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Sub-Chronic and chronic studies of effects of concurrent administration of paracetamol and aqueous extract of *Hibiscus sabdariffa* Linn Calyx on paracetamol hepatotoxicity in mice

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Abstract

This study evaluated the effects of concurrent administration of paracetamol and aqueous extract of *Hibiscus sabdariffa* Linn calyx on paracetamol-associated hepatotoxicity in mice under sub-chronic and chronic conditions. It comprised of four groups of five mice each to reflect control, extract only, paracetamol only and concurrent administration of paracetamol and extract. Paracetamol (500mg/kg body weight) and *Hibiscus sabdariffa* Linn calyx extract (250mg/kg body weight) were administered orally to experimental mice. Paracetamol toxicity was evidenced by significant increases ($P \leq 0.05$) in ALT, AST, ALP and GGT activities, total cholesterol and bilirubin levels, and decreased serum total protein and albumin levels. Also found were significant decreases ($P \leq 0.05$) in catalase and SOD activities, GSH level, and increase ($P \leq 0.05$) in MDA level, relative to control. Concurrent administration of paracetamol and extract proved effective in ameliorating paracetamol-associated liver injury in mice particularly at the sub-chronic phase where the values of many of the parameters returned to control.

Keywords: paracetamol; *Hibiscus sabdariffa* Linn calyx; sub-chronic, chronic, concurrent administration; liver

Introduction

Paracetamol is a mild analgesic used in the treatment of headaches and other minor pains. It is a major ingredient in numerous cold and flu remedies^[1]. The recommended maximum daily dose for healthy adults is 3 grams. Higher doses lead to increasing risk of toxicity^[2]. Due to the ease of its availability, misuse or over - use may not be inevitable and could lead to liver and kidney injuries³. Intentional overdosing (self-poisoning with suicidal intent) is frequently implicated in paracetamol toxicity^[3].

The mechanism of action of paracetamol is not completely understood to date. The inhibition of cyclooxygenase (COX) has been proposed as a possible mechanism, with findings suggesting a high selectivity for COX-2^[4]. Therefore it does not significantly inhibit the production of the pro-clotting thromboxanes^[4]. While it has analgesic and anti-pyretic properties comparable to those of aspirin or other NSAIDs, its peripheral anti-inflammatory activity is usually limited by several factors, one of which is the high level of peroxides present in inflammatory lesions. However, in some circumstances, even peripheral anti-inflammatory activity comparable to NSAIDs can be observed^[4].

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^[5].

Hibiscus sabdariffa Linn which is known as Red Sorrel or Roselle in English is taken as a common local drink popularly known as Zobo in Nigeria. It is an annual dicotyledonous herbaceous tropical plant belonging to the family Malvaceae^[6]. The calyx of the plant has been reported to contain protein (1.9 g / 100 g), fat (0.1 g / 100 g), carbohydrates (12.3 g / 100 g), fibre (2.3 g / 100 g), vitamin C (14 mg / 100 g), β -carotene (300 μ g / 100 g), calcium (1.72 mg / 100 g) and iron (57 mg / 100 g)⁷. Also reported is the presence of antioxidants such as anthocyanin, quercetin and protocatechuic acid^[8, 9] reported the presence of arachidic acid, β -sitosterol, delphinidin, gossypetin and hibiscetin.

The aim of the study was to evaluate the effects of concurrent administration of paracetamol and aqueous extract of *Hibiscus sabdariffa* Linn calyx on hepatotoxicity induced in mice by paracetamol under sub-chronic and chronic exposures.

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Materials and Methods

Materials

Chemicals and Reagents

All chemicals and reagents used were of analytical grade.

Plant material

The calyces of *Hibiscus sabdariffa* Linn were purchased from Karu market, Abuja, Nigeria. The plant was identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin City by Mr. Joseph Erhabor. Subsequently a voucher specimen of the plant (voucher number UBHm 0261) was deposited at the Herbarium, University of Benin, Benin City.

Experimental animals

A total of 40 mice of both sexes (27 - 32g) were used for this study. They were obtained from a breeder in Benin City and housed in wooden cages in controlled laboratory conditions in the animal house of the Department of Biochemistry, University of Benin. The mice were allowed two weeks for acclimatization before commencement of any treatment during which they had free access to tap water and food (Growers mash, Bendel Feeds and Flour Mills Ltd, Ewu, Edo State).

Methods

LD50

The oral LD₅₀ of the calyx extract was determined using the method of Lorke, 1983^[10].

