Rotary evaporator and the resultant extract stored at 4°C throughout the experiment. Percentage yield was calculated using the formula:

$$\% \text{ Yield (w/w)} = a / b \times 100$$

where a is the weight of extract and b is the weight of the plant material.

Procurement and management of experimental animals

Eighty adult male albino rats weighing between 120 and 295 g were procured from the Genetics and Experimental Animal Breeding Laboratory of Zoology and Environmental Biology Department, University of Nigeria, Nsukka. The rats had no history of drug consumption (that is, they have not been used for any investigation). They were kept in stainless wire rat cages equipped with drinkers and fecal collecting trays, in a clean and fly proof experimental animal house. The rats were fed with commercial growers chick mash (18 % crude protein) made by Vital Feeds, Nigeria Limited and clean drinking water. They were allowed to acclimatize 14 days before the start of the experiment. The rats were allowed unhindered access to food and water. The fecal droppings in the tray were removed daily.

Experimental design:

Eighty adult male albino rats were randomly assigned to five groups of sixteen rats each and kept according to their groups in stainless wire rat cages. Group I served as the control. They received commercial growers chick mash (18% crude protein) and water. Groups II to V represented the experimental groups. They were fed commercial growers chick mash (18% crude protein), water and the extract daily. Groups II, III, IV and V were orally administered 100, 200, 400 and 800 mg/kg body weight of the methanolic calyx extract of *H. sabdariffa*, respectively for 28 days.

Determination of body weight and weight loss

All the rats were weighed using a Mettler, electronic balance PC 2000 at day 0. Four rats from each group were randomly selected and weighed before collection of blood samples at days 7, 14, 21 and 28. The weight values obtained at days 7, 14, 21 and 28 of the experiment were subtracted from the weight of the each rat at day 0 in order to enable us monitor the effect of the extract on the experimental animals.

Collection of blood sample

About 5 ml of the blood samples was collected from each of the anaesthetized rats using the ocular puncture method described by Hoff (2000). The samples were allowed to clot for about 30 min and subsequently centrifuged at 2000 rpm for 10 min. The sera obtained were used to estimate the levels of total cholesterol, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Determination of liver marker enzymes parameters

Serum AST and ALT were determined using method of Reitman and Frankel (1957). The serum ALP was measured using the method of King and Armstrong (1934) and total cholesterol determined using the method of Roeschlau et al. (1974). All liver marker enzymes were measured using Randox commercial enzyme kit.

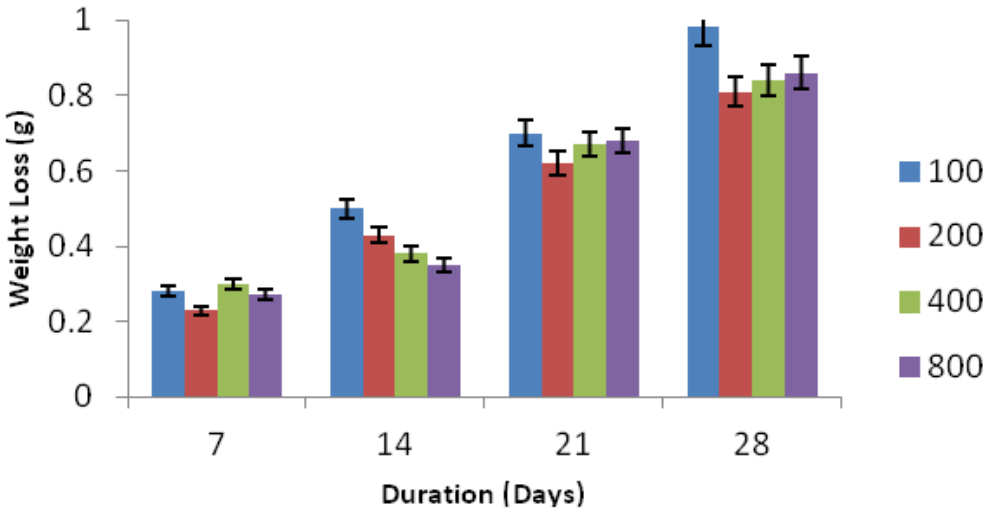

Figure 1. Effects of *H. sabdariffa* on the body weight showing time-dependent decrease in the body weights of all the treated groups when compared to the control.

