Archives of Current Research International

10(4): 1-6, 2017; Article no.ACRI.37777
ISSN: 2454-7077

Modulations of *Hibiscus sabdariffa* Extract on Ethanol Induced Hepatotoxicity

R. N. Ativie[1], A. A. Aigbiremolen[2], R. E. Ubom[3], V. O. Oseh[4], O. M. Odigie[4*] and J. C. Igweh[4]

[1]Department of Medical Rehabilitation and Technology, Faculty of Health Sciences, University of Nigeria, Enugu Campus, Enugu State, Nigeria.
[2]Department of Pharmacology and Therapeutics, Faculty of Clinical Sciences, College of Medicine, Ambrose Ali University, Ekpoma, Edo State, Nigeria.
[3]Department of Human Physiology, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria.
[4]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors RNA and AAA carried out the bench work and statistical analysis. Authors REU, VOO and OMO wrote the first draft of the manuscript and managed the literature searches. Author JCI designed and supervised the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACRI/2017/37777
Editor(s):
(1) Magdalena Valsikova, Professor, Horticulture and Landscape Engineering, Slovak University of Agriculrure, Nitra, Slovakia.
Reviewers:
(1) Ioana Stanciu, University of Bucharest, Romania.
(2) Noriah Bidin, Universiti Teknologi Malaysia, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history/21948

Original Research Article

Received 28th October 2017
Accepted 7th November 2017
Published 17th November 2017

ABSTRACT

Commonly known as "zobo", *Hibiscus sabdariffa* has been used in different parts of the world for medicinal and nutritional purposes. This work studied its activity on the liver in its course of daily usage as a refreshing social drink. The methanol extract of the dried plant's petals was prepared by a 96 hours cold maceration; following which an acute toxicity testing was found to be safe (LD_{50} > 5000 mg/kg). The extract was evaporated with Soxhlet evaporator. It was later reconstituted and orally administered at graded doses (20, 40, 80 and 160 mg/kg) via orogastric cannula to 30 albino rats (180-200 g; n = 6), with one group serving as control. This administration occurred after

**Corresponding author: Email: osgiedeprof@gmail.com, osgiedeprof@yahoo.com;*

ethanol was locally used to induce hepatotoxicity in rats. After six weeks of drug administration, the rats were sacrificed by cervical dislocation and blood samples were collected for assays. The results showed a significant dose-dependent decrease in serum alanine aminotransferase, aspartate aminotransferase and Bilirubin at a dose 160 mg/kg. Thus, it is suggested that the use of extracts of *Hibiscus sabdariffa* for a long period of time should be encouraged.

Keywords: Liver; hepatoprotective; hepatotoxicity; Hibiscus sabdariffa; methanolic extract.

1. INTRODUCTION

One of the most important organs in the human body, the Liver helps in the maintenance of normal body functions, homeostatic control of metabolites, as well as detoxification of harmful substance. To this point, its hepatoprotective effects, as well as its extent of protection from toxic agents that may damage it are essential [1]. Being a center for alcohol metabolism, several studies have implicated alcohol for the elevation of liver enzymes, resulting in deleterious damage(s) to the hepatic parenchyma cells [2].

Hepatotoxicity can also be caused by a variety of toxic agents like chemicals, severe septicemia, as well as drugs. Also implicated are deficiencies in specific food factors such as cystine, tocopherol, thiamine and selenium [2,3,4]. The use of medicinal herbs for curing diseases has been well documented. According to the world health organization (WHO), 80% of the world's population use plant-based remedies as their primary form of health care [5].

*Hibiscus Sabdariffa*is, an example of such herbs is a shrub belonging to the *Malvaceae* family. It is called "roselle" in English and "zobo" in Nigeria. In recent times, several studies have been carried out on the plant to ascertain its antihypertensive [5,6], anticancer [7], antioxidant [8], and antihyperlipidemic [9] effects on the human body. This study aimed at determining the hepatoprotective effect of the methanolic extract of *Hibiscus Sabdariffa,* using Wistar rats as experimental model.

2. METHODOLOGY

2.1 Plant Sample Collection and Identification

Fresh plants of *Hibiscus sabdariffa* used in this study were gotten from the metropolitan market in Kaduna, Nigeria. The calyces were identified and authenticated by taxonomists in the Bio-resources Development and Conservation Program center, Nsuka.

