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Amelioration of paracetamol-induced nephrotoxicity in mice by aqueous extract from the calyx of *Hibiscus sabdariffa* Linn.

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ABSTRACT: The impact of co-administration of paracetamol and aqueous extract of *Hibiscus sabdariffa* Linn calyx on nephrotoxicity prompted by paracetamol, under acute and sub-acute treatments was assessed. Four groups, each of them having 5 mice were involved in this study: control, extract-treated, paracetamol-treated and co-treatment with extract and drug. The extract and drug were given by oral route (250mg/kg and 500mg/kg respectively) to the animals. Treatment with paracetamol significantly increased ($P \leq 0.05$) creatinine and urea, and reduced bicarbonate and sodium levels when compared to the control group. Also significantly reduced ($P \leq 0.05$) were superoxide dismutase and catalase activities alongside the level of reduced glutathione, while the value of malondialdehyde was significantly increased ($P \leq 0.05$). Co-administration was seen to attenuate the changes brought about by paracetamol in the parameters studied. Assessment of histopathology of kidney segments indicated that treatment with the drug caused acute tubular necrosis and necrotizing pyelitis while co-treatment of drug with extract provided protection. Co- administration of paracetamol and extract was shown to enhance kidney function in response to toxicity caused by paracetamol in mice. The source of this amelioration may be due to the antioxidant components of the extract which have been widely reported.

Keywords: Acute; co-administration; *Hibiscus sabdariffa* Linn; nephrotoxicity; paracetamol; sub-acute.

Introduction

Paracetamol, also called acetaminophen, is a drug commonly used as painkiller and to manage fever (Aghababian, 2010). It is usually found in medications for flu (Hanna and Zylicz, 2013). Since the drug is easily obtainable in most drug outlets, abuse is prevalent and has been found to cause hepatic and renal damages (Gunnel *et al.,* 2000). Andersson *et al.,* (2011) gave a clue about how paracetamol manages pain. They discovered that N – acetyl – *p* - benzoquinoneimine (NAPQI) (paracetamol metabolite),

prevails on transient receptor potential ankyrin subtype 1 (TRPA1) in the spinal cord to subdue the sensory information coming from the surface of the dorsal horn, to relieve ache.

Metabolism of paracetamol following ingestion takes place largely in the liver by glucuronidation (approximately 63%) and sulphation (34%). At therapeutic doses of paracetamol, NAPQI is cleared by glutathione (Dahlin *et al.,* 1984). NAPQI has been held accountable for harmful effects of paracetamol (Waring, 2012). When the drug is taken in excess, intracellular glutathione levels are diminished and NAPQI increases leading to death of tissue (Bessems and Vermeulen, 2001). This also leads to proliferation of reactive species. The harmful effects of Paracetamol include severe tubular necrosis, which is the key source of kidney failure (Blantz, 1996).

There has been growing interest in the intake of plants and plant products due to their health benefits and lower adverse reactions when compared to orthodox treatments (Hu *et al.,* 2003). *Hibiscus sabdariffa* Linn is one of the plants which have attracted interest due to the bioactive agents found in it. *Hibiscus sabdariffa* Linn is called Roselle and well known for a drink Nigerians call Zobo. Its calyces have been found to contain nutrients such as protein, fat, carbohydrates, fiber, vitamin C, β-carotene, calcium and iron (Ismail *et al.,* 2008). Also reportedly found in the calyces are antioxidants which include anthocyanin, quercetin and protocatechuic acid (Hirunpanich *et al.,* 2005).

This study aimed to elucidate the effects of acute and sub-acute co-treatment of paracetamol and *Hibiscus sabdariffa* Linn calyx extract on nephrotoxicity brought about by treatment with paracetamol, using the mice as experimental model.

Materials and Methods

Materials

Reagents and chemicals used in this study were of analytical grade.

Plant

Hibiscus sabdariffa Linn calyces were obtained from Karu market, Abuja, FCT. Identification of the plant was carried out at the Department of Plant Biology and Biotechnology, University of Benin, Benin City by Mr. Joseph Erhabor. Thereafter, a sample (identification number UBHm 0261) was placed at the Herbarium, University of Benin, Benin City.

