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Original Research Article

Pre-treatment with *Hibiscus Sabdariffa* Linn calyx Extract is Protective Against Sub-Chronic Paracetamol Hepatotoxicity in Mice

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ABSTRACT

Many plant parts have been found to have efficient protective and ameliorative effects against drug induced liver injury. The present study aimed to investigate the prophylactic effects of *Hibiscus sabdariffa* Linn calyx aqueous extract (HSCE) on sub chronic paracetamol induced hepatotoxicity in mice. Twenty mice were randomly distributed into four groups of five mice each. The control and HSCE only group were given aqueous DMSO and HSCE (250 mg/kg b. wt.) respectively. Hepatotoxicity was induced in the mice by oral administration of paracetamol at a sub chronic dose (500 mg/kg b. wt.) in paracetamol only group. The pretreatment group were administered HSCE at zero time and paracetamol (500 mg/kg b. wt.) 8 h later. The blood and liver were collected and subjected to biochemical analysis after 8 weeks. The administration of paracetamol significantly ($P \leq 0.05$) induced hepatotoxicity as evidenced by the increase in the level of liver marker enzymes and significant ($P \leq 0.05$) decrease in the level of protein and albumin. Pretreatment with HSCE significantly ($P \leq 0.05$) reduced serum levels of elevated liver enzyme markers. As regards to oxidative stress markers, pretreatment with HSCE significantly ($P \leq 0.05$) decreased the level of malondialdehyde and increased the activities of superoxide dismutase and catalase. The hepatoprotective activity of HSCE was also confirmed by histopathological findings. These results suggest that pretreatment with HSCE has protective effect against paracetamol induced hepatotoxicity in mice.

Keywords: Drug induced liver injury, *Hibiscus sabdariffa* Linn, oxidative stress, paracetamol.

Introduction

The role of the liver in the detoxification of drugs and xenobiotics renders it susceptible to drug induced injury. Paracetamol is an antipyretic and analgesic drug used in the treatment of pain and fever, and is considered safe when administered at therapeutic doses. However, paracetamol overdose can cause liver injury, and is the main cause of acute liver failure in many countries.[1] In paracetamol overdose, the reactive intermediate metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) is produced in excess, resulting in intracellular reduced glutathione (GSH) depletion since GSH reacts with NAPQI to form GSH adduct that is excreted in bile.[2] Once GSH is depleted, excess NAPQI reacts with cellular proteins to form protein adducts which mediate oxidative stress, cellular injuries and consequently hepatotoxicity.

Hibiscus sabdariffa Linn is an annual herbaceous plant that is abundant in Africa and Asia. The plant has numerous biological and pharmacological actions. It was shown to have anti-inflammatory activity,[3] hypo-cholesterolemic activity,[4] antibacterial,[5] antidiabetic, [6] and antihypertensive effect.[7]

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Phytochemical investigation of the extract of *Hibiscus sabdariffa* L. revealed the presence of anthocyanins, flavonoids and polyphenols.[8] Paracetamol is a widely used over-the-counter analgesic and antipyretic. It is also a major ingredient in cold and flu medications. The drug is readily available which makes misuse or over-use inevitable. One approach for reducing harm done by paracetamol overdose is selling paracetamol pre-combined in tablets either with an emetic[9] or an antidote. This study therefore investigated the prophylactic effects of aqueous extract of *Hibiscus sabdariffa* L. calyx against sub chronic paracetamol hepatotoxicity, using the mice as experimental model.

Materials and Methods

Plant material

Dry calyces of *Hibiscus sabdariffa* were purchased from Karu market, Abuja, Nigeria (June, 2013). Taxonomic authentication of the plant was done by Mr. Joseph Erhabor at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria (Voucher number UBHm 0261, July 2013).