Preparation of plant extract

Dried calyces of *Hibiscus sabdariffa* Linn were pulverized into fine coarse powder. The powder was soaked in distilled water (1:3w/v) for 24 hours at 4°C^[11]. The cold extract was filtered and doses corresponding to 250 mg/kg^[12] were prepared.

Paracetamol preparation

Paracetamol base powder (Huang Gang Yin He Aati Pharmaceutical Co. Ltd. China) was provided by Late Dr. G. C. Josephs (Department of Pharmaceutical Microbiology and Biotechnology, University of Benin, Benin City, Nigeria). The drug was first dissolved in dimethyl sulfoxide (DMSO) (2.5% aqueous solution of DMSO), then mixed with distilled water to make up the required quantity and administered orally (500 mg / kg bd wt) to the mice by means of gavage.

Experimental design and treatment schedule

This study was divided into two (2) categories namely: sub-chronic (repeated exposure for 8 weeks) and chronic (repeated exposure for 16 weeks)^[13]. Each category comprised of four (4) groups to reflect paracetamol and extract free (control), extract only, paracetamol only and concurrent administration of paracetamol and extract. Each group comprised of 5 mice. The drug was administered orally (500 mg/kg body weight) to experimental mice^[14]. *Hibiscus sabdariffa* Linn calyx extract (HSCE) was also administered orally at 250 mg/kg^[12].

Sub-chronic study

A total of 20 mice were used in this study, treated as described above. They were randomly divided into four groups. Group 1 served as control and was not treated with paracetamol and extract but received aqueous DMSO. Group 2 received only extract. Group 3 received only paracetamol while in group 4, the drug and extract were administered concurrently. All treatments were given once daily for eight (8) weeks. At the end of the treatment period, each mouse was sacrificed.

Chronic study

A total of 20 mice were used in this study, treated as described above. They were randomly divided into four groups. Group 1 served as control and was not treated with paracetamol and extract but received aqueous DMSO. Group 2 received only extract. Group 3 received only paracetamol while in group 4, the drug and extract were administered concurrently. All treatments were given once daily for sixteen (16) weeks. At the end of the treatment period, each mouse was sacrificed.

Collection and preparation of samples for analyses

The mice were sedated with chloroform and blood collected by heart puncture into plain sample bottles. Blood samples were allowed to clot and thereafter centrifuged at 4,000 rpm for 10 minutes to separate sera. The samples were kept at -20 °C until required for the assays. The liver was also excised and washed in ice cold saline. Portions of the liver from a mouse in appropriate groups were fixed for histopathological examinations while a known weight of the organ was homogenized in phosphate buffered saline (PBS) 50mM pH 7.4, centrifuged at 3500rpm for 15 minutes and the resultant supernatant used for biochemical assays.

Biochemical analyses

Biochemical analyses that were carried out on serum include alanine aminotransferase (ALT)^[15], aspartate aminotransferase (AST)^[15], alkaline phosphatase (ALP)^[16], gamma glutamyl transferase (GGT)^[17], total cholesterol^[18], albumin^[19], total protein^[20] and bilirubin (total and direct)^[21]. Reduced glutathione (GSH)^[22], malondialdehyde (MDA)^[23], superoxide dismutase (SOD)^[24] and catalase^[25] were assayed for in liver homogenate supernatant.

Histopathological examination

Fixed tissue (liver) sections were processed for histopathological examination. The samples were sectioned, stained with Haematoxylin and Eosin, and examined under light microscope.

Statistical analysis

The experimental results were expressed as mean ± S.E.M. They were analyzed for statistical significance by one way ANOVA and mean values that were significantly different from each other were identified by the Duncan's multiple range test. $P \leq .05$ was considered significant.

Results

LD50

The oral LD₅₀ of the calyx extract was determined to be over 5000mg/kg body weight.

Effects of HSCE on sub - chronic paracetamol exposure

Liver function parameters

Table 1 shows results for liver function tests carried out in serum. After treatment for 8 weeks, paracetamol significantly increased ($P \leq .05$) ALT, AST, ALP, GGT, cholesterol, total and direct bilirubin, while albumin and total protein decreased, relative to control. Albumin level was significantly increased ($P \leq .05$) in the extract - only treated group, relative to control. Concurrent administration of paracetamol and extract significantly reduced ($P \leq .05$) ALT, AST, cholesterol, GGT, ALP, total and direct bilirubin levels, and significantly increased total protein and albumin, relative to paracetamol – only group.