Animals

Forty mice weighing between 27 and 32g, gotten from a breeder in Benin City, were used for this study. They were kept in cages made of wood in the Department of Biochemistry, University of Benin animal house. They were allowed two weeks for adaptation before the start of study. Also, unrestricted access to tap water and food (Growers mash, Bendel Feeds and Flour Mills Ltd, Ewu, Edo State) was granted to them.

Ethical Approval

This study was done in line with the conventional procedures recognized 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.

Plant extract preparation

Dry calyces of *Hibiscus sabdariffa* Linn were crushed into suitable particles, which was left to soak in distilled water (1:3w/v) for 24 hours at 4°C (Dafalla and Mustafa, 1996). The solution was sifted and doses equivalent to 250 mg/kg (Dahiru *et al.,* 2003) were prepared.

Preparation of paracetamol

Paracetamol base powder (Huang Gang Yin He Aati Pharmaceutical Co. Ltd. China) was made available by Late by Dr. G. C. Josephs (Department of Pharmaceutical Microbiology and Biotechnology, University of Benin). The powder was dissolved completely in dimethyl sulfoxide (DMSO) (2.5% aqueous solution of DMSO), after which distilled water was added to make up the necessary measure.

Experimental design and treatment schedule

This work comprised of two (2) categories: acute (contact for not more than 24 hours) and sub-acute (contact for 4 weeks). Each category was made up of four (4) groups: control, extract only, paracetamol only and concurrent administration of paracetamol and extract. There were 5 mice in each group. Paracetamol was administered orally (500 mg/kg body weight); *Hibiscus sabdariffa* Linn calyx extract (HSCE) was also administered orally at 250 mg/kg.

Acute Study

Twenty mice which were distributed into four groups were used in this study. Group 1, the control was administered aqueous DMSO. Animals in the 2nd group were given extract only (zero time and 8h later). The 3rd group received paracetamol only (zero time and 8h later), and in the 4th group, there was concurrent administration of drug and extract (zero time and 8h later). At the end of the treatments, the mice were sacrificed in 24 hours.

Sub-acute Study

Twenty mice, grouped into four were also used in this study. The control, group 1 was given only aqueous DMSO. Group 2 was given extract only once a day, group 3 paracetamol only once a day and group 4 received concurrent administration of paracetamol and extract once a day. The treatment lasted for 4 weeks after which all the animals were sacrificed.

Collection and preparation of samples for analyses

After sedating the mice with chloroform, blood was taken by heart puncture and placed in plain sample bottles. Clotted blood samples were centrifuged at 4,000 rpm for 10 minutes in order to obtain sera which were stored at -20º. The kidney was also removed and rinsed in very cold saline. Kidneys from mice in applicable groups were fixed for histopathological investigations. Also for biochemical analyses, a measured weight of the tissue was homogenized in phosphate buffered saline (PBS) 50mM pH 7.4, centrifuged at 3500rpm for 15 minutes to obtain the supernatant which was employed.

Biochemical analyses

Analyses conducted on serum include creatinine (Bartels *et al.*, 1972), urea (Weatherburn, 1967), bicarbonate (Tietz *et al.*, 1986), sodium ion (Maruna, 1958; Trinder, 1951), chloride (Skeggs and Hochstrasser, 1964) and potassium (Terri and Sesin, 1958). Reduced glutathione (GSH) (Tietz, 1969), malondialdehyde (MDA) (Buege and Aust, 1978), superoxide dismutase (SOD) (Misra and Fridovich, 1972) and catalase (Cohen *et al.*, 1970) were carried out on kidney homogenate supernatant.

Statistical analysis

Data obtained were presented as mean ± S.E.M. Analysis for significance was done by one way ANOVA and mean values that differed significantly were identified using the Duncan's multiple range test. $P \leq 0.05$ was considered significant.

Results

Effects of HSCE on acute paracetamol exposure

Kidney function parameters

Table 1 shows results for tests conducted on serum to ascertain kidney function. Creatinine and urea were significantly increased ($P \leq 0.05$) while bicarbonate and sodium were significantly decreased ($P \leq 0.05$) in the groups administered only paracetamol, relative to control. Co – administration of extract and paracetamol significantly reduced ($P \leq 0.05$) creatinine and urea while bicarbonate was significantly increased ($P \leq 0.05$), relative to paracetamol only group.