Experimental animals

Twenty healthy adult mice weighing 27 to 32g were used for this study. The animals were housed in hygienic wooden cages and maintained under standard environmental conditions. They were allowed to acclimatize for two weeks and fed with standard pellet (Growers mash, Bendel Feeds and Flour Mills Ltd, Ewu, Edo State) and water *ad libitum.* The study was conducted in accordance with the conventional procedure accepted by the National Institute of Health Guide for the

Care and Use of Laboratory Animals, and approved by the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Methods

Preparation of plant extract

The dried calyces *of Hibiscus sabdariffa* were pulverized to fine coarse powder and soaked in distilled water (1.3w/v) for 24 h at 4°C.[10] The cold extract was then filtered and doses corresponding to 250 mg/kg prepared.[11]

Preparation of Paracetamol sample

Paracetamol base powder (Huang Gang Yin Aati Pharmaceutical Co. Ltd. China) was first dissolved in DMSO (2.5% aqueous solution of DMSO) and then brought up to the required concentration with distilled water. The dose administered was 500 mg/kg b. wt.[12]

Experimental design and treatment arrangement

The mice were randomly distributed into four groups consisting of five mice each as follows:

Group 1 served as the control and received aqueous DMSO, Group 2 received *Hibiscus sabdariffa* calyx extract (HSCE) only, Group 3 received paracetamol only while Group 4 received HSCE at zero time and paracetamol 8 h later. Paracetamol (500mg/kg b.wt.)[12] and HSCE (250 mg/kg) [11] were administered orally for 8 weeks. At the end of the treatment period, serum was obtained and kept at -20^{0}C for biochemical assays. The liver from the animals were rapidly excised and washed in very cold saline. Liver from mice in applicable groups were fixed for histopathological investigations while part of the tissue was weighed and homogenized in phosphate buffer saline (PBS) 50mM pH 7.4, and supernatant obtained by centrifuging at 3500 rpm for 15 min.

Biochemical analyses

The serum was used to analyze liver marker enzymes such as alanine aminotransferases (ALT) and aspartate aminotransferases (AST),[13] alkaline phosphatase (ALP)[14] and gamma glutamyl transferase (GGT).[15] The levels of total cholesterol,[16] albumin,[17] total protein (TP),[18] total bilirubin and direct bilirubin,[19] were also analyzed. The liver homogenate was used to determine the levels of malondialdehyde,[22] activity of superoxide dismutase (SOD)[20] and catalase,[21] and reduced glutathione (GSH) [23] levels.

Statistical analysis

The results are expressed as mean ± S.E.M (n=5). Tests of statistical significance were carried out using one-way ANOVA, followed by Duncan's multiple range tests where $P \leq 0.05$ was considered statistically significant.

Results and Discussion

The liver is a complex organ which is important for survival. The liver can become injured by clinically useful drugs when they are converted to reactive intermediate metabolites like free radicals or electrophiles that have the ability to alter the structure of cellular macromolecules.[24] Paracetamol is an analgesic that helps to relieve pain and fever at therapeutic dose. However, at high levels, paracetamol is oxidized by the cytochrome P450 system, producing excess NAPQI, a reactive intermediate specie. Conjugation of NAPQI with glutathione results in depletion of glutathione stores and formation of protein adducts in the mitochondria, thus initiating oxidative and nitrosative stress.[25] An increase in serum enzyme markers represents biochemical evidence of hepatic injury.

In this present study, the results of liver function tests carried out in serum as presented in Table 1 show significant increase ($P<0.05$) in serum markers (ALT, AST, cholesterol, GGT, total bilirubin and direct bilirubin) and significant decrease ($P<0.05$) in levels of total protein and albumin, compared to the control group, after 8 weeks of paracetamol administration. This result is in accordance with previous studies reporting hepatotoxicity of paracetamol.[26] Pretreatment with aqueous HSCE (250 mg/kg b. wt.) at zero time followed by paracetamol administration significantly ($P<0.05$) lowered the levels of the serum markers towards normalcy as compared with paracetamol-only group. The results suggest that HSCE exhibits hepatoprotective properties, reduced the progression of hepatic injury by preserving the structural integrity of hepatocellular membranes and preventing the breakdown of proteins.

