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Research Article

Effects of Concurrent Administration of Paracetamol and Aqueous Extract of *Hibiscus Sabdariffa* Linn Calyx on Paracetamol Hepatotoxicity in Mice.

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ABSTRACT

Paracetamol is a cheap but effective analgesic and antipyretic commonly obtained over the counter. The effects of concurrent administration of paracetamol and aqueous extract of *Hibiscus sabdariffa* Linn calyx on liver injury in mice (induced by paracetamol) under acute and sub-acute conditions were investigated. Paracetamol toxicity was evidenced by significant increases ($P \leq 0.05$) in ALT, AST, ALP and GGT activities as well as total cholesterol level, and decreased serum total protein and albumin levels, relative to control, under acute and sub-acute exposures. There were significant decreases ($P \leq 0.05$) in catalase activity and GSH level, but an increase ($P \leq 0.05$) in MDA level, relative to control, under acute and sub-acute exposures. Concurrent administration of paracetamol with extract significantly reversed the drug-induced alterations in liver function indices. Histopathological examination of liver sections showed paracetamol brought about macrovesicular steatosis, hepatitis and hepatocyte destruction, while concurrent administration of drug with extract produced profound protective action. This study revealed that the extract is effective in ameliorating paracetamol-associated liver injury when administered concurrently in mice. The results suggest the basis of this protection may not be unconnected with the phenolic and other antioxidant bioactive agents reportedly present in the extract such as anthocyanins, protocatechuic acid, quercetin and vitamin C.

Keywords: Paracetamol; *Hibiscus sabdariffa* Linn calyx; acute; sub-acute; concurrent administration; liver.

INTRODUCTION

Paracetamol is a widely used over the counter analgesic and antipyretic [1]. It is a mild analgesic commonly used for the relief of minor aches and pains. It is also a major ingredient in various cold and flu remedies. In the case of patients in whom salicylates and other non - steroidal anti - inflammatory drugs (NSAIDs) are contraindicated, paracetamol is a substitute for acetanilide and phenacetin, as the analgesic - antipyretic of choice. Such cases include people with bronchial asthma, peptic ulcer, hemophilia, salicylate-sensitized, children

under 12 years of age and pregnant or breastfeeding women [2].

The metabolic biotransformation of paracetamol predominantly proceeds through phase II pathways in the liver [3]. The main reaction is hepatic conjugation with glucuronic acid, accounting for about 60% of renally excreted metabolites [3]. Conjugation with sulphate contributes about 35% to urinary metabolites, about 3% of paracetamol is excreted as mercapturic acid, while less than 5% of it is excreted unchanged in urine [3]. The conjugates are not likely to cause organ damage as they are not chemically reactive. However mercapturic acid is formed via the conjugation reaction between N-acetyl–p-benzoquinoneimine (NAP - QI), a cytotoxic electrophile that binds to cellular proteins, and sulphydryl group in glutathione [3]. Glutathione plays an essential role in protecting hepatocytes from injury by chemically reactive metabolites. When low doses of paracetamol are administered, NAPQI favorably combines with glutathione. As the dose is increased, the availability of glutathione in tissues decreases until, at a certain threshold dose, accumulation of NAPQI results in a sharp increase in binding to sulphydryl group in hepatocytes leading to the formation of adducts, arylation of hepatic cellular macromolecules and ultimately centrilobular necrosis ensues [3]. Therefore, glutathione provides the cell with a means of preventing NAPQI from attaining a critical, effective concentration at therapeutic doses of paracetamol. The paracetamol - glutathione conjugate that is formed in the liver is converted to mercapturic acid in the kidneys and excreted in urine [3]. Besides damage to the liver, paracetamol can also induce damage to the kidney medulla. Prostaglandin synthetase, which is more predominant than cytochrome P450 in the kidneys, is involved in the formation of NAPQI via an intermediate free radical, N - acetylbenzosemiquinoneimine which could bind to renal proteins [3].