Statistical analysis

The computer software, statistical package for social sciences (SPSS) version 20.0 for windows was used for the statistical analysis. The data obtained were subjected to one-way analysis of variance (ANOVA) and the differences in means between groups separated using Duncan's New Multiple Range Test. The results were presented as the mean ± standard error of the mean (SEM). Differences in the means were considered significant at the probability values less than 5% ($p<0.05$).

RESULTS

The effect of methanolic extract of H. sabdariffa on body weight was presented in Figure 1. The result showed a significant ($p<0.05$) time-dependent decrease in the body weights of all the treated groups when compared with the control, with 100 mg/kg causing significant ($p < 0.05$) decrease at weeks 2 and 4, respectively when compared with other treatment doses.

Figure 2 shows the results the effects of the extract on serum cholesterol. It was observed that the extract significantly ($p<0.05$) precipitated both dose-dependent and time-dependent decrease in the serum cholesterol as compared to the control, except at the dose of 400 mg/kg by day 21 where a significant ($p<0.05$) increase in the serum cholesterol was observed.

The effect of methanolic extract of H. sabdariffa on the liver marker enzymes is shown in Table 1. The result showed that beyond day 7, the extract elicited a concentration and time dependent significant ($p>0.05$) increase in the liver enzyme markers as compared to the control, except a slight variation at day 21, where 400 mg/kg of the extract significantly ($p>0.05$) reduced the ALT and AST values.

Further evidence from the histopathology of the liver showed that sections from the untreated group (control) had normal hepatic lobules with cords of normal hepatocytes radiating around a central vein and the bile duct, hepatic artery and the portal vein situated at the periphery of the hepatic lobules (Figure 3). In the treatment groups, histopathological changes were observed in all the different dose groups from the first week to the fourth week. In the groups treated with 100 and 200 mg/kg body weight of the extract, a moderate multifocal hepatocellular vacuolar degeneration with infiltration of mononuclear and polymorphonuclear leucocytes into the periportal areas were observed at the end of the first week (Figure 4). Similar histopathological lesions with a consistency in severity was observed at the ends of the second, third and fourth week post treatment. However, there seemed to be a change in the inflammatory cell population, from polymorphonuclear to mononuclear cell dominated population.

In the groups treated with 400 and 800 mg/kg body weight of the extract, widespread vacuolar hepatocellular degeneration was observed, with multifocal areas of hepatocellular coagulative necrosis and infiltration of mononuclear and polymorphonuclear leucocytes were observed from week one to week four (Figure 5). A summary of the histopathological studies of the liver of rats treated with methanolic calyx extract of H. sabdariffa is shown in Table 2.

DISCUSSION

This study evaluated the effects of *H. sabdariffa* methanolic calyx extract on the body weight, cholesterol and liver marker enzymes activities of normal male albino rats. The decrease in the values on body weight when