Oxidative stress is an important mechanism in the development of paracetamol hepatotoxicity. Free radicals such as superoxide anion and hydrogen peroxide produced by oxidative stress provoke the formation of lipid peroxidation product like MDA.[27] Results for antioxidants and lipid peroxidation tests carried out in liver homogenates from this study as presented in Table 2 showed a significant increase ($P<0.05$) in MDA and decrease in reduced glutathione level in the group treated with paracetamol only compared to the control group. The activities of oxidative stress marker enzymes SOD and catalase were also significantly reduced ($P<0.05$). This result agrees with previous report of a significant increase in MDA and significant decrease in SOD catalase and glutathione following paracetamol overdose.[28] Glutathione is among the first line of defense against paracetamol induced liver injury. It scavenges free radicals and can also serve as cofactor, thus enabling effective functioning of other antioxidants such as glutathione peroxidase and glutathione-s-transferase.[29] When antioxidants are depleted, opportunities for lipid peroxidation are enhanced.

Pre-treatment with HSCE before paracetamol significantly decreased ($P<0.05$) MDA level and enhanced reduced glutathione level compared to the paracetamol only group. In addition, prophylactic treatment with HSCE significantly reduced tissue damage indicated by increase in the activities of SOD and catalase as compared to the paracetamol only group. SOD and catalase are important enzymes that play an indispensable role in the antioxidant defense system. SOD catalyzes the conversion of superoxide to hydrogen peroxide and oxygen while catalase degrades hydrogen peroxide to oxygen and water.[30]

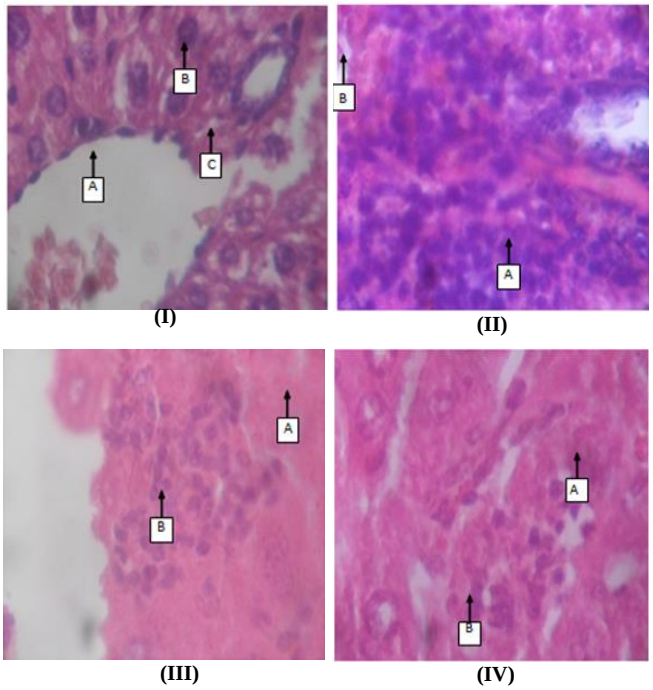

Figure 1: Photomicrograph of liver tissue from mice (H & E, x400). (i) Control [portal vein (A), hepatocytes (B) sinusoids (C)] (ii) HSCE-only [moderate periportal lymphocytosis (A), mild portal congestion (B)] (iii) Paracetamol-only [necrotic hepatocytes (A) surrounded by moderate infiltrates of acute inflammatory cells (B)] (iv) HSCE at zero time and paracetamol 8 h later [necrotic hepatocytes (A) with focal infiltrates of acute inflammatory cells (B)].