Hibiscus sabdariffa Linn is an annual dicotyledonous herbaceous tropical plant that belongs to the family Malvaceae [4]. Scientific studies have revealed that there are elements of truth in some of the folkloric claims that its consumption has health benefits. The benefits may not be unconnected with its antioxidant effect [5][6], anti-inflammatory activity [7], anti-hypertensive effect [8][9], anti-diarrheal activity [10] and anti-mutagenic activity [11].

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), ß-carotene (300 µg / 100 g), calcium (1.72 mg / 100 g) and iron (57 mg / 100 g) [12]. Also reported is the presence of antioxidants such as anthocyanin, quercetin and protocatechuic acid [5]. [13] reported the presence of arachidic acid, ß-sitosterol, delphinidin, gossypetin and hibiscetin.

The aim of this 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 acute and sub-acute exposures.

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).

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.

METHODS

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 [7]. The cold extract was filtered and doses corresponding to 250 mg/kg [14] 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: acute (exposure for less than 24 hours) and sub-acute (repeated exposure for 4 weeks) [15]. 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 [16]. *Hibiscus sabdariffa* Linn calyx extract (HSCE) was also administered orally at 250 mg/kg [14].

Acute 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 at zero time and 8 h later. Also, all mice used in this study were sacrificed in less than 24 hours.

Sub - acute 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 once daily for 4 weeks. Group 3 received only paracetamol once daily for 4 weeks while group 4 was concurrently administered paracetamol and extract once daily for 4 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^{0}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) [17], aspartate aminotransferase (AST) [17], alkaline phosphatase (ALP) [18], gamma glutamyl transferase (GGT) [19], total cholesterol [20], albumin [21], total protein [22] and bilirubin (total and direct) [23]. Reduced glutathione (GSH) [24], malondialdehyde (MDA) [25], superoxide dismutase (SOD) [26] and catalase [27] 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 ≤ 0.05 was considered significant.

RESULTS

Effects of aqueous HSCE on acute paracetamol exposure

Liver function parameters

Table 1 shows results for liver function tests carried out on sera samples. Paracetamol significantly increased (P ≤ 0.05) the activities of ALT, AST, ALP, GGT and total cholesterol level but significantly reduced (P ≤ 0.05) total protein and albumin levels, relative to control. Concurrent administration of paracetamol and extract significantly reduced (P ≤ 0.05) the activities of ALT, AST, ALP, GGT, and total cholesterol level while albumin and total protein levels were significantly increased (P ≤ 0.05) relative to paracetamol only group.

Table 1: Effects of HSCE on liver function parameters in mice' serum on acute paracetamol exposure:

Biochemical parameter (serum)	Control	Extract (zero time and 8h later)	Paracetamol (zero time and 8h later)	Concurrent administration (zero time and 8h later)
ALT (U/I)	8.32±0.32b	7.68±0.32b	12.48±0.32a	7.36±0.39b
AST (U/I)	53.20±0.29c	53.27±0.32c	79.03±0.07a	54.60±0.19b
TOTAL PROTEIN (g/dl)	6.40±0.22a	6.42±0.18a	5.04±0.10c	5.68±0.15b
ALBUMIN (g/dL)	3.12±0.08a	3.34±0.12a	2.52±0.02b	3.34±0.12a
TOTAL CHOLESTEROL(mmol/L)	8.44±0.19b	7.92±0.20b	10.48±0.39a	8.57±0.37b
ALP (IU/L)	42.23±0.29c	42.23±0.29c	63.93±0.59a	46.33±0.36b
GGT (U/I)	20.84±2.32b	20.84±2.32b	39.37±2.84a	23.16±0.00b
TOTAL BILIRUBIN (mg/dL)	0.30±0.02a	0.30±0.02a	0.36±0.03a	0.30±0.02a
DIRECT BILIRUBIN (mg/dL)	0.17±0.03a	0.17±0.03a	0.26±0.03a	0.17±0.03a

Values are Mean ± SEM (n=5)
Values with different letters within a row differ significantly from each other (P ≤ 0.05).
Values with same letters within a row do not differ significantly from each other (P ≥ 0.05)

Antioxidants and lipid peroxidation in the liver

Table 2 shows results for antioxidants and lipid peroxidation assays carried out on liver homogenate supernatant. Paracetamol significantly increased (P ≤ 0.05) MDA level in mouse liver while catalase activity and GSH concentration were significantly reduced (P ≤ 0.05), relative to control. Concurrent administration of paracetamol and extract significantly decreased (P ≤ 0.05) MDA level while catalase activity and GSH concentration were significantly increased (P ≤ 0.05), relative to paracetamol only group.

