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*Short Communication*

# Effect of Methanolic Extract of *Hibiscus sabdariffa* in Ethanol-Induced Hepatotoxicity

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**ABSTRACT**

The liver is one of the most important organs in the body which aids in the maintenance of normal body functions. It is the center of alcohol metabolism and alcohol has been implicated in the elevation of liver enzymes and damage to the parenchyma cells of the liver. Plants had been used for medicinal purposes long before recorded history and many people worldwide rely on herbal medicines for some part of their primary health care. The objective of this study was to evaluate the activity of *Hibiscus sabdariffa* on the liver of rats following repeated administration of ethanol. Hepatotoxicity was induced on the rats using ethanol and the levels of serum enzymes such as serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), total and conjugated bilirubin were estimated. The levels of hepatic enzymes ( SGOT, SGPT and bilirubin) in the serum were significantly increased ($P < 0.05$) in rats treated with ethanol when compared with the control ( Pre phase). In the post phase, administration of different doses of ethanol extract significantly reduced the levels of increased hepatic serum enzymes when compared with the control. Methanolic extract of *Hibiscus sabdariffa* seems to have hepatoprotective effect on ethanol induced liver damage. However, similar study needs to be carried out on human to prove the authenticity of hepatoprotective effect of Hibiscus Sabdariffa.

Keywords; *Hibiscus Sabdariffa*, hepatoprotective, SGOT, SGPT and bilirubin.

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## INTRODUCTION

The liver plays a central role in the metabolism and excretion of xenobiotics which makes it highly susceptible to their adverse and toxic effects. Hepatotoxicity refers to liver dysfunction, liver injury or liver damage that is associated with an overload of drugs, alcohol or xenobiotics (Navarro and Senior, 2006). An association between liver disease and heavy alcohol consumption was recognized more than 200 years ago (Smart and Mann, 1992). Long-term heavy alcohol use is the most prevalent single cause of illness and death from liver disease (National Center for Health Statistics. Health, United States, 1993). The liver is particularly susceptible to alcohol-related injury because it is the primary site of alcohol metabolism. As alcohol is broken down in the liver, a number of potentially dangerous by-products are generated, such as acetaldehyde and highly reactive molecules called free radicals. These products contribute to alcohol-induced liver damage. Elevations in serum enzyme levels (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase) were taken as indicators of liver injury, whereas increases in both total and conjugated bilirubin levels were measures of overall liver function (Rosalski and McIntyre, 1999).

*Hibiscus sabdariffa* is a shrub belonging to Malvaceae family. It is called roselle in English and zobo in Nigeria. *Hibiscus sabdariffa* is an erect, mostly branched, annual shrub. Stem reddish in colour and up to 3.5 m tall, with a deep penetrating tap root. The leaf is reported to contain protein, fat, carbohydrate, fiber, ash, calcium, phosphorus, iron, thiamin, β-carotene, riboflavin, niacin and ascorbic acid (Dake and Atchley, 1984). *Hibiscus sabdariffa* has many medicinal applications. For example, in China it is used to treat hypertension, pyrexia, liver damage and leukaemia due to its high content of protocatechuic acid (Tseng et al, 2000). Roselle can prevent cancer, low blood pressure, improve the digestive system in human and used as an effective treatment for patients

with kidney stone due to its uricosuric effect (Muhammad and Shakib, 1995). Many studies have been carried out on roselle such as its antihypertensive (Bako and Dawu, 2009), anticancer and antioxidant (Chang and Huang, 2009) activities but this study was to determine the hepatoprotective effect of *Hibiscus sabdariffa* on the liver of rats following repeated administration of ethanol.

## MATERIALS AND METHODS

**Collection and Authentication of plant materials.:** Fresh plants of *Hibiscus sabdariffa* used in this study were commercial samples purchased from the metropolitan market in Kaduna, Nigeria. The calyces were subsequently identified and authenticated by Mr. A. Ozioko of the Bioresources Development and Conservation Programme centre, University of Nigeria, Nsukka.

**Preparation of plant extract.:** The floral parts of *Hibiscus sabdariffa* was sun dried and mechanically reduced to fine powder. The fine powder was extracted by soaking in methanol for 48 hours. The extracts were filtered using Whatman filter paper no. 1 followed by evaporation at 45°C in a water bath and subsequently air dried.

**Phytochemical Analysis.:** The standard methods of Trease and Evans (1989) were used in the analysis of the phytochemical components of floral parts of Hibiscus Sabdariffa. The constituents analysed for were alkaloids, saponins, tannins, anthraquinones, cardiac glycoside, steroids and flavonoids.

**Experimental Animals.:** The animal study was conducted in accordance with the protocols approved by the local experimental animal ethics committee. Adult male Wistar rats (weight = 134 ± 5g) bred at the animal house of the Department of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, were housed in clean stainless steel cages and in well ventilated facility with free access to standard feed and drinking tap water. The animals were kept at room temperature (28±2°C) with a 12 h daylight/ dark cycle under humid tropical conditions for a period of 2 week.