2.2 Preparation of Plant Extract

The floral parts of *Hibiscus sabdariffa* were sun-dried for two weeks, and pulverized to fine powder using a mortar and pestle. One (1) kg of the powder was then macerated with 300 ml of methanol (Analytical grade from BDCP) for 48 hours at room temperature. The solutions obtained were separately sieved using a sieve net of 150 µ pore size filter paper. Extraction was done with a Soxhlet apparatus, and the extract obtained was evaporated to dryness using rotary evaporator (Buchi R-210). The dried extract obtained was dissolved in distilled water and administered to the animals.

2.3 Phytochemical Analysis

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

2.4 Acute Toxicity Studies (LD_{50})

The acute toxicity test was carried out according to the method described by Lorke [10].

2.5 Ethical Approval

Prior to investigation, ethical clearance was obtained from the Research and Ethics committee of the college of Health Sciences, University of Nigeria, Enugu campus, Enugu State, Nigeria. Global best practices were strictly adhered followed while sacrificing animals for the study.

2.6 Experimental Animals

A total of thirty-six (36) male rats were used for the study, amongst which six were randomly selected for the determination of the lethal dose (LD_{50}) of *Hibiscus sabdariffa*. These animals were obtained from the animal house of the Department of Pharmacology & Toxicology,

Faculty of Pharmaceutical Sciences, University of Nigeria and housed in metallic cages with access to water *ad libitum.* The decision to use 36 animals for this study was guided by a key principle that governs the ethical use of animals in research, testing and teaching. This principle is that, no animal life is wasted. The number of animals used in each project must be the minimum necessary to obtain valid and meaningful results (National Research Council, 1996). More so, from the research advisory guide on animal experiments, "25 is the least forgiving number that guarantees the largest possible sample size" [11].

2.7 Animal Grouping and Administration of Extract

Thirty male rats (180-200 g) were divided into 5 groups (n=6). All animals received 20% ethanol orally every day, using orogastric cannula for a period of six weeks. Group I animals (Control) were administered with 20% ethanol and distilled water Groups II-V Received 20, 40, 80 and 160mg/kg of the extract respectively. After six weeks treatment period, the animals were fasted overnight and sacrificed by cervical dislocation. Laparotomy was then conducted to access the internal organs. Blood sample was collected by cardiac puncture and centrifuged to obtain for serum ALT, AST and Total bilirubin levels.

2.8 Analysis of Sample

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities were carried out according to the enzymatic methods described by Reitman and Frankel [12]. Aspartate amino transferase (AST) activity was determined according to the enzymatic methods described by Karmen et al. [13]. Total serum bilirubin level was determined according to the method described by Walters and Gerarde [14].

2.9 Statistical Analysis

Data obtained were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA) using the SPSS software (version 21).

P-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

See Figs. 1-3 (below) for graphical representation of obtained results in this study.

3.2 Discussion

The liver is the major organ of xenobiotic metabolism and is prone to xenobiotic injuries [15,16]. Various mechanisms of hepatotoxicity have been proposed, and its relevance in clinical research has been reported by researchers [17], [18,19].

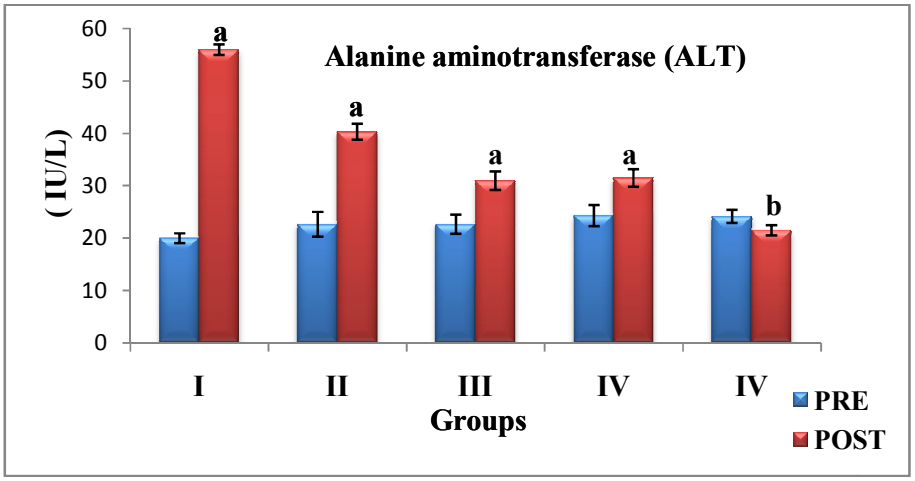

Fig. 1. Showing Effect of methanolic extract of *Hibiscus sabdariffa* on serum Alanine aminotransferase (ALT)