Table 1: Effects of aqueous HSCE on kidney function parameters in serum of mice 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)
Creatinine (mg/dL)	9.10±0.00b	9.36±0.26b	10.92±0.32a	9.62±0.32b
Urea (mmol/L)	5.96±0.26b	6.16±0.20b	9.59±0.30a	6.14±0.20b
Bicarbonate (mmol/L)	44.26±0.14a	44.13±0.17a	41.83±0.17b	44.13±0.17a
Sodium (mEq/L)	182.93±0.39a	182.93±0.39a	174.41±0.49c	180.55±0.35b
Chloride (mEq/L)	92.44±0.16a	92.50±0.22a	93.08±0.22a	92.57±0.12a
Potassium (mEq/L)	3.90±0.06a	3.87±0.05a	4.00±0.06a	3.88±0.07a

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 kidney

Table 2 shows results for antioxidant and lipid peroxidation assays carried out in the kidney. Relative to control, MDA was significantly increased ($P \leq 0.05$) in the paracetamol treated group. SOD was significantly reduced ($P \leq 0.05$) in the paracetamol only while co – administration significantly increased ($P \leq 0.05$) SOD, relative to paracetamol only group. Paracetamol also significantly ($P \leq 0.05$) reduced GSH concentration relative to control, while co – administration significantly increased ($P \leq 0.05$) GSH concentration relative to paracetamol only group.

Table 2: Effects of aqueous HSCE on antioxidants and lipid peroxidation in the kidney of mice on acute paracetamol exposure

Biochemical parameter (kidney)	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	0.09±0.00a	0.07±0.00b
SOD (Units/mg tissue)	0.06±0.00a	0.06±0.00a	0.05±0.00b	0.06±0.00a
CAT (Units/g tissue)	7.31±0.00a	7.44±0.00a	5.85±0.00c	6.79±0.00b
GSH (mmol/L)	0.07±0.00a	0.07±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 ($P \leq 0.05$).

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

The control mouse showed normal architecture of the kidney, composed of glomerulus (A) and tubules (B), separated by interstitial space (C) (plate 1). The mouse that received extract only at zero time and 8h later showed mild interstitial congestion (A) (plate 2). The mouse that received paracetamol only at same time interval showed focal cloudy swelling of tubular epithelial cells (A) and narrowing of lumen (B) (plate 3), while the mouse that received extract and paracetamol simultaneously (co-administration) at same time interval showed patent tubular lumen (A) (plate 4).

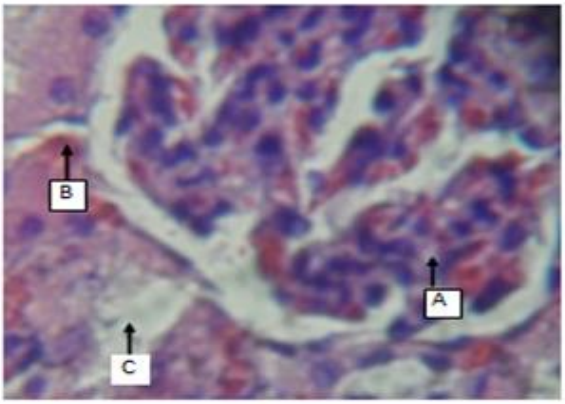

Plate 1: Photomicrograph of control mouse' kidney (H & E, x400).

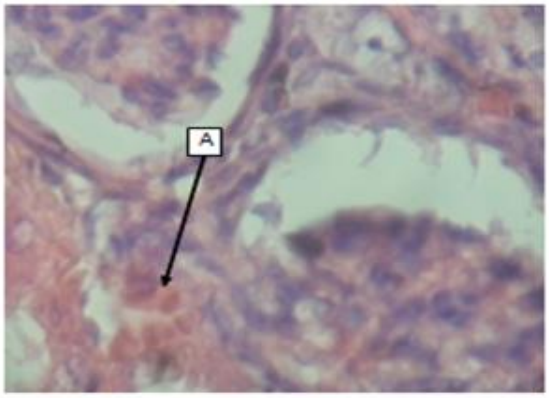

Plate 2: Photomicrograph of kidney from mouse given extract at zero time and 8h later (H & E, x400).