**Acute Toxicity study.:** The acute toxicity study was carried out following the method described by Lorke (1983) The groups were continuously observed for mortality and behavioral changes during the first 24hours and then daily for 2 weeks.

**Hepatoprotective Activity:** Male wistar rats were divided into five groups having six animals in each. All animals received 20% ethanol every other day. In addition, while animals in group 1 (negative control) were administered with 20% ethanol and distilled water, the animals in the treatment phase (groups II-V) also received 20, 40, 80 and 160 mg/kg body weight of the extract respectively. All substances were administered by gastric intubation daily for six weeks. During this period the animal were observed daily for clinical and toxic signs. Blood samples were collected from each animal before the commencement of dosing (day 0) as the pre reading (basal reading) and also at the end (day 42) as the post reading for biochemical serum analysis. The blood samples were allowed to clot after standing for 10 min. at ambient temperature. Thereafter, the serum was separated by centrifugation at 3000 x g for 10 min. The serum was aspirated individually and stored in deep freezer for Biochemical assays.

**Biochemical assays:** Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) were determined using colorimetric method (Reitman and Frankel, 1957), serum alkaline phosphatase using Bessy-Lowry method and serum bilirubin (total and conjugated) using Powel method (Powel, 1994).

**Statistical analysis.:** The results were expressed as mean ± standard error mean. The statistical significance was assessed using one way analysis of variance (ANOVA)

## RESULTS.

**Phytochemical Analysis.:** The result of phytochemical screening of the methanolic plant extract of *Hibiscus sabdariffa* leaves revealed the presence of saponins, tannins, cardiac glycosides, flavonoids steroidal ring, steroid and terpenes. Alkaloids and anthraquinones were however absent.

**Acute Toxicity.:** The animals were generally dull and still after administration of extract, but became normal after about 30 minutes to 1 hour. No death was also recorded in any of the treatment groups. Hence the $LD_{50}$ of *Hibiscus sabdariffa* was estimated to be greater than 2000mg/kg body weight.

**Hepatoprotective Activity.:** The result of hepatoprotective activity of methanolic extract of *Hibiscus sabdariffa* on ethanol treated rats is summarized in table 1. In the pre phase, the hepatic enzymes SGOT, SGPT and bilirubin in serum were significantly increased ($p < 0.05$) in ethanol treated rats when compared with the control. In the post phase, the hepatic enzymes SGOT, SGPT and bilirubin in serum were significantly reduced in ethanol and *Hibiscus sabdariffa* treated rats when compared with the control.

## DISCUSSION

Medicinal plants are widely used by the populations of underdeveloped countries as an alternative therapy (Bako and Dawud, 2009). In Africa, hundreds of plants are used traditionally for the management and/or for enhancing the liver function. Unfortunately, only a few of such African medicinal plant have received scientific scrutiny (Bako and Dawud, 2009). The result of this present study showed that methanolic extract of *Hibiscus sabdariffa* had a hepatoprotective effect in an ethanol induced hepatotoxicity when compared to the basal readings in a dose dependent manner.

Increase in the levels of liver enzymes: SGOT, ALP, SGPT and bilirubin are actually considered to be most relevant indicators of hepatic injury (Agunwa, 2004). The levels of these enzymes can be used to: detect the presence of liver disease, distinguish among different types of liver disorders, measure the extent of known liver damage and follow treatment response (Ndu, 2007).

**Table 1**
Mean liver enzyme levels (±SEM)

| Groups | SGPT (IU/L) | | SGOT (IU/L) | | TB (mg/dl) | | CB (mg/dl) | |
|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| I (20% Ethanol + water) | 20.00 ±.93 | 56.00 ±1.01* | 20.66 ±1.22 | 35.66 ±1.38* | 0.78 ±.05 | 1.31 ±.025* | 0.15 ±.01 | 0.27 ±.009* |
| II (20% Ethanol + extract (20mg/kg) | 22.66 ±2.36 | 40.33 ±1.52* | 21.33 ±2.68 | 36.33 ±.71* | 0.48 ±.07 | 0.90 ±.027* | 0.16 ±.027 | 0.26 ±.018* |
| III (20% Ethanol + extract (40mg/kg) | 22.66 ±1.85 | 31.00 ±1.77* | 26.16 ±2.68 | 33.33 ±.98* | 0.83 ±0.5 | 1.13 ±.025* | 0.13 ±.009 | 0.23 ±.004* |
| IV (20% Ethanol + extract (80mg/kg) | 24.33 ±2.02 | 31.50 ±1.66* | 28.66 ±2.02 | 37.66 ±1.08* | 0.66 ±.08 | 0.92 ±.027* | 0.15 ±.018 | 0.24 ±.021* |
| V (20% Ethanol + extract (160mg/kg) | 24.16 ±1.27 | 21.50 ±.99 | 22.00 ±2.95 | 21.50 ±.76 | 0.67 ±.09 | 0.53 ±.054 | 0.16 ±.018 | 0.15 ±.19 |

*Significantly different at P<0.05 relative to PRE values.