Table 2: Effects of HSCE on antioxidants and lipid peroxidation in mice' liver on acute paracetamol exposure:

Biochemical parameter (liver)	Control	Extract (zero time and 8h later)	Paracetamol (zero time and 8h later)	Concurrent administration (zero time and 8h later)
MDA (mol/g tissue)	0.07±0.00b	0.07±0.00b	0.10±0.00a	0.07±0.00b
SOD (Units/mg tissue)	0.05±0.00a	0.05±0.00a	0.05±0.00a	0.05±0.00a
CAT (Units/g tissue)	7.65±0.00a	7.80±0.00a	5.79±0.00c	7.17±0.00b
GSH (mmol/L)	0.08±0.00a	0.08±0.00a	0.06±0.00b	0.07±0.00a

Values are Mean ± SEM (n=5)
Values with different letters within a row differ significantly from each other (P ≤ 0.05)
Values with same letters within a row do not differ significantly from each other (P ≥ 0.05)

Liver ultrastructure of mice exposed to concurrent administration of paracetamol and extract at the acute phase.

The control mouse showed normal architechure of the liver, composed of portal vein (A) and hepatocytes (B), separated by sinusoids (C) (plate 1). The mouse that received extract only at zero time and 8h later showed mild periportal lymphocytosis (A) and portal dilatation (B) (plate 2). The mouse that received paracetamol at same time interval showed mild hepatocyte fat vacuolation (A) and portal congestion (B) (plate 3), while the mouse that received paracetamol and extract concurrently showed mild kupffer cell activation (A) (plate 4).

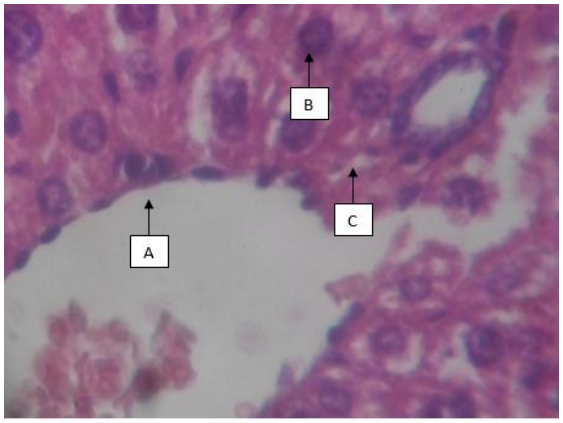

Plate 1: Photomicrograph of control mouse' liver (H & E, x400)

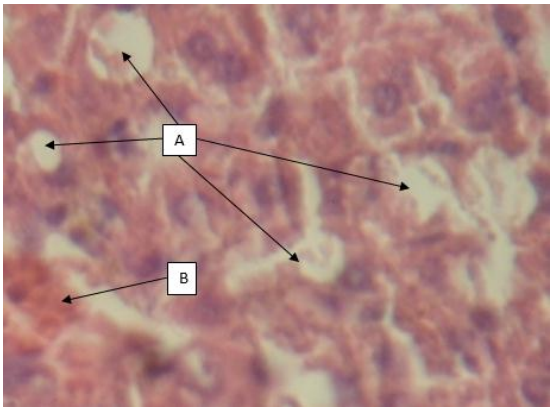

Plate 3: Photomicrograph of liver from mouse given paracetamol at zero time and 8h later (H & E, x400)