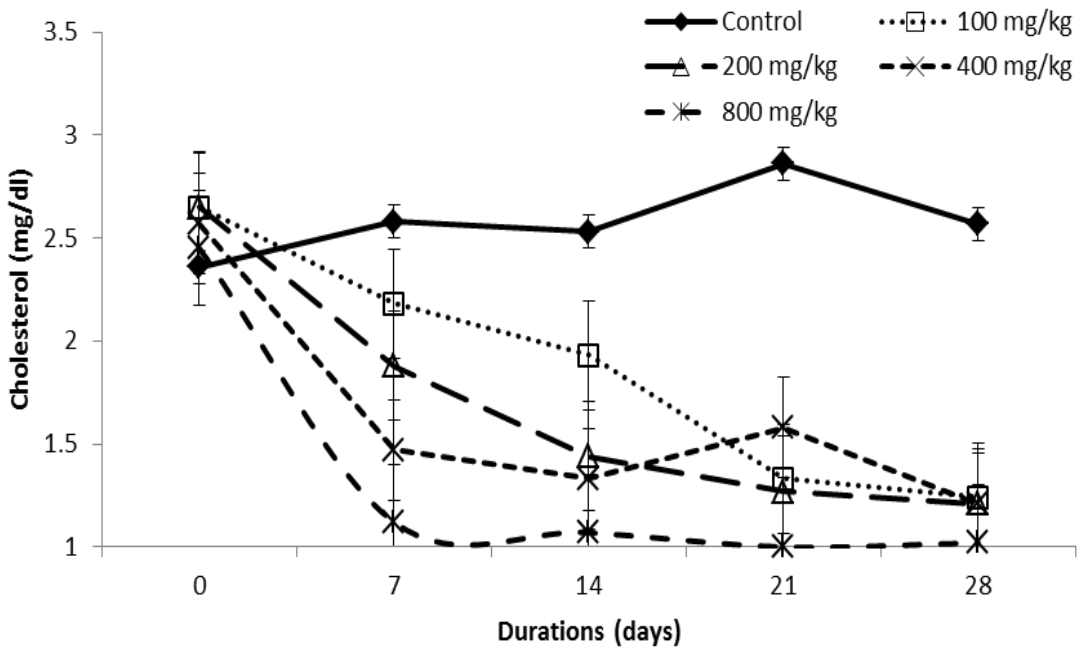

Figure 2. Effects of *H. sabdariffa* on the serum cholesterol showing dose-dependent and time-dependent decrease in the serum cholesterol as compared with the control.

Table 1. Effects of the methanolic extracts of *H. sabdariffa* on ALT, AST and ALP of albino rats.

Parameter	Concentrations (mg/kg)	Duration (Days)				
		0	7	14	21	28
ALT(U/L)	Control	4.00±0.99^{a1}	9.55±0.33^{a2}	9.00±2.31^{a2}	11.33±1.76^{a23}	14.67±1.45^{a3}
	100	4.00±0.99^{a1}	9.67±0.33^{a12}	13.67±3.84^{b2}	26.33±1.86^{b3}	31.00±3.51^{b3}
	200	4.01±0.99^{a1}	9.33±0.33^{a1}	20.33±3.33^{bc2}	35.33±1.20^{c3}	39.33±1.76^{bc3}
	400	4.01±0.99^{a1}	9.67±0.33^{a2}	25.00±2.31^{cd3}	9.67±0.33^{a2}	41.67±0.88^{c4}
	800	4.02±0.99^{a1}	9.33±0.33^{a1}	31.33±1.86^{d12}	43.67±0.88^{d3}	54.33±4.81^{d4}
AST(U/L)	Control	5.00±0.00^{a1}	7.67±0.33^{a12}	8.00±0.38^{a23}	10.00±1.73^{a23}	12.00±1.16^{a3}
	100	5.00±0.00^{a1}	7.33±0.33^{a12}	10.00±0.58^{b2}	16.33±1.20^{b3}	23.67±1.86^{b4}
	200	5.02±0.00^{a1}	7.67±0.33^{a1}	15.33±0.88^{c2}	27.33±0.88^{c3}	35.67±1.45^{c4}
	400	5.00±0.00^{a1}	7.33±0.33^{a2}	18.33±0.67^{c3}	8.33±0.33^{a2}	40.33±0.67^{d4}
	800	5.01±0.00^{a1}	7.67±0.33^{a2}	25.00±0.58^{d3}	37.00±1.00^{d4}	42.67±1.20^{d4}
ALP(U/L)	Control	20.01±0.01^{a1}	47.67±0.33^{a3}	26.67±0.33^{a2}	23.67±2.03^{a12}	26.67±3.33^{a2}
	100	20.01±0.01^{a1}	47.00±0.00^{a2}	33.33±1.76^{b2}	43.33±2.76^{b3}	58.33±6.39^{b4}
	200	20.01±0.01^{a1}	47.00±0.00^{a2}	45.33±1.73^{c2}	46.67±1.20^{b2}	67.67±4.33^{bc3}
	400	20.02±0.01^{a1}	47.67±0.33^{a2}	46.33±1.03^{c2}	47.67±1.33^{b2}	79.33±1.45^{cd3}
	800	20.01±0.01^{a1}	47.33±0.33^{a2}	53.00±3.51^{d23}	60.33±4.84^{c3}	86.33±1.20^{d4}