Table 1: Effects of aqueous HSCE on liver function parameters in serum of mice on sub – chronic paracetamol exposure:

Biochemical parameter (serum)	Control	Extract only	Paracetamol only	Concurrent administration of paracetamol and extract
ALT (U/I)	8.32±0.32d	8.32±0.32d	14.08±0.32a	7.36±0.39d
AST (U/I)	54.39±0.34e	53.83±0.28e	84.63±0.26a	56.42±0.26d
TP (g/dL)	7.98±0.15a	8.43±0.18a	5.19±0.05e	7.69±0.18b
ALBUMIN(g/dL)	3.21±0.05b	3.65±0.05a	1.94±.04e	2.71±0.04c
CHOLESTEROL (mmol/L)	8.90±0.10b	8.70±0.10b	10.00±0.20a	8.80±0.16b
ALP (IU/L)	42.61±0.36e	43.19±.29e	76.52±0.29a	48.70±0.36d
GGT (U/I)	20.84±2.32c	20.84±2.32c	48.64±2.32a	23.16±0.00c
TOTALBILIRUBIN (mg/dL)	0.34±0.02b	0.32±.00b	0.61±0.03a	0.36±0.03b
DIRECTBILIRUBIN (mg/dL)	0.17±0.03b	0.17±.03b	0.32±0.03a	0.20±0.04b

Values are Mean ± SEM (n=5)

Values with different letters within a row differ significantly from each other ($P \leq 0.05$).

Antioxidants and lipid peroxidation in the liver

Table 2 shows results for antioxidant and lipid peroxidation assay carried out in the liver. After treatment for 8 weeks, paracetamol significantly increased ($P \leq 0.05$) MDA level while SOD and catalase activities, and GSH level were reduced, relative to control. Concurrent administration of paracetamol

and extract significantly increased GSH level, and SOD and catalase activities, while MDA level was significantly reduced ($P \leq 0.05$), relative to paracetamol – only group. Administration of extract significantly increased ($P \leq 0.05$) GSH level, relative to control.

Table 2: Effects of aqueous HSCE on antioxidants and lipid peroxidation in the liver of mice on sub - chronic paracetamol exposure

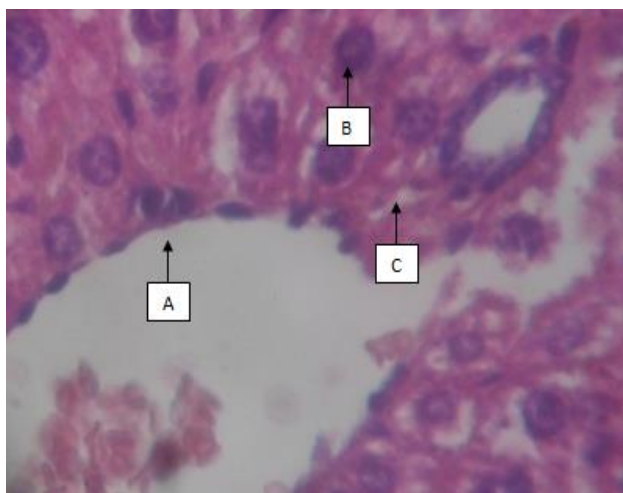
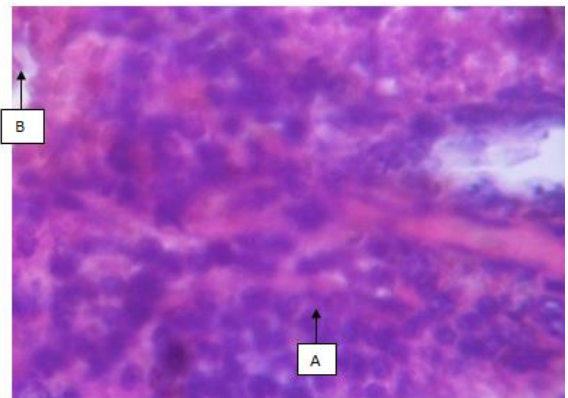
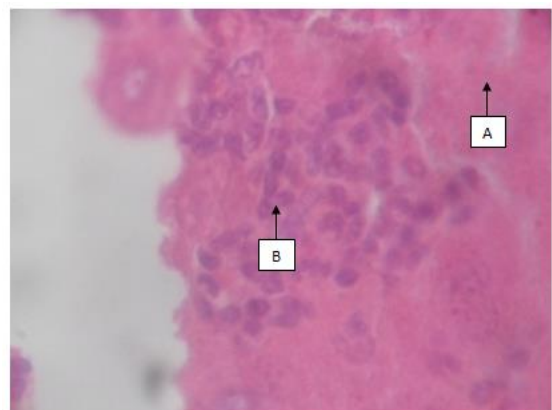
Biochemical parameter (liver)	Control	Extract only	Paracetamol only	Concurrent administration of paracetamol and extract
MDA (mol/g tissue)	0.07±0.00c	0.07±0.00c	0.10±0.00a	0.07±0.00c
SOD (Units/mg tissue)	0.05±0.00a	0.05±0.00a	0.03±0.00c	0.05±0.00a
CATALASE (Units/g tissue)	7.30±0.07a	7.22±.04a	5.44±0.11d	7.10±0.02b
REDUCED GLUTATHIONE (mmol/L)	0.09±0.00b	0.10±0.00a	0.06±0.00d	0.09±0.00b