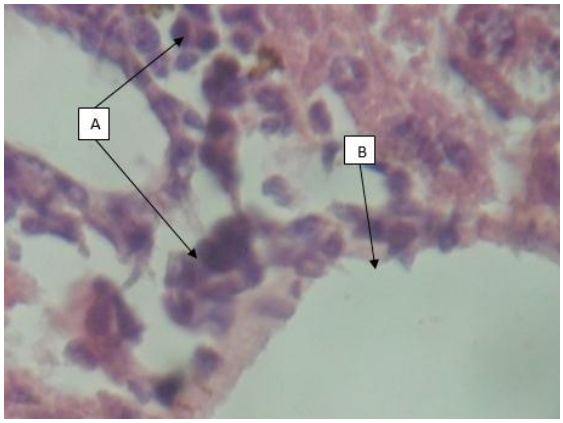

Plate 2: Photomicrograph of liver from mouse given extract at zero time and 8h later (H & E, x400)

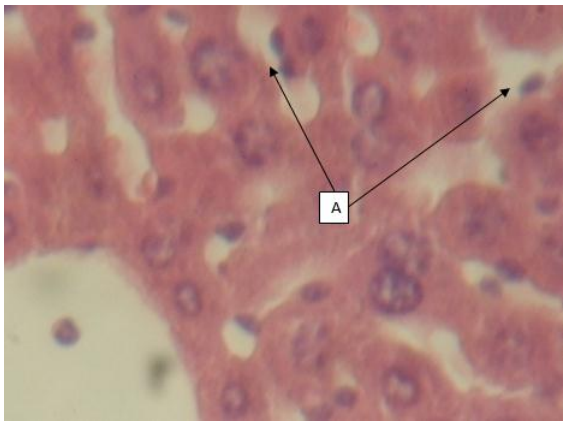

Plate 4: Photomicrograph of mouse' liver concurrently administered paracetamol and extract, zero time and 8h later (H & E, x400)

Effects of aqueous HSCE on sub - acute paracetamol exposure

Liver function parameters

Table 3 shows results for liver function tests carried out on sera samples. Paracetamol significantly increased ($P \leq 0.05$) the activities of ALT, AST, ALP and GGT, the levels of cholesterol and total bilirubin and significantly reduced ($P \leq 0.05$) albumin and total protein levels, relative to control. Concurrent administration of paracetamol and extract significantly reduced ($P \leq 0.05$) the activities of ALT, AST, ALP and GGT, the levels of cholesterol and total bilirubin while albumin and total protein levels were significantly increased ($P \leq 0.05$), relative to paracetamol only group.

Table 3: Effects of HSCE on liver function parameters in mice' serum on sub-acute paracetamol exposure:

Biochemical parameter (serum)	Control	Extract only	Paracetamol only	Concurrent administration of paracetamol and extract
ALT (U/I)	8.32±0.32b	8.32±0.32b	12.16±0.39a	8.64±0.39b
AST (U/I)	53.13±0.26c	52.70±0.20c	77.35±0.35a	61.53±0.26b
TOTAL PROTEIN (g/dL)	6.44±0.03a	6.46±0.02a	5.28±0.14b	6.42±0.06a
ALBUMIN (g/dL)	3.57±0.05a	3.66±0.05a	2.68±0.04b	3.59±0.04a
CHOLESTEROL (mmol/L)	8.88±0.03c	8.75±0.07c	13.73±0.07a	10.64±0.13b
ALP (IU/L)	41.23±0.29c	41.01±0.03c	68.13±0.36a	42.42±0.02b
GGT (U/I)	23.16±0.00b	25.48±2.32b	37.06±2.32a	27.79±2.84b
TOTALBILIRUBIN (mg/dL)	0.34±0.02b	0.32±0.00b	0.45±0.02a	0.34±0.02b
DIRECTBILIRUBIN (mg/dL)	0.17±0.03a	0.17±0.03a	0.26±0.03a	0.17±0.03a

Values are Mean ± SEM (n=5)
Values with different letters within a row differ significantly from each other ($P \leq 0.05$)
Values with same letters within a row do not differ significantly from each other ($P \geq 0.05$)

Antioxidants and lipid peroxidation in the liver

Table 4 shows results for antioxidants and lipid peroxidation assays carried out on liver homogenate supernatant. Paracetamol significantly increased ($P \leq 0.05$) MDA level while SOD and catalase activities, and GSH concentration were significantly reduced ($P \leq 0.05$), relative to control. Concurrent administration of paracetamol and extract significantly reduced ($P \leq 0.05$) MDA level while catalase activity and GSH concentration were significantly increased ($P \leq 0.05$), relative to paracetamol – only group. Also, administration of extract alone caused significant increase ($P \leq 0.05$) in catalase activity relative to control.