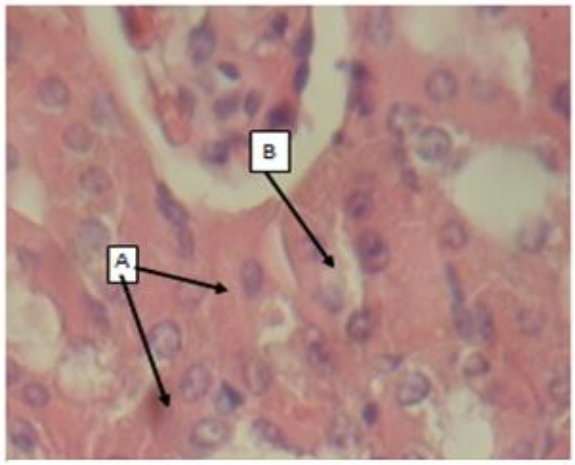

Plate 3: Photomicrograph of kidney from mouse given paracetamol at zero time and 8h later (H & E, x400).

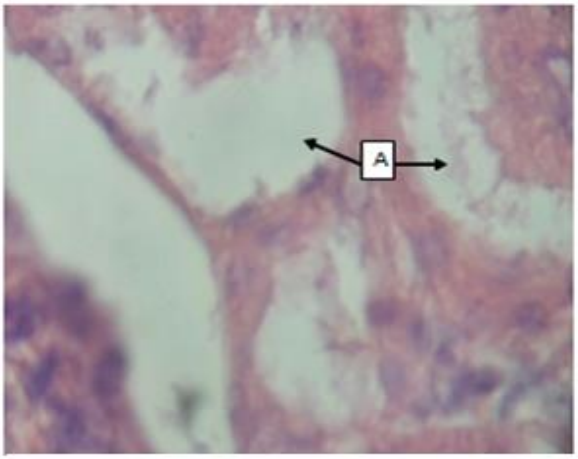

Plate 4: Photomicrograph of kidney from mouse co-administered paracetamol and extract at zero time and 8h later (H & E, x400).

Effects of HSCE on sub-acute paracetamol exposure

Kidney function parameters

Table 3 shows results for tests conducted in serum to assess kidney function. Paracetamol significantly increased ($P \leq 0.05$) urea, creatinine and chloride levels while bicarbonate and sodium were reduced, relative to control. Co - administration of extract and paracetamol significantly reduced ($P \leq 0.05$) creatinine and chloride while bicarbonate and sodium were increased, relative to paracetamol - only group.

Table 3: Effects of aqueous HSCE on kidney function parameters in serum of mice on sub – acute paracetamol exposure

Biochemical parameter (serum)	Control	Extract only	Paracetamol only	Co-administration of paracetamol and extract
Creatinine (mg/dL)	9.07±0.23b	9.07±0.23b	11.16±0.19a	9.64±0.19b
Urea (mmol/L)	5.76±0.03c	5.73±0.04c	9.42±0.23a	6.16±0.05b
Bicarbonate (mmol/L)	43.54±0.17a	43.71±0.00a	41.31±0.17b	43.85±0.14a
Sodium (mEq/L)	185.07±0.48a	184.68±0.48a	155.93±0.25b	185.46±0.39a
Chloride (mEq/L)	92.50±0.16b	92.69±0.06b	93.40±0.22a	92.76±0.08b
Potassium (mEq/L)	3.90±0.05a	3.90±0.04a	4.01±0.06a	3.90±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 kidney

Table 4 shows results for antioxidants and lipid peroxidation assays carried out in the kidney. Paracetamol significantly increased ($P \leq 0.05$) MDA while SOD and catalase activities, and GSH level were reduced, relative to control. Co – administration of paracetamol and extract significantly increased ($P \leq 0.05$) catalase activity and GSH level, relative to paracetamol – only group. Also, co-administration significantly decreased MDA relative to paracetamol only group, though still significantly higher than the control. For catalase, co-administration returned values to control while treatment with extract caused a significant increase.

Table 4: Effects of aqueous HSCE on antioxidants and lipid peroxidation in the kidney of mice on sub - acute paracetamol exposure:

Biochemical parameter (serum)	Control	Extract only	Paracetamol only	Co-administration of paracetamol and extract
MDA (mol/g tissue)	0.07±0.00c	0.06±0.00c	0.12±0.00a	0.08±0.00b
SOD (Units/mg tissue)	0.06±0.00a	0.06±0.00a	0.04±0.00c	0.05±0.00b
CATALASE Units/g tissue)	7.25±0.04b	7.73±0.01a	5.21±0.03c	7.15±0.04b
REDUCED GLUTATHIONE (mmol/L)	0.07±0.00a	0.07±0.00a	0.05±0.00b	0.07±0.00a

Values are Mean ± SEM (n=5)
Values with different letters within a row differ significantly from each other ($P \leq 0.05$).