Values are Mean ± SEM (n=5)

Values with different letters within a row differ significantly from each other ($P \leq 0.05$).

Liver ultrastructure of mice exposed to extract and paracetamol concurrently

The control mouse showed normal architecture of the liver, composed of portal vein (A) and hepatocytes (B), separated by sinusoids (C) (plate 1). The mouse that received extract only showed moderate periportal lymphocytosis (A), and mild portal congestion (B) (plate 2). The mouse that received paracetamol only showed necrotic hepatocytes (A), surrounded by moderate infiltrates of acute inflammatory cells (B) (plate 3), while the mouse that received extract and paracetamol concurrently showed mild portal congestion (A), mild periportal lymphocytosis (B) and viable hepatocytes (C) (plate 4).

**Plate 1:** Photomicrograph of control mouse' liver (H & E, x400).**Plate 2:** Photomicrograph of liver from mouse treated with extract only for 8 weeks (H & E, x400).**Plate 3:** Photomicrograph of liver from mouse treated with paracetamol only for 8 weeks (H & E, x400).

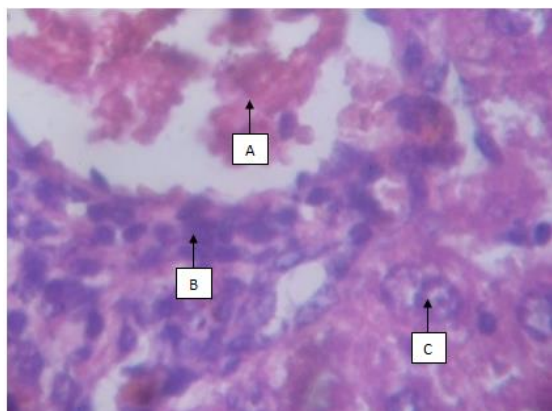


Plate 4: Photomicrograph of liver from mouse concurrently administered paracetamol and extract for 8 weeks (H & E, x400).

Effects of HSCE on chronic paracetamol exposure Liver function parameters

Table 3 shows the results for liver function tests carried out in serum. After 16 weeks, paracetamol caused a significant increase ($P \leq 0.05$) in the levels of ALT, AST, cholesterol, ALP, GGT, total bilirubin and direct bilirubin and reduction in levels of total protein and albumin, relative to control. Administration of extract significantly ($P \leq 0.05$) increased total protein and albumin levels, relative to control. Concurrent administration of paracetamol and extract significantly reduced ($P \leq 0.05$) ALT, AST, cholesterol, GGT, ALP, total and direct bilirubin levels, and significantly increased total protein and albumin, relative to paracetamol – only group.

Table 3: Effects of aqueous HSCE on liver function parameters in serum of mice on chronic paracetamol exposure:

Biochemical parameter (serum)	Control	Extract only	Paracetamol only	Concurrent administration of paracetamol and extract
ALT (U/I)	8.64±0.39d	8.96±0.39d	17.28±0.78a	11.84±0.64c
AST (U/I)	53.90±0.86e	53.20±0.70e	91.70±0.70a	57.40±0.86d
Total Protein (g/dL)	6.88±0.08b	7.28±0.08a	5.24±0.10e	6.24±0.10c
Albumin (g/dL)	3.26±0.03b	3.65±0.04a	2.34±0.05f	3.02±0.03c
Cholesterol (mmol/L)	8.38±0.12c	8.29±0.10c	12.62±0.10a	8.38±0.12c
ALP (IU/L)	41.59±0.72d	42.18±0.59d	77.59±0.36a	55.46±0.36c
GGT (U/I)	23.16±0.00d	20.84±2.32d	53.27±2.84a	32.42±2.32c
Total Bilirubin (mg/dL)	0.34±0.02d	0.32±0.00d	0.65±0.00a	0.36±0.03c
Direct Bilirubin (mg/dL)	0.17±0.03b	0.17±0.03b	0.32±0.03a	0.20±0.04b

Values are Mean ± SEM (n=5)

Values with different letters within a row differ significantly from each other ($P \leq 0.05$).