Values are expressed as Mean ± Standard Error of Mean (SEM), n=6. (a) Significant increase when compared with Post values. (b) Significant decrease when compared with Pre values

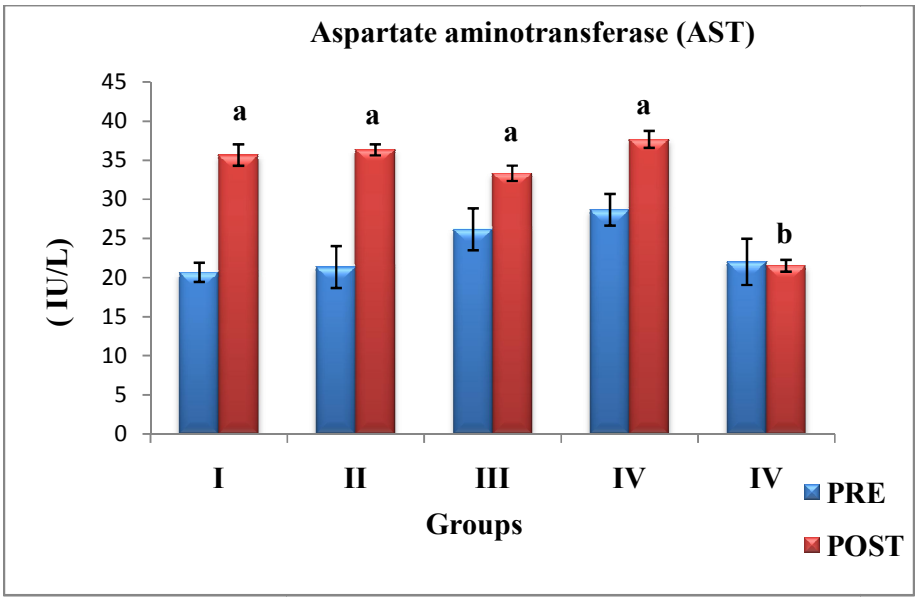

Fig. 2. Showing Effect of methanolic extract of *Hibiscus sabdariffa* on serum Aspartate aminotransferase (AST)

Values are expressed as Mean ± Standard Error of the Mean (SEM), n=6. (a) Significant increase when compared with Pre values. (b) Significant decrease when compared with Pre values

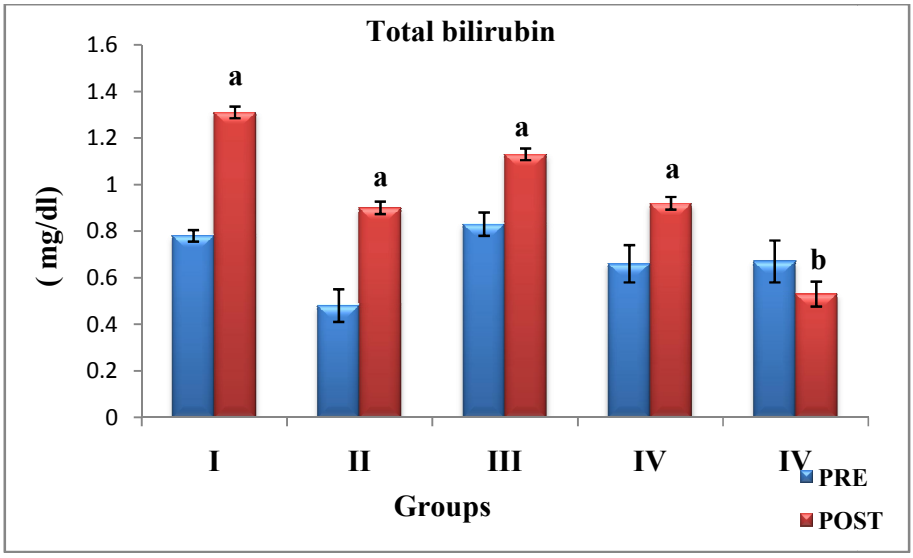

Fig. 3. Showing Effect of methanolic extract of Hibiscus sabdariffa on serum Total bilirubin