Table 4: Effects of HSCE on antioxidants and lipid peroxidation in mice' liver on sub – acute 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	0.12±0.00a	0.08±0.00b
SOD (Units/mg tissue)	0.05±0.00a	0.05±0.00a	0.04±0.00b	0.04±0.00b
CATALASE (Units/g tissue)	7.14±0.03b	7.47±0.05a	5.56±0.03c	7.06±0.02b
REDUCED GLUTATHIONE (mmol/L)	0.08±0.00a	0.08±0.00a	0.06±0.00b	0.08±0.00a

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 paracetamol and extract concurrently at the sub-acute phase.

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

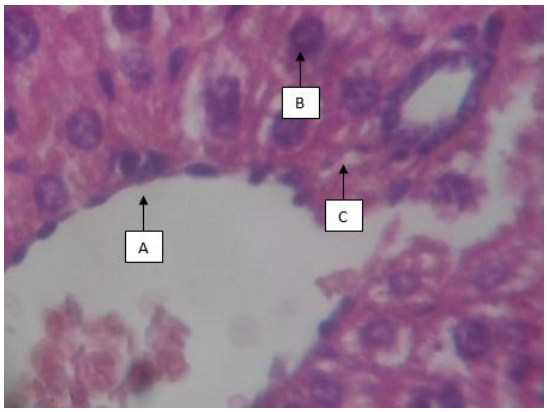

Plate 5: Photomicrograph of control mouse' liver (H & E, x400)

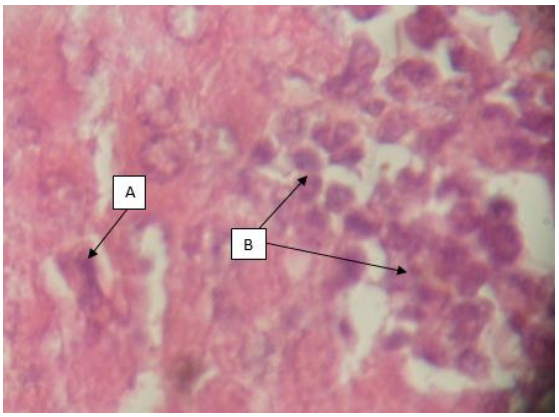

Plate 7: Photomicrograph of liver from mouse given paracetamol only for 4 weeks (H & E, x400)

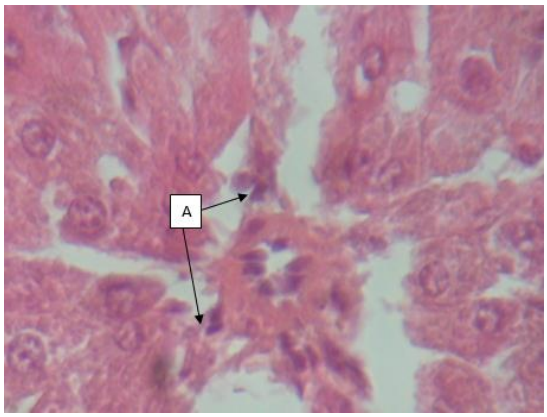

Plate 6: Photomicrograph of liver from mouse given extract only for 4 weeks (H & E, x400)

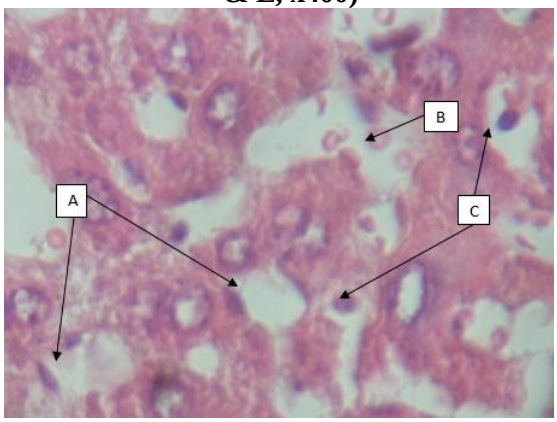

Plate 8: Photomicrograph of liver from mouse concurrently administered paracetamol and extract for 4 weeks (H & E, x400)