Antioxidants and lipid peroxidation in the liver

Table 4 shows results for antioxidant and lipid peroxidation assay carried out in liver. Paracetamol significantly increased ($P \leq 0.05$) MDA level while SOD and catalase activities, and GSH level were reduced, relative to control. Concurrent

administration of paracetamol and extract significantly increased ($P \leq 0.05$) catalase activity, while MDA level was significantly reduced ($P \leq 0.05$), relative to paracetamol – only group.

Table 4: Effects of aqueous HSCE on antioxidants and lipid peroxidation in the liver of mice on chronic paracetamol exposure

Biochemical parameter (liver)	Control	Extract only	Paracetamol only	Concurrent administration of paracetamol and extract
MDA (mol/g tissue)	0.08±0.00d	0.08±0.00d	0.13±0.00a	0.10±0.00c
SOD (Units/mg tissue)	0.06±0.00a	0.06±0.00a	0.04±0.00b	0.04±0.00b
Catalase (Units/g tissue)	7.95±0.24a	8.44±0.30a	5.08±0.14d	6.26±0.01b
Reduced Glutathione (mmol/L)	0.08±0.00a	0.08±0.00a	0.05±0.00b	0.05±0.00b

Values are Mean ± SEM (n=5)

Values with different letters within a row differ significantly from each other ($P \leq 0.05$).

Liver ultrastructure of mice exposed to concurrent administration of extract and paracetamol

The control mouse showed normal architecture of the liver, composed of portal vein (A) and hepatocytes (B), separated by sinusoids (C) (plate 5). The mouse that received extract only showed moderate Kupffer cell activation (A) (plate 6). The mouse that received paracetamol only showed portal congestion (A) and moderate infiltrates of inflammatory cells (B) (periportal hepatitis) (plate 7), while the mouse that received extract and paracetamol concurrently showed mild vascular congestion (A) and focal lymphocytosis (B) (hepatitis) (plate 8).

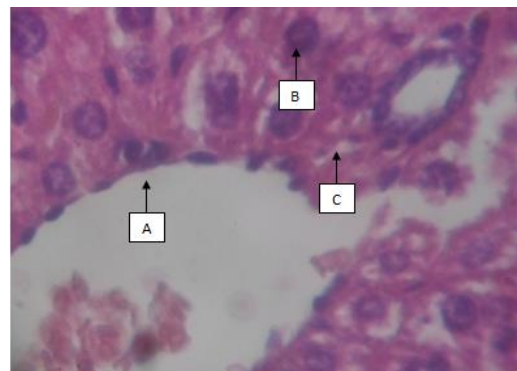


Plate 5: Photomicrograph of control mouse liver (H & E, x400).

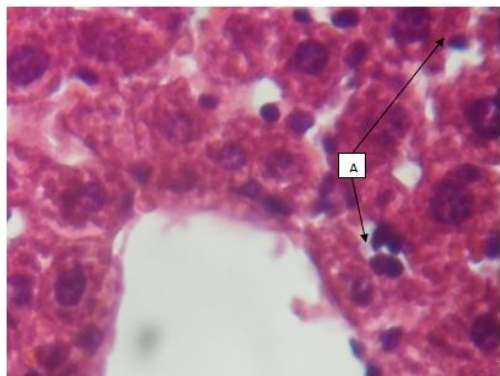


Plate 6: Photomicrograph of liver from mouse given extract only for 16 weeks (H & E, x400).

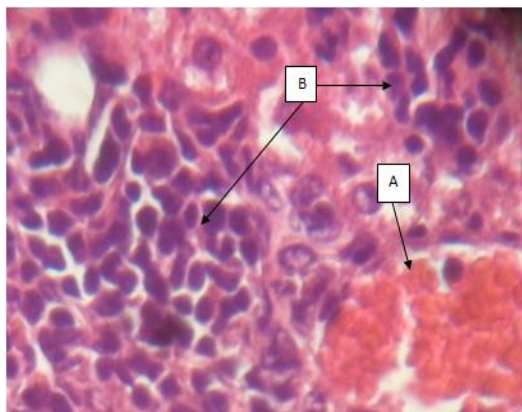


Plate 7: Photomicrograph of liver from mouse given paracetamol only for 16 weeks (H & E, x400).

