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# EFFECTS OF CHRONIC EXPOSURE TO PARACETAMOL AND *HIBISCUS SABDARIFFA* LINN CALYX EXTRACT ON THE KIDNEY OF MICE.

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**ABSTRACT**
This study was conducted to evaluate the effects of concurrent administration of aqueous extract of *Hibiscus sabdariffa* Linn calyx and paracetamol on kidney injury induced by chronic exposure to paracetamol in mice. The study was carried out over 16 weeks. The animals were grouped into 4 of 5 mice each: control (paracetamol and extract free), extract only, paracetamol only and concurrent administration of paracetamol with extract. The drug and extract were administered orally at 500 and 250 mg/kg body weight respectively by means of an oral gavage. In order to assess kidney function, electrolytes (sodium, bicarbonate, potassium and chloride), urea and creatinine were assessed in serum. Malondialdehyde (MDA) and antioxidant status (superoxide dismutase and catalase activities, and reduced glutathione level) were estimated in kidney homogenate. Histopathological examinations of kidney sections were also carried out on the mice. Paracetamol toxicity was confirmed by significant increase ($P \leq 0.05$) in urea and creatinine levels along with altered electrolyte concentrations. Concurrent administration of paracetamol with aqueous extract of *Hibiscus sabdariffa* Linn calyx significantly ($P \leq 0.05$) reversed the drug-induced alterations close to control, in most of the parameters assayed. The antioxidant activity of the extract is most likely due to its strong scavenging effect on reactive oxygen species and free radicals.

**Keywords:** Chronic exposure, concurrent administration, *Hibiscus sabdariffa* Linn calyx extract, kidney, paracetamol.

## INTRODUCTION

Paracetamol is the most frequently used over-the-counter (OTC) medication, having been discovered over 100 years ago, with its use as OTC medication beginning in the 1960s (Cranswick and Coghlan, 2000). Paracetamol is a mild analgesic used in the treatment of headaches and other minor pains. It is an effective analgesic and antipyretic (Lee, 2017). Paracetamol is easily available, hence, ill-use is expected and usually causes damage to the liver and kidneys. Intentional poisoning is commonly associated with paracetamol toxicity (Gunnell *et al.*, 2000). Paracetamol is a commonly used antipyretic agent which, in high doses, causes renal tubular damage and uremia. An approach by which damage caused by paracetamol poisoning could be lessened is vending paracetamol mixed with emetics or antidotes (Dargan and Jones, 2003).

*Hibiscus sabdariffa* Linn which is also called Roselle, is a model produce for countries which are developing since it grows easily. In Nigeria, it is well known for a drink called zobo. It is also used in the pharmaceutical and food industries (Da-Costa-Rocha *et al.*, 2014). The calyces of the plant have been reported to contain valuable nutrients like protein, fat, carbohydrates, fibre, vitamin C, β-carotene, calcium and iron (Ismail *et al.*, 2008). Hirunpanich, *et al*., (2005) also reported the presence of antioxidants like anthocyanin, quercetin and protocatechuic acid.

This study was undertaken to determine the effects that will be observed on the kidney of mice due to chronic exposure to paracetamol and concurrent treatment with aqueous extract of *Hibiscus sabdariffa* Linn calyx.

## MATERIALS AND METHODS

### Plant

The calyces of *Hibiscus sabdariffa* Linn were obtained from a popular market in the Federal Capital Territory, Abuja. Identification of the plant was then carried out at the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The calyx extract was prepared in accordance with our previously used method (Orji and Obi, 2018).

### Animals

A total of 20 mice weighing 27-32 g, supplied by a breeder in Benin City were used. They were kept in cages made of wood in the Department of Biochemistry, University of Benin animal house and allowed two weeks for adaptation before

commencing the study. The study was conducted in line with the conventional procedures recognized by National Institute of Health Guide for the Care and Use of Laboratory Animals.

**Paracetamol**

Paracetamol powder (Huang Gang Yin He Aati Pharmaceutical Co. Ltd. China) was dissolved in dimethyl sulfoxide (DMSO) after which distilled water was added to make up the required measure.

**Experimental design and treatment schedule**

In this study, 4 groups of 5 mice each were used. The groups include: control, extract only, paracetamol only and concurrent administration of paracetamol and extract. Paracetamol was administered orally (500 mg/kg body weight); the *Hibiscus sabdariffa* Linn calyx extract (HSCE) was also administered orally at 250 mg/kg, for 16 weeks.

**Biochemical analyses**

Blood was collected by heart puncture and placed in appropriate sample bottles. The kidneys were also excised for biochemical analysis and histopathological investigations. Electrolytes, urea and creatinine concentrations were assessed in serum, employing standard procedures which were described in the assay kits. Malondialdehyde (MDA) (Buege and Aust, 1978), reduced glutathione (GSH) (Tietz, 1969), superoxide dismutase (SOD) (Misra and Fridovich, 1972) and catalase (Cohen *et al.*, 1970) were carried out on kidney homogenate.