Values expressed as Mean ± SEM. Mean values in a column with different alphabets are significantly different (p=0.05). Mean values in a row with different figures are significantly different ($p < 0.05$).

compared with the treated and control groups suggests that *H. sabdariffa* possesses anti-obesity property. This result agrees with that of Carvajal-Zarrabal et al. (2009), who observed a drastic loss of weight among animals treated with various concentrations of *H. sabdariffa* extracts. In contrast however, Olatunji et al. (2005) observed no significant decrease in body weight among rats that were chronically treated with 25 and 50 mg/kg

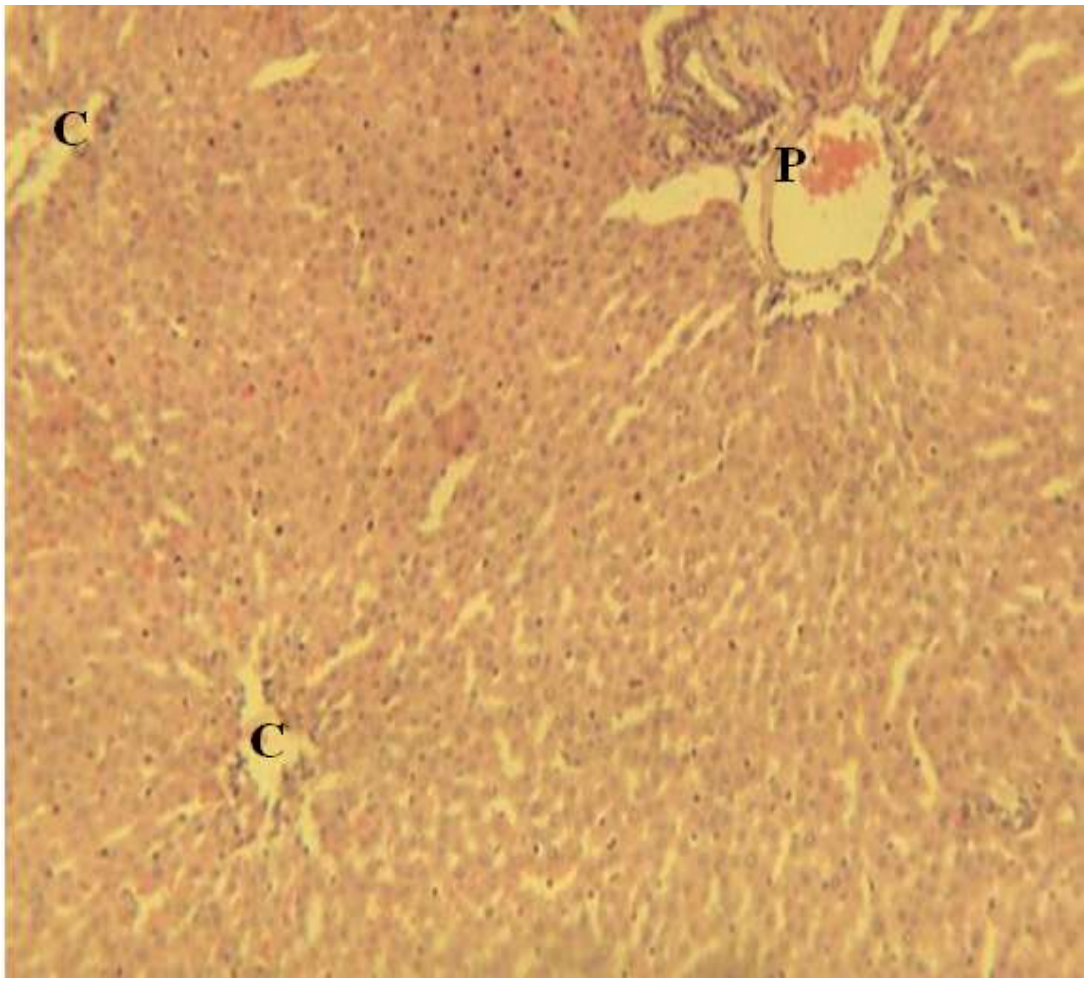

Figure 3. A photomicrograph of the liver from the control group with normal liver architecture. Central vein (C), Portal triad (P), H&E X 40.

body weight of *H. sabdariffa* extracts. Carvajal-Zarrabal et al. (2009) noted that such weight decreases might have been as a result of dietary palatability problem when *H sabariffa* concentration was increases. The loss of appetite in the treated animal models due to daily administration of *H. sabdariffa* extracts had previously been suggested (Abubakar et al., 2010; Orisakwe et al., 2003). This reduction in the body weight could also be directly attributed to the observed hypocholesterolemic property of this extract, owing to the fact that cholesterol significantly contributes to the total fat content of an individual.