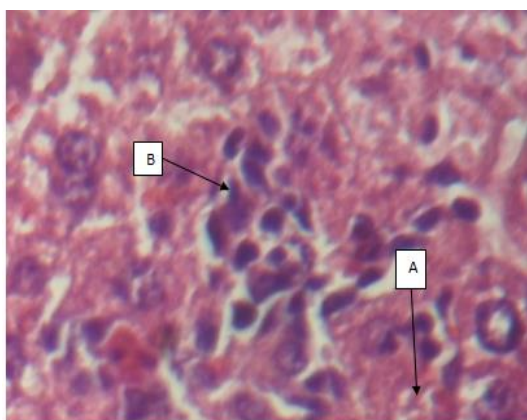


Plate 8: Photomicrograph of liver from mouse concurrently administered paracetamol and extract for 16 weeks (H & E, x400).

Discussion

Medicinal plants are mostly used by under developed countries as alternative therapy [26]. A good number of them are used traditionally in Africa for the management of liver function. Unfortunately, only a few of such African medicinal plants have received scientific scrutiny [26], one of which is *Hibiscus sabdariffa* Linn. This study evaluated the effects of concurrent administration of paracetamol and aqueous extract of *Hibiscus sabdariffa* Linn calyx on liver injury induced in mice by paracetamol under sub-chronic and chronic exposures. In both sub-chronic and chronic phases of this study, paracetamol significantly ($P \leq 0.05$) increased serum cholesterol, direct and total bilirubin levels as well as ALT, AST, ALP and GGT activities while total protein and albumin levels were significantly reduced ($P \leq 0.05$), relative to control (Tables 1 & 3). The increased serum levels of hepatic marker enzymes have

been attributed to liver injury, because these enzymes are in the cytoplasm of the cell and are released into circulation as a result of cell membrane injury [27]. Reduction in total protein level has been reported in previous studies [28]. The metabolism of excess paracetamol in the liver leads to the formation of excess free radicals and the reactive metabolite, n-acetyl-p-benzoquinonimine (NAPQI). The metabolite is believed to form covalent bond with protein thiol groups. Consequently there is disruption of the synthesis of protein and hence reduced total protein level [29]. These results show that paracetamol induced liver dysfunction which is in accordance with previous studies [28]. This damage to the liver was further depicted by the depletion in the levels of GSH, SOD and catalase activities in the liver and increase in MDA. The antioxidant enzymes and GSH, with other peroxidases constitute a supportive team against reactive oxygen species [30, 31] hence the depletion of GSH and catalase could enhance lipid peroxidation [32].

The results showed that paracetamol-induced hepatotoxicity in mice was counteracted by the concurrent administration of paracetamol and HSCE as the values obtained for ALT, cholesterol, GGT, total and direct bilirubin, MDA, SOD and GSH in the sub-chronic phase were not different from control while the remaining parameters (AST, total protein, albumin, alkaline phosphatase and catalase) had results near control and significantly different from the paracetamol-only group. Chronic phase also produced some results near control with only cholesterol and direct bilirubin having similar values as the control group. The mice were more responsive to the treatment in sub-chronic phase.

Interestingly, administration of HSCE alone significantly increased serum albumin level in sub-chronic phase, while serum albumin and total protein levels were significantly increased in chronic phase. This was also reported earlier by [28] who opined that this increase is necessary in forming and repairing new and damaged cells and tissues.

Increase in tissue marker enzymes in serum is usually due to leakage, caused by toxicant – induced membrane damage. So reduction in the level of these enzymes in the serum consequent upon extract administration is an indication that the mechanism for membrane damage was interfered with by the extract. When concurrently administered with paracetamol, the process of ROS production and membrane damage was likely impaired by the bioactive principles in the extract which include protocatechuic acid and anthocyanin [33, 34]. One other possible explanation is that the extract chelates [35] essential metal ions required for proper enzyme activity. In this way, the activity of the enzymes in the extract-treated animals is lowered. There is evidence that metabolism of paracetamol is associated with increased cellular level of hydrogen peroxide production [36]. Hydrogen peroxide is a ready substrate for Fenton and Haber-Weiss reactions which are facilitated by the presence of Fe^{2+} and Cu^{2+} . [37]. It has been reported that anthocyanins are likely to chelate cations [38]. Evidently, if anthocyanins present in *Hibiscus sabdariffa* Linn calyx extract chelate Fe^{2+} and Cu^{2+} , they will impair the Fenton and Haber-Weiss enhanced production of OH \cdot radical and consequently diminish OH \cdot radical associated tissue injury. This is therefore another possible mechanism by which the extract counteracted paracetamol-induced liver injury in mice as demonstrated in this study.