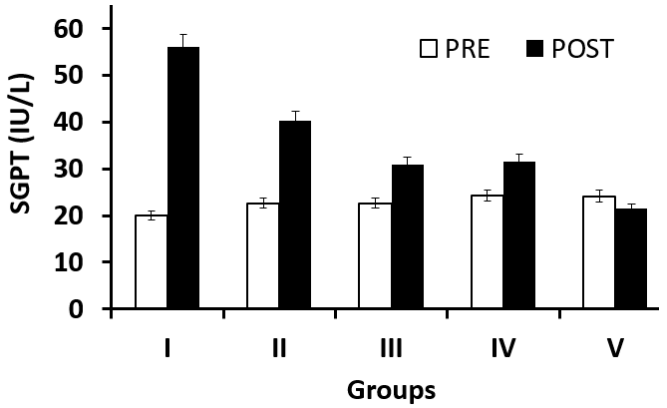


**Figure 1.**
Mean values of serum glutamic pyruvic transaminase (SGPT)

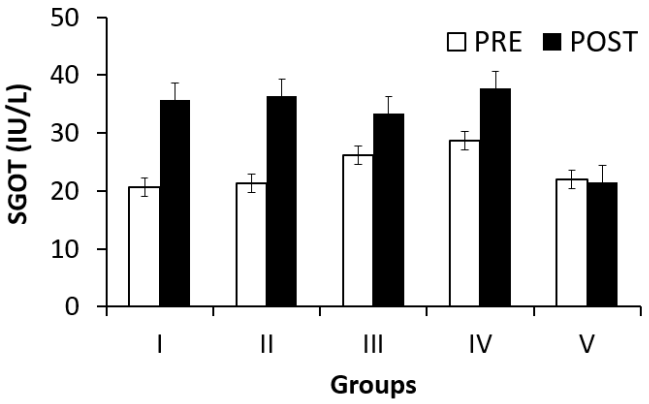


**Figure 2.**
Mean values of glutamic oxaloacetic transaminase (SGOT)

Comparison in between the levels of the enzymes showed that the methanolic extract of *Hibiscus sabdariffa* is more pronounced in Serum glutamic pyruvic transaminase (SGPT) than in glutamic oxaloacetic transaminase (SGOT) and serum bilirubin.

The calyces of *Hibiscus sabdariffa* are rich in vitamin C (Zhou et al, 2006) , hence the reduction in the liver damages may be ascribed partly to this natural antioxidant which also functions in the conversion of α tocopheroxy radical to α tocopherol (Packer and Kagan, 2006). Anthocyanins are ubiquitous in the calyces of *Hibiscus sabdariffa* thus this property of the extract may also be attributed partly to those flavonoids which are known to be potent antioxidants and free radical scavengers (Zhou et al, 2006).

In conclusion, the result of this present study showed that *Hibiscus sabdariffa* has hepatoprotective effect however; further work should focus on elucidating the actual protective mode of the extract. Also, since there are various phytochemicals in the calyces, proper fractionation of the extracts should be done and each fraction subjected to both in vitro and in vivo studies.

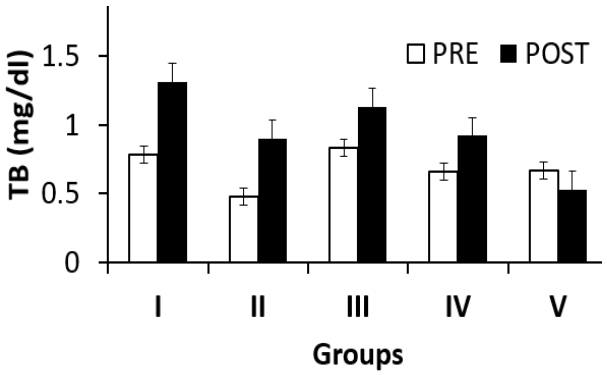


**Figure 3.**
Mean values of total bilirubin (TB)

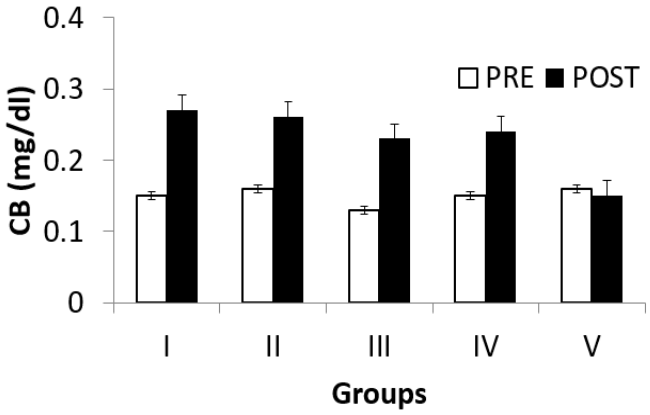


**Figure 4.**
Mean values of conjugated bilirubin (CB)

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