The result on the effects of the extracts of *H. sabdariffa* on serum cholesterol showed that the extracts of *H. sabdariffa* are capable of dropping the serum cholesterol levels of treated rats on a dose and time dependent fashion. This observation on the hypocholesterolemic ability of *H. sabdariffa* is consistent with the report of Tzu-Li et al. (2007) who observed that the consumption of *H. sabdariffa* extracts can significantly decrease serum cholesterol levels in human beings. This view was corroborated by the results of Olatunji et al., (2005). Lin et al., (2007) also observed that the level of serum cholesterol among treated individuals decreased significantly. Similarly, Chen et al. (2003) reported on its anti-atherosclerotic property. This hypocholesterolemic effect has been attributed to its abundant antioxidant composition (Andersen et al., 2002).

Study of enzymological and biochemical profile of blood are commonly used as indicators to access the functional status of the animal health and the internal environment of the organism (Rehman et al., 2006). According to Sood (2006) an elevation above normal in the level of liver marker enzymes indicates damage or inflammation of the hepatocytes. The significant increase obtained on the liver marker enzyme tends to suggest liver dysfunction in the experimental animals (Wells et al., 1986). The observed elevation in the liver marker enzymes after day 7 is consistent with earlier reports which showed that a prolonged usage of *H. sabdariffa*