Hepatotoxicity induces depletion of GSH [39] as it is required in the detoxification of toxic compounds. In this study, GSH level was significantly reduced in the paracetamol - treated mice, relative to the control group. It was reduced due to GSH involvement in the conjugation events of the detoxification

process²⁹. GSH level in the cell is usually depleted by N-acetyl-p-benzoquinoneimine (NAPQI) in the event of paracetamol toxicity, with which it forms an excretable complex. The present study has demonstrated that concurrent administration of paracetamol and extract increased GSH level that was decreased in the paracetamol only group to values similar to that of control in the sub-chronic phase. The bioactive agents in the extract which are known antioxidants (anthocyanins, ascorbic acid, quercetin and protocatechuic acid) probably enhanced cellular level of GSH, either by inducing its biosynthesis or by sparing it.

Histopathological examination of liver sections showed that chronic and sub-chronic administration of paracetamol induced necrosis and periportal hepatitis (Plates 3 & 7), while concurrent administration of the drug with extract revealed the protective action of the extract which was more pronounced in the sub-chronic phase (plate 4).

Summary and conclusion

Results showed that paracetamol had toxic effects on the liver, evidenced by changes in tissue GSH and MDA levels as well as SOD and catalase activities. These alterations were reversed by *H. sabdariffa* Linn calyx extract. The extract (250mg/kg) was found to be effective in ameliorating paracetamol induced tissue damage and altered biochemical parameters without itself causing damage.

In conclusion, aqueous extract of *Hibiscus sabdariffa* Linn calyx is hepatoprotective, with this effect being more pronounced at the sub-chronic phase, as shown by the values of the various parameters examined in mice in which paracetamol and extract were concurrently administered.

Conflict Of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This work was carried out in accordance with the standard protocols established by National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by Ethic Committee of the Faculty of Pharmacy, University of Benin, Benin city, Nigeria.

References

- Hanna M, Zylicz Z. Cancer Pain. Springer, 2013; vii:17.
- Daly FF, Fountain JS, Murray L, Graudins A, Buckley NA. Guidelines for the management of paracetamol poisoning in Australia and New Zealand-explanation and elaboration. A consensus statement from clinical toxicologists consulting to the Australasian poisons information centres. The Medical journal of Australia. 2008; 188(5):296-301.
- Gunnell D, Murray V, Hawton K. Use of paracetamol (acetaminophen) for suicide and nonfatal poisoning: worldwide patterns of use and misuse. Suicide & life-threatening behavior. 2000; 30(4):313-26.
- Hinz B, Cheremina O, Brune K. Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. Federation of American Societies for Experimental biology (FASEB) Journal. 2008; 22:383-390.
- Navarro VJ, Senior JR. Drug-related hepatotoxicity. N Engl J Med. 2006; 354:731-739.
- Gautam RD. Sorrel- a lesser known source of medicinal soft drink and food in India. Nat Prod Rad. 2004; 3(5):338-342.
- Ismail A, Ikram EHK, Nazri HSM. Roselle (*Hibiscus sabdariffa* L.) seeds nutritional composition, protein quality and health benefits. Food. 2008; 2(1):1-16.
- Hirunpanich V, Utaipat A, Morales NP, Bunyapraphatsara N, Sato H. Antioxidant effects of aqueous extracts from dried calyx of *Hibiscus sabdariffa* L. (Roselle) *in vitro* using rat low-density lipoprotein (LDL). Biol. Pharm. Bull. 2005; 28:481-484.
- Gaet N. *Hibiscus sabdariffa* L. In: Iva A. ed. Medicinal plants of the world. New York: Human Press, 1999, 165-170.
- Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology 1983; 54(4):275-87.
- Dafallah AA, Mustafa Z. Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. Am J Clin Med. 1996; 24:263-9.
- Dahiru D, Obi OJ, Umaru H. Effect of *Hibiscus sabdariffa* Linn calyx extract on carbon tetrachloride induced liver damage. Biokemistri. 2003; 15:27-33.
- David LE, Steven GG. Principles of Toxicology. In: Casarett & Doull's Essentials of Toxicology. Curtis DK, John BW, editors. 2nd ed. New York: McGraw-Hill, 2010, 9.
- Tabarak M, Devendra KP, Nitu D. Ameliorative potential of aqueous root extract of *Withania somnifera* against paracetamol induced liver damage in mice. Pharmacologia. 2013; 4(2):89-94.
- Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957; 28:56-63.
- Tietz NW. Fundamentals of clinical chemistry. 2nd ed., WB Saunders Company, Philadelphia, 1976, 897.
- Szasz G. A kinetic photometric method for serum gamma glutamyl transpeptidase. Clin Chem. 1969; 22:124-136.
- Trinder P. Enzymatic end point method. Ann Clin Biochem. 1969; 6:24.
- Grant GH. Amino acids and proteins. In: Fundamentals of clinical chemistry. Tietz NW ed., WB Saunders Company, Philadelphia USA, 1987, 328-329.
- Tietz NW. Clinical guide to laboratory tests. 3rd ed., WB Saunders Company, Philadelphia, 1995, 518-519.
- Jendrassik L, Groff P. Determination of direct and total bilirubin in serum. Biochem. 1938; 297:81-89.
- Tietz F. Enzymic method for quantitative determination of nanogram amount of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem. 1969; 27:502-22.
- Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52:302-310.
- Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay of superoxide dismutase. J Biol Chem. 1972; 247:3170-3175.
- Cohen G, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. Anal Biochem. 1970; 34:30-38.
- Bako CV, Dawud G. Drug induced liver disease. Marcel Dekker Inc. New York, USA, 2009, 773.
- Brent JA, Rumack BH. Role of free radicals in toxic hepatic injury II. J Clin Toxicol. 1993; 31:173-196.
- Ahmad-Raus R, Jamal P, Mohd-Isa ES. *Hibiscus sabdariffa* L. aqueous extract prevents progression of acute liver injury induced by acetaminophine. Pertanika Journal of Tropical Agricultural Science. 2012; 35(3):511-520.
- Parkinson A. Biotransformation of xenobiotics. In: Casarett and Doull's Toxicology: The basic science of Poisons. 5th ed. New York, McGraw-Hill, 1996, 14.