DISCUSSION

The consumption of botanicals as alternative medicine has been encouraged because they are relatively cheap and are believed to contribute significantly to the improvement of human health, in terms of prevention and cure of various human disorders. They are also reported to present fewer side effects when compared to orthodox drugs [28]. Among these botanicals of interest is *Hibiscus sabdariffa* Linn which is known as Red Sorrel or Roselle in English and taken as a common local drink popularly known as Zobo in Nigeria. In both acute and sub-acute phases of the study, paracetamol significantly ($P \leq 0.05$) increased serum cholesterol level 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). These were restored close to control on concurrent administration of the drug and extract. The hypocholesterolemic effect of the extract seen in this study is in agreement with earlier studies [29]. They noted that HSCE administration significantly reduced serum cholesterol. In another study, [30] found that the aqueous extract of HSC at 250 mg / kg was able to significantly reduce stress - induced rise in serum cholesterol. This ability may not be unconnected with its chemical constituents such as ascorbic acid [12], saponins [31], ß-sitosterol [13] and quercetin [4], which are believed to possess anti-cholesterolic effect. [32] reported that ß - sitosterol lowers blood cholesterol level by inhibiting intestinal absorption of dietary cholesterol and reabsorption of bile acids. 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 [33].

Although paracetamol is considered safe at therapeutic doses, in overdose, it produces a centrilobular hepatic necrosis that could be fatal [34]. [35] noted that in some individuals,

paracetamol toxicity can result from normal use. They opined that this may be due to individual differences in the expression and activity of certain enzymes in one of the metabolic pathways that handle paracetamol. In this study, indices of liver function in the serum were significantly altered by treatment with paracetamol, 500 mg / kg body weight, twice a day. [36] also reported a significant increase ($P \leq 0.05$) in serum ALT and AST on administration of a single dose of 500 mg / kg body weight of paracetamol to wistar rats.

Antioxidants and lipid peroxidation assays were also carried out in this study. In both phases of the study, the results were remarkable as paracetamol significantly ($P \leq 0.05$) increased MDA in mice liver relative to control (Tables 2 & 4). Concurrent administration of paracetamol and extract reversed the values to that of control in the acute phase (Table 2) and close to control in the sub-acute phase (Table 4). The increased MDA level confirms high lipid peroxidation activity in the affected liver. Interestingly, this increased level was counteracted by giving *H. sabdariffa* extract, which indicates anti - oxidative potency. Reduced MDA levels can be attributed to the polyphenolic nature of the anthocyanin component of *H. sabdariffa* calyx extract. In their presence, abstractable hydrogen atom(s) is obtained from them thereby sparing membrane polyunsaturated fatty acid hydrogen, since loss of membrane polyunsaturated fatty acid hydrogen initiates lipid peroxidation and culminates in MDA. So the presence of the extract caused lower MDA level. This is possible in view of the fact that the extract is rich in anthocyanin [37].

Hepatotoxicity induces depletion of GSH [38] 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 (Tables 2 & 4). It was reduced possibly due to GSH involvement in the conjugation events of the detoxification process [3]. 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. 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. This possibility is conceived because ordinarily, 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, but the findings in this study show that GSH level was enhanced when extract and paracetamol were given to the same set of mice. The above agents are rich in hydroxyl groups and phenolic hydroxyl groups are good hydrogen donors which react with ROS. By so doing, they break the cycle of reaction by which new radicals are produced and propagated. These bioactive agents in the extract may also chelate metal ions (Fe^{2+} and Cu^{2+}) involved in the production of $OH\cdot$ via Fenton and Haber-Weiss reactions, thereby reducing free radical damage [39].