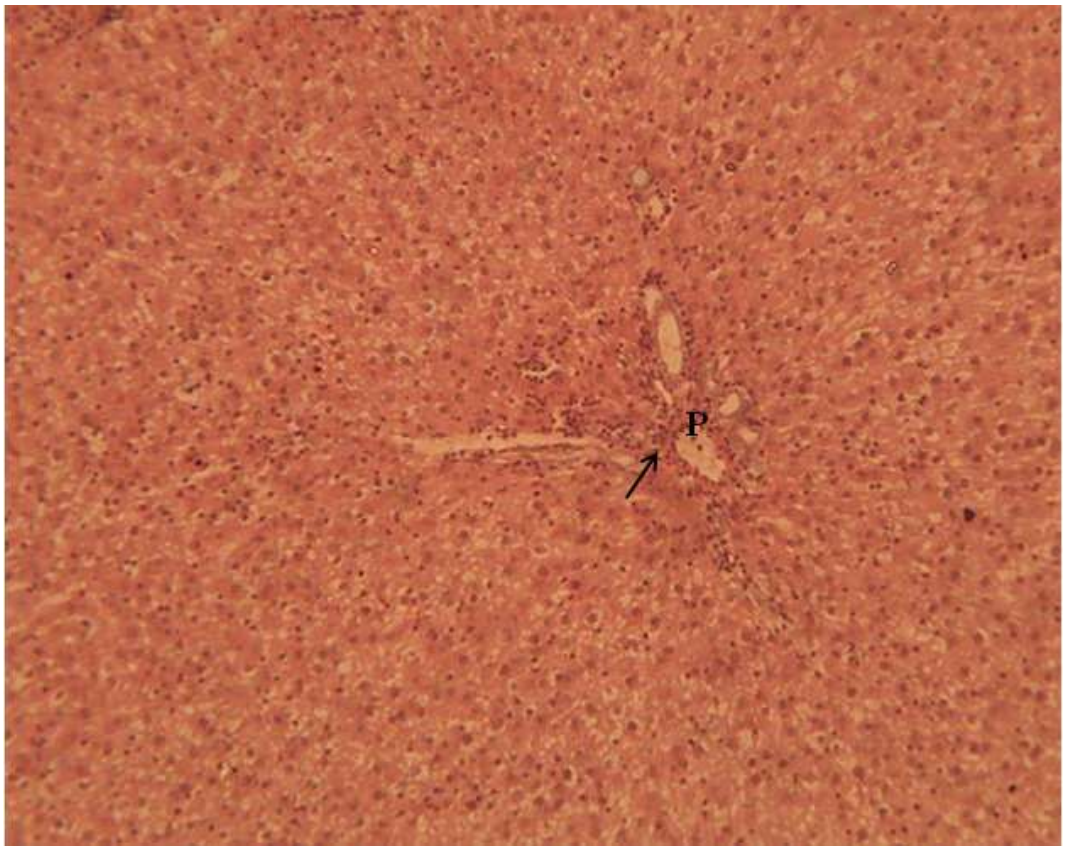

Figure 4. A section of the liver from group 2 and 3 (100 and 200 mg/kg) showing a moderate widespread vacuolar degeneration of the hepatocytes and moderate periportal infiltration of mononuclear leucocytes (arrow). P: Portal area; H&E x100

Table 2. A summary of the histopathological studies of the liver of rats treated with methanolic calyx extract of *Hibiscus sabdariffa*.

Week	Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
1	-	+	+	++	++
2	-	+	+	++	++
3	-	+	+	++	++
4	-	+	+	++	++

+: Mild lesion; ++: Moderate lesion; -: No visible lesion.

aqueous and methanolic calyx extracts could cause liver injury in experimental animals even at dose levels as low as 150 to 180 mg/kg (Akindahunsi and Olaleye, 2003; Ali et al., 2005). This supports the findings of Morton (1987) who reported that *H. sabdariffa* calyx had been analyzed to contain phytic acid, tannin and glycosides such as delphinidin-3-monoglucoside and delphinidin which are toxic to animal and human tissues at high doses. The present observation however is at variance with report of Prommetta et al. (2006) who observed that doses of *H. sabdariffa* ranging from 250 to 1000 mg/kg/day, did not elicit any adverse effect on several important organs such as liver, kidney and the blood system. Reports abound on the hepato-protective effects of *H. sabdariffa* extracts (Tseng et al., 1997; Farombi, 2003). The observed increase in the activities of these marker enzymes following the administration of *H. sabdariffa* extracts may be a unique adaptation by the liver to the assault from the plant extract or as a result of fresh synthesis of the enzyme molecules following extract administration (Yakubu et al., 2007). It seems also plausible that the effect of the extracts on the activities of Aspartate and