30. Bandhopadhyay U, Das D, Banerjee KR. Reactive oxygen 52 species: Oxidative damage and pathogenesis. *Curr. Sci.* 1999; 77:658-665.
31. Tabatabaie T, Floyd RA. Susceptibility of glutathione peroxidase and glutathione reductase to oxidative damage and the protective effect of spin trapping agents. *Archiv. Biochem. Biophys.* 1994; 314:112-119.
32. Anandan R, Deepa-Rekha R, Devaki T. Protective effect of *Picrorrhiza kurroa* on mitochondrial glutathione antioxidant system in D-galactosamine-induced hepatitis in rats. *Curr. Sci.* 1999; 76:1543-1545.
33. Tseng TH, Wang CJ, Kao ES, Chu HY. Hibiscus protocatechuic acid protects against oxidative damage induced by tertbutylhydroperoxide in rat primary hepatocytes. *Chemico-Biological Interactions.* 1996; 101:137-148.
34. Wang CJ, Wang JM, Lin WL, Chu CY, Chou FP. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food and Chemical Toxicology.* 2000; 38:411-6.
35. Ling-Peng D, Xin-Jia D, Hai-Hu M. Antioxidative and chelating properties of anthocyanins in *Azolla imbricata* induced by cadmium. *Polish Journal of Environmental Studies.* 2012; 21(4):837-844.
36. Jamil I, Symonds A, Lynch S, Alalami O. Divergent effects of paracetamol on reactive oxygen intermediate and reactive nitrogen intermediate production by U937 cells. *Int. J. Mol. Med.* 1999; 4(3):309-12.
37. Aruoma OI. Free radicals and antioxidant strategies in sports. *Journal of Nutritional Biochemistry.* 1994; 5:370-381.
38. Edward K, Anna K, Pawel F, Maria K. Effect of anthocyanin on selected biochemical parameters in rats exposed to cadmium. *Acta Biochimica Polonica.* 2003; 50:2/2003.
39. Ohta Y, Sasaki E, Nishida K, Kobayashi T, Nagata M. Preventive effect of Dai-saiko-to (Da-Chai-Hu-Tang) extracts in disrupted hepatic active oxygen metabolism in rats with carbon tetrachloride-induced liver injury. *American Journal Chinese Medicine.* 1995; 23:53-64.