Kidney ultrastructure of mice exposed to concurrent administration of paracetamol and extract at the sub-acute phase.

The control mouse showed normal architecture of the kidney, composed of glomerulus (A) and tubules (B), separated by interstitial space (C) (plate 5). The mouse that received extract only showed unremarkable glomerulus (A) and tubules (B) (plate 6). The mouse that received paracetamol only showed patchy tubular cloudy swelling (A) and focal infiltrates of inflammatory cells (B) (necrotizing pyelitis) (plate 7) while the mouse that received extract and paracetamol simultaneously (co-administration) showed patchy tubular cell cloudy swelling (A) and interstitial congestion (B) (plate 8).

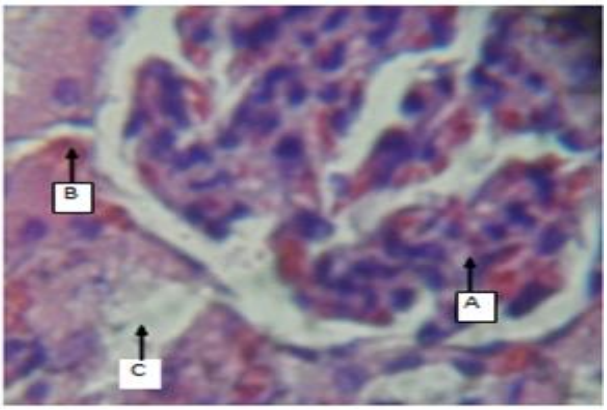

Plate 5: Photomicrograph of control mouse' kidney (H & E, x400).

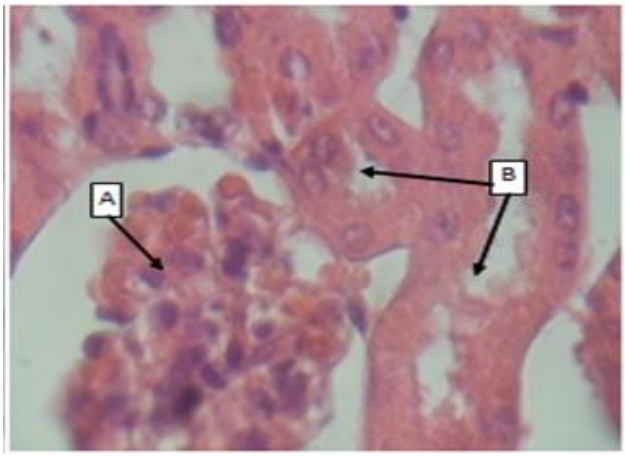

Plate 6: Photomicrograph of kidney from mouse given extract only for 4 weeks (H & E, x400).

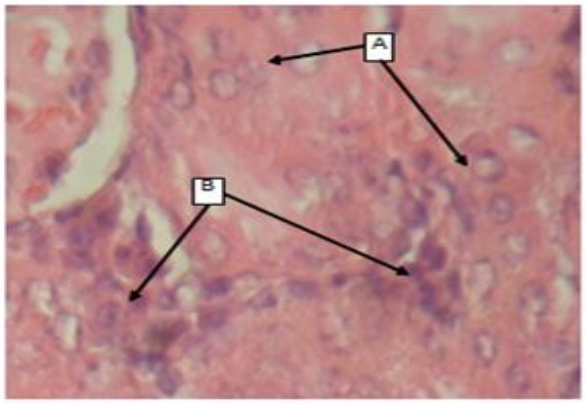

Plate 7: Photomicrograph of kidney from mouse given paracetamol only for 4 weeks (H & E, x400).