Histopathological examination of liver sections showed that acute administration of paracetamol induced patchy hepatocyte fat vacuolation (macrovesicular steatosis) (Plate 3), while sub-acute administration of paracetamol induced moderate vascular congestion and portal hepatitis (Plate 7). It was also found that the extract activated local immune system of the mouse (Plate 6). Concurrent administration of the drug with the extract revealed the protective action of the later (Plate 4).

SUMMARY AND CONCLUSION

The concern still remains that the extensive use of paracetamol as an analgesic commonly obtained over the counter could lead to severe toxicological effects in man. Hence, the effects of this drug on the liver were investigated in mice at different levels of usage viz, acute and sub – acute to mimic different regimens of use by man. A possible toxicity preventive approach in consumption of *Hibiscus sabdariffa* Linn calyx extract was modelled. Results showed that paracetamol had toxic effects on the liver. This was evidenced by significant changes in tissue GSH and MDA levels. These alterations were reversed by *H. sabdariffa* Linn calyx extract. The extract (250 mg / kg) was found to be effective in ameliorating paracetamol induced tissue damage and altered biochemical parameters without itself causing damage. [14]

reported on the effect of the extract on peroxidative tissue damage. They found that the extract used at 250 mg / kg was more effective than 500 mg / kg in mitigating carbon tetrachloride induced liver injury.

In conclusion, aqueous extract of *Hibiscus sabdariffa* Linn calyx is hepatoprotective as shown by the values of the various parameters examined in mice in which paracetamol and the extract were concurrently administered.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Aghababian RV. Essentials of emergency medicine. Burlington, MA: Jones and Bartlett Publishers; 2010, p 814.
2. Marta J, Jerzy ZN. Paracetamol: mechanism of action, applications and safety concern. Acta Pol Pharm 2014; 71(1):11-23.
3. Parkinson A. Biotransformation of xenobiotics. In: Casarett and Doull's Toxicology, The basic science of Poisons, New York, McGraw-Hill, 1996; p 14.
4. Gautam RD. Sorrel- a lesser known source of medicinal soft drink and food in India. Nat Prod Rad 2004; 3(5):338-342.
5. Hirunpanich V, Utaipat A, Morales NP, Bunyapraphatsara N, Sato H. Antioxidant effects of aqueous extracts from dried calyx of *Hibiscus sabdariffa* L. (Roselle) *invitro* using rat low-density lipoprotein (LDL). Biol Pharm Bull 2005; 28:481-484.
6. Ologundudu A, Obi FO. Prevention of 2, 4-dinitrophenylhydrazine-induced tissue damage in rabbits by orally administered decoction of dried flower of *Hibiscus sabdariffa* Linn. J Med Sci 2005; 5(3):208-211.
7. Dafallah AA, Mustafa Z. Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibicus sabdariffa*. Am J Clin Med 1996; 24:263-269.
8. Adegunloye BJ, Omoniyi JO, Owolabi OA, Ajagbonna OP, Sofola OA. Mechanisms of blood pressure lowering effect of the calyx of *Hibiscus sabdariffa* in rats. Afr J Med Sci 1996; 25:235-238.
9. Onyeneke PC, Ajani EO, Ameh DA, Gamaniel KS. Anti-hypertensive effect of Roselle (*Hibiscus sabdariffa*) calyx infusion in spontaneously hypertensive rats and comparison of its toxicity with that in wistar rats. Cell Biochem Funct 1999; 17:199-206.
10. Salah AM, Gathumbi J, Vierling W. Inhibition of intestinal motility by methanol extracts of *Hibiscus sabdariffa* L. (Malvaceae) in rats. Phytother Res 2002; 16:283-285.
11. Chewonarim T, Kinouchi T, Kataoka K, Arimochi H, Kuwahara T. Effect of Roselle *(Hibiscus Sabdariffa* Linn) a Thai medicinal plant, on the mutagenicity of various known mutagens in *Salmonella typhimurium* and formation of aberrant crypt foci induced by colon carcinogens azoxymethane and 2-amino-1-methyl 6-phenylimidazopyridine in F344 rats. Food Chem Toxicol 1999; 37: 591-601.
12. Ismail A, Ikram EHK, Nazri HSM. Roselle (*Hibiscus sabdariffa* L.) seeds nutritional composition, protein quality and health benefits. Food 2008; 2(1):1-16.
13. Gaet N. *Hibiscus sabdariffa* L. Medicinal plants of the world, New York: Human Press; 1999, p 165.
14. Dahiru D, Obi OJ, Umaru H. Effect of *Hibiscus sabdariffa* Linn calyx extract on carbon tetrachloride induced liver damage. Biokemistri 2003; 15:27-33.
15. David LE, Steven GG. Principles of Toxicology. In: Casarett & Doull's Essentials of Toxicology. New York: McGraw-Hill; 2010, p 9.
16. 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.
17. 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.
18. Tietz NW. Fundamentals of clinical chemistry. 2nd ed. Philadelphia: WB Saunders Company; 1976, p 897.
19. Szasz G. A kinetic photometric method for serum gamma glutamyl transpeptidase. Clin Chem 1969; 22:124-136.
20. Trinder P. Enzymatic end point method. Ann Clin Biochem 1969; 6: 24.
21. Grant GH. Amino acids and proteins. Fundamentals of clinical chemistry.