Values are expressed as Mean ± Standard Error of the Mean (SEM), n=6. (a) Significant increase when compared with Pre values. (b) Significant decrease when compared with Pre values

Results of this present study shows that, the methanolic extract of *Hibiscus Sabdariffa* has a hepatoprotective effect in ethanol induced hepatotoxicity, with significant statistical difference ($P < 0.05$) when compared to the basal readings in a dose-dependent manner as shown in Figs. 1- 3. This is reflected in post levels of the enzymes, which significantly decreased as compared with the basal readings (Pre) at the end of the sixth week. The hepatoprotective properties of *Hibiscus sabdariffa* observed in this study may be

attributed to the major phytochemicals present in the plant which have also been reported by various authors [20-24].

The mechanism of this action is not fully understood. However, it could be suggested that the phytochemicals in the extract reduced the lipid peroxidation induced by ethanol by acting synergistically to sequester the free filterable platinum. Hence, making it less available for cellular damage. Also, from the results, the bioactivity of the extract is dose dependent. *Hibiscus sabdariffa* is a rich source of vitamin C [25]; hence the reduction in the liver damages may be ascribed partly to this natural antioxidant and free radical scavenging activities of *Hibiscus sabdariffa,* which also functions in the conversion of a tocopheroxy radical to α-tocopherol [26]. This is in line with previous findings of Zhou et al. 2006 Also, comparison between the levels of the enzymes showed *Hibiscus Sabdariffa* extract activity to be more pronounced in ALT than in AST and bilirubin. Thus, in the management of liver disease, *Hibiscus Sabdariffa* extract may probably be effective in those that are characterized by the elevation of ALT.

Increase in the levels of liver enzymes ALT, AST, and Bilirubin are actually considered to be most relevant indicators of hepatic injury [3]. The levels of these enzymes can be used to detect the presence of liver disease, distinguish among different types of liver disorders, gauge the extent of known liver damage, and follow the response to treatment [27]. The protective effect of aqueous extract of *Hibiscus Sabdarriffa* on paracetamol induced hepatotoxicity in rats has also been reported [6]. Significant reduction in lipid peroxidation in carbon tetrachloride induced liver damage has been reported with *Hibiscus Sabdarriffa* extract [27]. However, prolonged usage of *Hibiscus Sabdarriffa* extract could cause kidney injury as observed in one study [6]. Medicinal plants are widely used by the populations of underdeveloped countries as alternative therapy [6]. In Africa, hundreds of plants are used traditionally for the management and/or for enhancing the liver function, with only a few of such African medicinal plants haven to receive scientific scrutiny [6].

Hibiscus sabdariffa extract is characterized by a very low degree of toxicity. The acute toxicity LD_{50} of *Hibiscus sabdariffa* extract in rats was found to be above 5000 mg/kg according to the method of [11].

4. CONCLUSION

Elevated levels of liver enzymes (ALT and AST) and reduced Bilirubin levels are observed in ethanol induces hepatotoxicity in experimental rats. *Hibiscus Sabdariffa* ameliorated the toxicities induced by ethanol probably due to the presence of the phytochemicals flavonoids and tannins which have been shown to have potent polyphenolic antioxidant property. The effect of *Hibiscus sabdariffa* on the liver is dose dependent and more effective at 160 mg/kg.