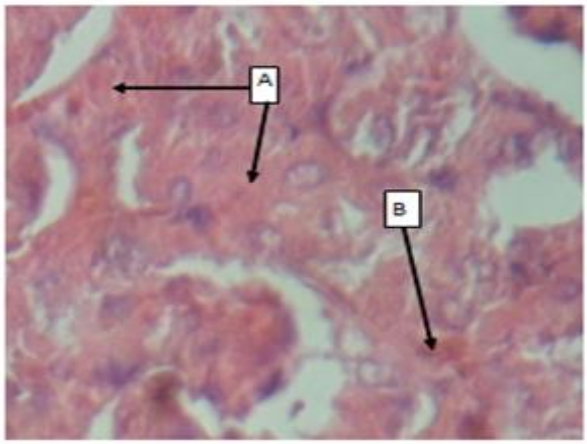

Plate 8: Photomicrograph of kidney from mouse co-administered paracetamol and extract for 4 weeks (H & E, x400).

Discussion

Paracetamol, also called acetaminophen, belongs to a subgroup of analgesics called aniline derivatives and is very effective in the relief of mild fever and pains. It is understandably very safe when given in the correct therapeutic dose but its overdose remains the major cause of liver injury and even death in many parts of the world among all drug toxicities (Linda *et al.*, 2009; Yayla *et al.*, 2014). Paracetamol undergoes metabolic activation by hepatic microsomal cytochrome P450 mixed function oxidase system (mainly the enzyme CYP2E1) to N-acetyl-P-benzoquinone imine (NAPQI). The active metabolite (being highly electrophilic) binds quickly to intracellular proteins, leading to both structural and functional changes (Monira *et al*., 2012). NAPQI is the active metabolite at the center of virtually all the metabolic disorders experienced when an overdose of acetaminophen occurs. The mechanism by which paracetamol ingestion causes nephrotoxicity is less described unlike its induction of hepatotoxicity. With reference to information from clinical and animal studies, some of the potential mechanisms of nephrotoxicity are the cytochrome P-450 pathway, prostaglandin synthesis and *N*-deacetylase enzymes

(Bessems and Vermeulen, 2001). These enzymes are very sensitive markers employed in the diagnosis of kidney diseases.

In the assessment of kidney injury, levels of urea and creatinine in serum should be first determined. From the results shown in Table 1 and Table 3 on the effects of HSCE on acute and sub-acute paracetamol exposure in kidney function parameters, Creatinine and urea were significantly increased ($P \leq 0.05$) on both acute and sub-acute paracetamol exposure. This finding was in tandem with the work of Dogukan *et al.,* (2016) who reported a significant increase in the urea and creatinine levels of rats exposed to paracetamol. These increased levels of urea and creatinine may be indicators of acute tubular necrosis (Adebayo *et al.,* 2003; Yakubu *et al.,* 2003). Bicarbonate and sodium were significantly decreased ($P \leq 0.05$) in the groups administered paracetamol only, relative to control in the acute and sub-acute paracetamol exposure. This was also similar to the work of Pakravan *et al.,* (2015) who reported an alteration in the electrolyte levels of rats exposed to acetaminophen at toxic levels. Co – administration of extract and paracetamol led to opposite effects for urea, creatinine and electrolytes as observed when only paracetamol was administered. These obvious differences could be as a result of the actions of the phytochemical constituents of the extract such as flavonoids in inhibiting the actions of the toxic metabolite NAPQI and also stabilizing the cell membranes of the intracellular proteins and other compounds (Parker *et al.,* 2017).

From the results shown in Table 2 and Table 4 on the effects of HSCE on acute and sub-acute paracetamol exposure in antioxidant and lipid peroxidation parameters, interesting observations were made. The generation of hydroxyl radical and other powerful radicals can initiate a chain reaction of lipid peroxidation in which polyunsaturated fatty acids are converted into lipid peroxides. Malondialdehyde (MDA) is a major indicator of lipid peroxidation (Nielsen *et al.,* 1997). In the present study, MDA was significantly increased ($P \leq 0.05$) compared to control in the paracetamol treated group for the acute and sub-acute paracetamol exposed group. This agreed with the work of Zoubair *et al.,* (2013) who reported increased MDA levels in oxidative stressed mice exposed to paracetamol and hydrogen peroxide. Increase in the levels of MDA could be as a result of cellular membrane damage initially caused by an increase in radical formation (Niedernhofer *et al.,* 2003), in this case, by the actions of the toxic metabolite NAPQI. However, treatment with *H. sabdariffa* aqueous extract caused a significant decrease ($p<0.05$) in the MDA concentration of the treatment group.