Philadelphia USA: WB Saunders Company; 1987, p 328.
22. Tietz NW. Clinical guide to laboratory tests. Philadelphia: WB Saunders Company; 1995, p 518.
23. Jendrassik L. Groff P. Determination of direct and total bilirubin in serum. Biochem 1938; 297:81-89.
24. 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; 27:502-522.
25. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52: 302-310.
26. 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.
27. Cohen G, Dembiee D, Marcus J. Measurement of catalase activity in tissue extracts. Anal Biochem 1970; 34: 30-38.
28. Hu X, Sato J, Oshida Y, Yu M, Bajotto G. Effect of Goshajinki-gan (Chinese herbal medicine) on insulin resistance in streptozotocin induced diabetic rats. Diabetes Res Clin Pract 2003; 59:103-111.
29. Emmanuel IN, Vincent CE, Anthony OE, Paul CE, Jude VE. Effects of *Hibiscus sabdariffa* Linn calyx aqueous extract on serum cholesterol, body weight and liver biomarkers of *Rattus Novergicus*. Int J Indigen Med Plants 2013; 46(4):1405.
30. Orji BO, Obi FO. A study on *Hibiscus sabdariffa* Linn calyx extract as a possible anti-stress agent in the rabbit as animal model. Nig J Life Sci 2013; 3(2):74-79.
31. Akanya HO, Oyeleke SB, Jigam AA, Lawal FF. Analysis of sorrel drink (soborodo). Nig J Biochem Mol Biol 1997; 12:77-82.
32. Matsuoka K, Nakazawa T, Honda C, Endo K, Tsukada M. Study of thermodynamic parameters for solubilization of plant sterol and stanol in bile salt micelles. Chem Phys Lipids 2008; 154 (2):87-93.
33. Brent JA, Rumack BH. Role of free radicals in toxic hepatic injury II. J Clin Toxicol 1993; 31:173-196.
34. Prescott LF. Kinetics and metabolism of paracetamol and phenacetin. Br J Clin Pharmacol 1980; 10: 2915-2985.
35. Vuppalanchi R, Liangpunsakul S, Chalasani N. Etiology of new-onset jaundice: how often is it caused by idiosyncratic drug-induced liver injury in the United States? Am J Gastroenterol 2007; 102 (3): 558-562;
36. Mohamed MS, Mohamed AN, Tamer AI. Immunohistochemical and molecular study on the protective effect of curcumin against hepatic toxicity induced by paracetamol in wistar rats. BMC Complem Altern M 2014; 14: 457.
37. Omotuyi IO, Elekofehiriti OO, Ejelonu OC, Obi FO. Mass spectra analysis of *H. sabdariffa* L. anthocyanidins and their in silico corticosteroid-binding globulin interactions. PhOL 2013; 1: 206-217.
38. 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 tetracloride-induced liver injury. Am J Chin Med 1995; 23: 53-64.
39. Aruoma OI. Free radicals and antioxidant strategies in sports. J Nutr Biochem 1994; 5: 370-381.

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