Table 1: Pretreatment with aqueous *Hibiscus sabdariffa* calyces extract (HSCE) on liver function parameters in serum of mice on sub-chronic paracetamol exposure

Groups	Biochemical Parameter (Serum)								
	ALT (U/I)	**AST (U/I)**	**TP (g/dL)**	**Albumin (g/dL)**	**Cholesterol (mmol/L)**	**ALP (IU/L)**	**GGT (U/I)**	**Total Bilirubin (mg/dL)**	**Direct Bilurubin (mg/dL)**
Control	8.32 ± 0.32^{c}	54.39 ± 0.34^{c}	7.98 ± 0.15^{a}	3.21 ± 0.05^{b}	8.90 ± 0.10^{b}	42.61 ± 0.36^{c}	20.84 ± 2.32^{c}	0.34 ± 0.02^{b}	0.17 ± 0.03^{b}
HSCE only	8.32 ± 0.32^{c}	53.83 ± 0.28^{c}	8.43 ± 0.18^{a}	3.65 ± 0.05^{a}	8.70 ± 0.10^{b}	43.19 ± 0.29^{c}	20.84 ± 2.32^{c}	0.32 ± 0.02^{b}	0.17 ± 0.03^{b}
Paracetamol only	14.08 ± 0.32^{a}	84.63 ± 0.26^{a}	5.19 ± 0.05^{c}	1.94 ± 0.04^{d}	10.00 ± 0.20^{a}	76.52 ± 0.29^{a}	48.64 ± 2.32^{a}	0.61 ± 0.03^{a}	0.32 ± 0.03^{a}
HSCE (zero time), then Paracetamol (8h later)	9.92 ± 0.32^{b}	67.76 ± 0.34^{b}	5.86 ± 0.03^{b}	2.29 ± 0.09^{c}	9.10 ± 0.20^{b}	70.43 ± 0.35^{b}	32.42 ± 2.32^{b}	0.39 ± 0.03^{b}	0.23 ± 0.04^{b}

Values are mean ± SEM, n=5 animals in each group, values with different letters in a column differ significantly from each other ($P \leq 0.05$)

Table 2: Pretreatment with aqueous *Hibiscus sabdariffa* calyx extract (HSCE) on antioxidant and lipid peroxidation in the liver of mice on sub-chronic paracetamol exposure

Groups	MDA (mol/g tissue)	SOD (Units/mg tissue)	Catalase (Units/g tissue)	Reduced Glutathione (mmol/L)
Control	0.07 ± 0.00^{c}	0.05 ± 0.00^{a}	7.30 ± 0.07^{a}	0.09 ± 0.00^{b}
HSCE only	0.07 ± 0.00^{c}	0.05 ± 0.00^{a}	$7.22 \pm .04^{a}$	0.10 ± 0.00^{a}
Paracetamol only	0.10 ± 0.00^{a}	0.03 ± 0.00^{c}	5.44 ± 0.11^{c}	0.06 ± 0.00^{d}
HSCE (zero time), then paracetamol (8h later)	0.08 ± 0.00^{b}	0.04 ± 0.00^{b}	6.83 ± 0.04^{b}	0.08 ± 0.00^{c}

Values are mean ± SEM, n=5 animals in each group, values with different letters in a column differ significantly from each other ($P \leq 0.05$).

Findings from this study corroborated previous reports that treatment with *Hibiscus sabdariffa* Linn extracts resulted in a decrease in oxidative damage.[31, 32] The ability of HSCE to lower MDA level may be attributed to its antioxidant potential. *Hibiscus sabdariffa* Linn has been reported to have biological and medicinal properties. Studies on phytochemical constituents of *Hibiscus sabdariffa* Linn calyx demonstrated the presence of many secondary metabolites like phenols, flavonoids, anthocyanins, gallic acid and quercetin.[8] HSCE induced hepatoprotective activity may possibly involve the synergistic action of these phytochemicals. These findings were further confirmed by histopathological observation of the liver sections (Figure 1) revealing that mice pretreated with HSCE showed reduced hepatic damage compared with the paracetamol only group.

Conclusion

Conclusively, the results from this study suggest that aqueous extract from the calyces of *Hibiscus sabdariffa* Linn exerted hepatoprotective activity against sub chronic paracetamol induced liver injury in mice. The protection provided may be due to its antioxidant properties.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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