Likewise, SOD, catalase and GSH were significantly reduced ($P \leq 0.05$) in the paracetamol only group for the both the acute and sub-acute exposure. Co – administration significantly increased ($P \leq 0.05$) SOD, catalase and GSH relative to paracetamol only group. This result is consistent with the finding of Parker *et al.,* (2017) who observed an increase in the above mentioned parameters following treatment with aqueous extracts of *H. sabdariffa* after acetaminophen-induction. The protective effect observed appears to be due to the antioxidant properties of this plant (Liu *et al*., 2006). It has been recorded that the aqueous extract of *Hibiscus sabdariffa* is enriched in high antioxidant constituents, mainly flavonoids and vitamin C (Hirunpanich *et al*., 2006), which serves as an antioxidant and a reductant (Wang *et al.,* 2000). The histological examination on the kidney tissue supported our results obtained.

In conclusion, administration of paracetamol at toxic levels has been shown to have deleterious effects on the kidney of mice. However, its co-administration with aqueous extract of *H. sabdariffa* has shown that the extract contains some compounds that are effective against the reactive oxygen species generated by paracetamol toxicity, thereby restoring the nephrotic structure and integration close to its original state.

References

Adebayo JO, Yakubu MT, Egwim CE, Owoyele BV, Enaibe BU. 2003. Effect of ethanolic extract of *Khaya senegalensis* on some biochemical parameters of rat kidney. Journal of Ethnopharmacology. 88: 69-72.

Aghababian RV. 2010. Essentials of Emergency Medicine. Jones and Bartlett Publishers. p. 814.

Andersson DA, Gentry C, Alenmyr L, Killander D. 2011. TRPA1 mediates spinal antinociception induced by acetaminophen and the cannabinoid Δ(9)-tetrahydrocannabiorcol. Nature Communications. 2:551.

Bartels H, Bohmer M, Heierli C. 1972. Serum creatinine determination without protein precipitation. Clin Chim Acta. 37:193-7.

Bessems JG, Vermeulen NP. 2001. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit Rev Toxicol. 31:55–38.

Blantz RC. 1996. Acetaminophen: acute and chronic effects on renal function. Am J Kidney Dis. 28:S3–S6.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. Methods in Enzymology.52:302-310.

Cohen G, Dembiee D, Marcus J. 1970. Measurement of catalase activity in tissue extracts. Analytical Biochemistry. 34: 30-38.

Dafallah AA, Mustafa Z. 1996. Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibicus sabdariffa*. American Journal of Clinical Medicine. 24:263-9.

Dahiru D, Obi OJ, Umaru H. 2003. Effect of Hibiscus sabdariffa Linn calyx extract on carbon tetrachloride induced liver damage. Biokemistri. 15:27-33.

Dahlin DC, Miwa GT, Lu AY, Nelson SD. 1984. *N*-Acetyl-*p*-benzoquinone imine: a cytochrome p-450-mediated oxidation product of acetaminophen. Proc Natl Acad Sci USA. 81:1327–1331.

Dogukan C, Yasin B, Nurcan KB, Esen SK, Hasan TA, Fatma BKO, Mevlut SK, Zekai H. 2016. Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of *Nigella sativa.* Pharmaceutical Biology. 54:10, 2082-2091.

Gunnell D, Murray V, Hawton K. 2000. Use of paracetamol (acetaminophen) for suicide and nonfatal poisoning: worldwide patterns of use and misuse. Suicide and Life- threatening Behavior. 30 (4): 313–26.

Hanna M, Zylicz Z. 2013. Cancer Pain. Springer. p. 17.

Hirunpanich V, Utaipat A, Morales NP, Bunyapraphatsara N, Sato H, Herunsale A, Suthisisang C. 2006. Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. J Ethnopharmacol. 103:252–260.

Hirunpanich V, Utaipat A, Morales NP, Bunyapraphatsara N, Sato H. 2005. Antioxidant effects of aqueous extracts from dried calyx of *Hibiscus sabdariffa* L. (Roselle) *in vitro* using rat low-density lipoprotein (LDL). Biological and Pharmaceutical Bulletin. 28:481-484.

Hu X, Sato J, Oshida Y, Yu M, Bajotto G. 2003. Effect of Goshajinki-gan (Chinese herbal medicine): Niu-che-sen-qi-wan) on insulin resistance in streptozotocin induced diabetic rats. Diabetes Reearch and Clinical Practice. 59:103-111.