5. PROSPECT FOR FURTHER STUDIES

Further work should be done to qualitatively determine the active principles responsible for the action of *Hibiscus Sabdariffa*. Also the molecular studies should be carried out to further determine the mechanism of action of *Hibiscus Sabdariffa*. Furthermore, *Hibiscus Sabdariffa* should be taking for protection of liver, when there is no immediate access to any health professional.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abo-Salem OM, Abd-Ellah MF, Ghonaim MM. Hepatoprotective activity of quercetin against acrylonitrileinduced hepatotoxicity in rats. J BiochemMol Toxicol. 2011; 25(6):386-92.
2. Agunwa CN. Therapeutic basis of Clinical Pharmacy in the Tropics, 3rd Ed. 2004;528-531.
3. Agunwa CN. Therapeutic basis of Clinical Pharmacy in the Tropics, 3rd Ed. 2004; 528-531.
4. Ali J, Sahil M. Muir's textbook of pathology 11th Ed. 2004;662.
5. Alison MR, Vig P, Russo F. Hepatic stem cells: from inside and outside the liver? Cell Proliferation. 2001;37:1.
6. Bako CV, Dawud G. Drug- induced liver disease. Marcel Dekker Inc. New York, U.S.A. 2009;773.
7. Bauer M. Hemeoxygenase in liver transplantation: Heme catabolism and metabolites in the search of function. Journal of Hepatology. 2003;38:286.

8. Chang V, Huang MN. An overview of *Flibsicus sabdariffa*. Natural Radiance Product. 2005;8(1):77-83.
9. Chau B, Chang V. Selected medicinal Plants of India. Bombayi Tata Press. 2005; 235-7.
10. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol. 1983;54: 275-287.
11. Kail RV, Siegel AW. Sex and hemispheric differences in the recall of verbal and spatial information. Cortex. 1998;14:557–563.
12. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetate and glutamate pyruvate transaminase. Am. J. Clin. Path. 1957;28:56-61.
13. Karmen A, Wroblewski F, LaDue JS. Transaminase activity in human blood. J. Clin. Invest. 1955;34:126.
14. Walters MI, Gerarde HW. An ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma. Microchem J. 1970;15:231.
15. Gagliano N, Grizzi F, Annoni G. Mechanism of aging and liver functions. Digest. Dis. Sci. 2007;25:118-123.
16. Sturgill MG, Lambert GH. Xenobioticsinduced hepatotoxicity; Mechanism of liver injury and method of monitoring hepatic function. Clin. Chem. 1997;43:1512-1526
17. Navarro VJ, Senior JR. Drug-related hepatotoxicity. The New England Journal of Medicine. 2006;354:731-9.
18. Russmann S, Kullak-Ublick GA, Grattagliano I. Current concepts of mechanisms in drug-induced hepatotoxicity. Current Medicinal Chemistry. 2009;16:3041-3.
19. Ozcelik E, Uslu S, Erkasap N, Karimi H. Protective effect of chitosan treatment against acetaminophen-induced hepato-toxicity. Kaohsiung Journal of Medical Sciences. 2014;30:286-90.
20. Obouayeba AP, Djyh NB, Diabaté S, Djaman AJ, N'guessan JD, Koné M. Phytochemical and antioxidant activity of Roselle (*Hibiscus sabdariffa* L.) petal extracts. Res J Pharm Biol Chem Sci. 2014;5:1453-65.
21. Wang CJ, Wang JM, Lin WL, Chu CY, Chou FP, Tseng TH. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. Food Chem Toxicol. 2003;38(5): 411-6.
22. Ologundudu A, Ologundudu AO, Ololade IA, Obi FO. The effect of *Hibiscus anthocyanins* on 2, 4-dinitrophenylhydrazine-induced hepato-toxicity in rabbits. Int J Phys Sci. 2009; 4:233-7.
23. Gulati RK, Agarwal S, Agrawal SS. Hepatoprotective studies on *Phyllanthus emblica* Linn. andquercetin. Indian J Exp Biol. 1995;33(4):261-8.
24. Oh H, Kim DH, Cho JH, Kim YC. Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from Equisetum arvense. J Ethnopharmacol. 2004;95(2-3): 421-4.
25. Zhou Q, Xie H, Zhang L, Stewart K, Gu X, Ryan SJ. cisTerpenones as an effective chemopreventive agent against aflatoxin B 1-induced cytotoxicity and TCDD induced P450 IA/B activity in HepG2 cells. Chem. Res. Toxicol. 2006;19:1415-1419.
26. Packer L, Kagan VE. Vitamin E: The antioxidant harvesting centre of membranes and lipoproteins. Marcel Dekker Inc., New York. 1993;131-139.
27. Ndu OO. Hepatoprotective Activity of Hibiscus Sabdariffa in Pracetamol Induced Hepatotoxicity. Pharmacology Journal. 2007;21:45.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21948