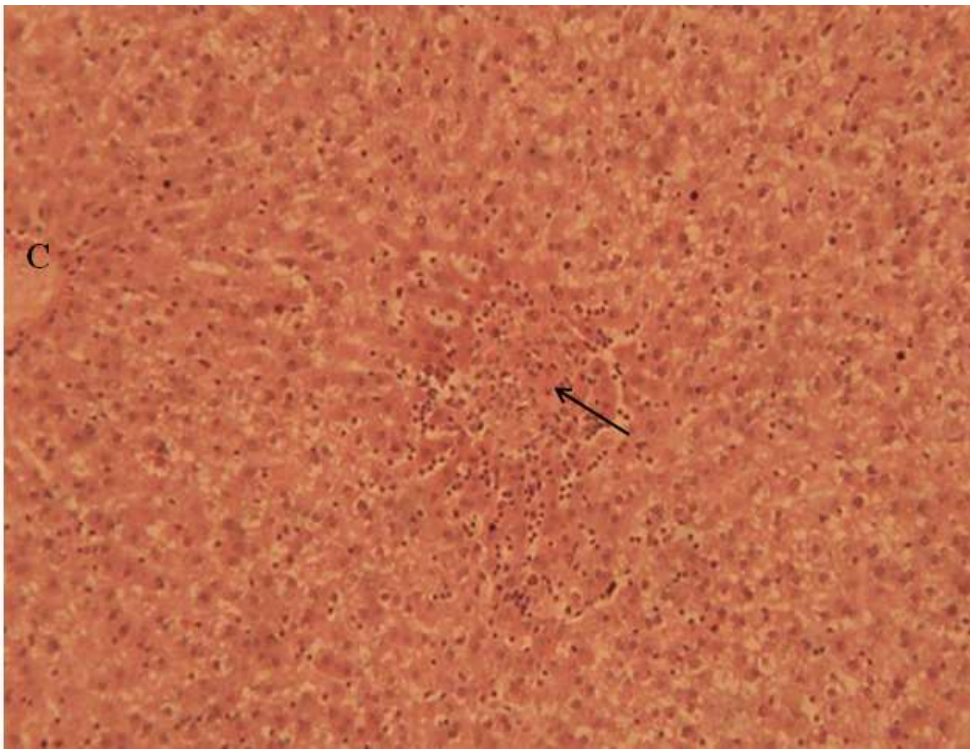

Figure 5. A section of the liver from group 4 and 5 (400 and 800 mg/kg) showing a widespread vacuolar degeneration of the hepatocytes and a focus of coagulative necrosis infiltrated by mononuclear leucocytes (arrow) in the midzonal area. C: Central vein. H&E X100

Alanine Aminotransferase may be the case of organ chain reactions. This is more so as the two enzymes have been found to be localized normally within the cells of the liver, heart, kidney, gill, muscle and other organs (Orisakwe et al., 2003). Regardless of the fact that these enzymes have been reported as important markers in assessing and monitoring liver damage, there is the need to correlate the mechanisms of the extract in the different tissues. This is a vital research area because major toxic effects of *H. sabdariffa* extracts have been reported in the kidneys and reproductive organs of male rats (Orisakwe et al., 2003).

The liver showed varying histopathological changes in hepatic histo-architecture ranging from inflammatory to degenerative and necrotic changes. Groups treated with 100 and 200 mg/kg body weight showed mild lesions from the first week to the fourth week; while the groups treated with 400 and 800 mg/kg body weight showed more severe lesions from the first week to the last week of the study. This revelation explains the basis for the increase in the serum ALT, AST and ALP.

Conclusion

The ability *of H. sabdariffa* extract to lower the total cholesterol level and body weight suggests its usefulness as a potential hypocholesterolemic and anti-obesity agent. However, its ability to cause an increase in the levels of liver marker enzymes (ALP, AST and ALT) tends to suggest a dysfunction in the coordinating physiology of the liver; hence, caution should be applied in its consumption. Alternatively, purification and extraction of the active hypocholesterolemic and anti-obesity principles can be made in order to exclude the hepatotoxic principle(s).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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