Ismail A, Ikram EHK, Nazri HSM. 2008. Roselle (*Hibiscus sabdariffa* L.) seeds nutritional composition, protein quality and health benefits. Food. 2(1): 1–16.

Linda J, Chun BS, Myon J. 2009. Acetaminophen hepatotoxicity and acute liver failure. J Clin Gastroenterol, 43:342-349.

Liu JY, Chen CC, Wang WH, Hsu JD, Yang MY, Wang CJ. 2006. The protective effects of *Hibiscus sabdariffa* extract on CCl4-induced liver fibrosis in rats. Food and Chemical Toxicology. 44 (3): 336-343.

Maruna RFL. 1958. Determination of serum sodium by magnesium uranyl acetate. Clinica Chimica Acta. 2: 581 – 585.

Misra HP, Fridovich I. 1972. The role of superoxide anion in the autooxidation of epinephrine and a simple assay of superoxide dismutase. Journal of Biological Chemistry. 247: 3170-3175.

Monira A, Kader AE, Naima ZM. 2012. Evaluation of protective and antioxidant activity of Thyme (*Thymus vulgaris*) extract on paracetamol-induced toxicity in rats. Australian Journal of Basic and Applied Sciences. 6(7):467-474.

Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. 200.3 Malondialdehyde a product of lipid peroxidation, is mutagenic in human cells. J Biol Chem. 278: 31426-31433.

Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. 1997. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clin Chem. 43.1209-1214.

Pakravan N, Shokrzadeh M, Akbari F, Shadboorestan A. 2015. Effect of a toxic dose of acetaminophen on electrolytes and histopathological changes in the Kidney. International Journal of Clinical Toxicology. 2: 64-70.

Parker EJ, Chuma SE, Chimere YU, Joshua OO, Prisca CO, Chinelo CN, Florence ON, Amaechi LO, Chibuike SU. 2017. Hepatocurative effect of aqueous extract of *Hibiscus sabdariffa* on some antioxidants and haematological indices of acetaminophen-challenged wistar albino rats. African Journal of Pharmacy and Pharmacology. 11(20): 250-259.

Skeggs LT, Hochstrasser HC. 1964. Thiocyanate (colorimetric) method of chloride estimation. Clinical Chemistry. 10: 918-920.

Terri AE, Sesin PG. 1958. Determination of serum potassium by using sodium tetraphenylboron method. American Journal of Clinical Pathology. 29: 86 – 90.

Tietz NW, Pruden EL, Siggard-Anderson O. 1986. Electrolytes, blood gases, and acid-base balance. In: Textbook of Clinical Chemistry- Tietz NW, editor. Saunders, Philadelphia. P. 1188.

Tietz F. 1969. Enzymic method for quantitative determination of nanogram amount of total and oxidized glutathione: applications to mammalian blood and other tissues. Analytical Biochemistry. 27:502-22.

Trinder P. 1951. A rapid method for the determination of sodium in serum. Analyst. 76: 596 –599.

Wang CJ, Wang JM, Lin WL, Chu CY, Chou FP, Tseng TH. 2000. Protective effect of *Hibiscus* anthocyanins against tertbutyl hydroperoxide-induced hepatic toxicity in rats. Food Chem Toxicol. 38:411–416.

Waring WS. 2012. Novel acetylcysteine regimens for treatment of paracetamol overdose. Ther Adv Drug Saf. 3:305–315.

Weatherburn MW. 1967. Colorimetric Urease-Berthelot method of urea estimation. Annals of Chemistry. 39: 971.

Yakubu MT, Bilbis LS, Lawal M, Akanji MA. 2003. Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. BIOKEMISTRI. 15(2): 50-56.

Yayla M, Halici Z, Unal B, Bayir Y, Akpinar E, Gocer F. 2014. Protective effect of et-1 receptor antagonist bosentan on paracetamol induced acute liver toxicity in rats. Eur J Pharmacol.
726:87–95.

Zoubair B, Azzahra LF, Fouzia H, Mohammed L, Brahim B, Noureddine B. 2013. Evaluation of Acetaminophen Effect on Oxidative Stressed Mice by Peroxide Hydrogen. American Journal of Biomedical Research. 1(4): 75-79.