**Statistical analysis**

The obtained data were presented as mean ± S.E.M. Analysis for significance was done by one way ANOVA and the mean values that differed significantly were identified using the Duncan's multiple range test. $P \leq 0.05$ was used to indicate significant level between treatments.

**RESULTS**

**Effects of chronic exposure to paracetamol and HSCE**

**Kidney function parameters**

Table 1 shows results for kidney function tests conducted in mice after treatment for 16 weeks. Paracetamol significantly increased creatinine and urea levels. Potassium and chloride levels also increased but sodium and bicarbonate levels significantly decreased. Concurrent administration of paracetamol with the extract led to significant decrease in parameters that were elevated by paracetamol but there was a significant increase in sodium and bicarbonate levels.

**Table 1: Effects of chronic exposure to paracetamol and HSCE in serum of mice**

| Biochemical parameter (serum) | Control | Extract only | Paracetamol only | Concurrent administration of paracetamol and extract |
|---|---|---|---|---|
| CREATININE (mg/dL) | 9.42±0.28c | 8.86±0.34c | 16.06±0.54a | 11.66±0.56b |
| UREA (mmol/L) | 5.76±0.06c | 5.52±0.18c | 15.53±0.06a | 7.31±0.07b |
| BICARBONATE (mmol/L) | 45.43±0.00a | 45.60±0.17a | 39.60±0.17c | 42.00±0.86b |
| SODIUM (mEq/L) | 183.16±0.42a | 183.51±0.34a | 149.57±0.00c | 169.55±0.75b |
| CHLORIDE (mEq/L) | 92.57±0.21c | 92.37±0.12c | 93.72±0.30a | 92.95±0.20b |
| POTASSIUM (mEq/L) | 3.92±0.04b | 3.91±0.07b | 4.14±0.08a | 3.94±0.03b |

Data presented as Mean ± SEM (n=5)

Letters of the alphabet which are not alike on a row are significantly different ($P \leq 0.05$).

**Antioxidants and lipid peroxidation in the kidney**

Table 2 shows the results for the antioxidant and lipid peroxidation studies carried out on the kidney. Administration of paracetamol led to a significant decrease in SOD, Catalase and reduced glutathione while there was increase in MDA levels. Concurrent administration of paracetamol with the extract led to an increase in the parameters that were decreased in paracetamol toxicity and a decrease in MDA levels.

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

| Biochemical parameter (kidney) | Control | Extract only | Paracetamol only | Concurrent administration of paracetamol and extract |
|---|---|---|---|---|
| MDA (mol/g tissue) | 0.07±0.00c | 0.07±0.00c | 0.13±0.00a | 0.10±0.00b |
| SOD (Units/mg tissue) | 0.07±0.00a | 0.07±0.00a | 0.04±0.00c | 0.05±0.00b |
| CATALASE(Units/g tissue) | 6.81±0.28a | 7.00±0.39a | 4.69±0.06b | 5.25±0.07b |
| REDUCED GLUTATHIONE (mmol/L) | 0.07±0.00b | 0.08±0.00a | 0.04±0.00d | 0.06±0.00c |

Data presented as Mean ± SEM (n=5)
Letters of the alphabet which are not alike on a row are significantly different ($P \leq 0.05$).

**Histopathological findings**

**The images below show the kidney ultrastructure of mice exposed to concurrent administration of paracetamol and extract.**
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 showed mild interstitial congestion (A) (plate 2). The mouse that received paracetamol only showed patchy tubular cloudy swelling (A) and mild infiltrates of inflammatory cells (necrotizing pyelitis) (B) (plate 3), while the mouse that received extract and paracetamol showed focal tubular swelling (A), mild interstitial congestion (B) and moderate infiltrates of inflammatory cells (C) (plate 4).

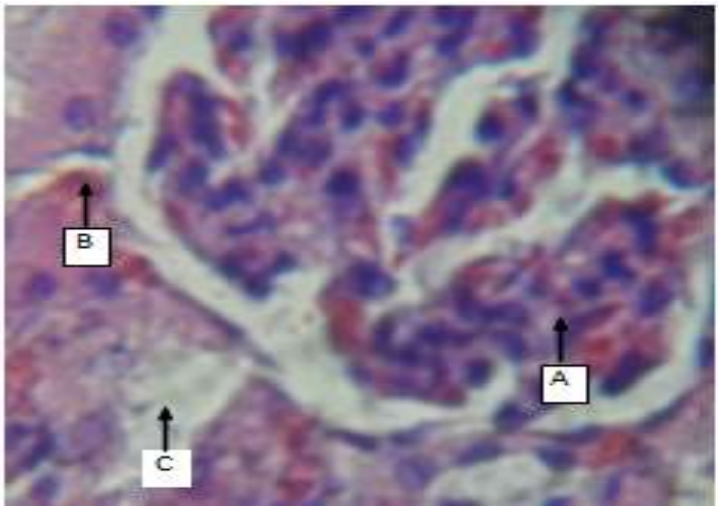


Plate 1: Control mouse' kidney (H & E, x400).

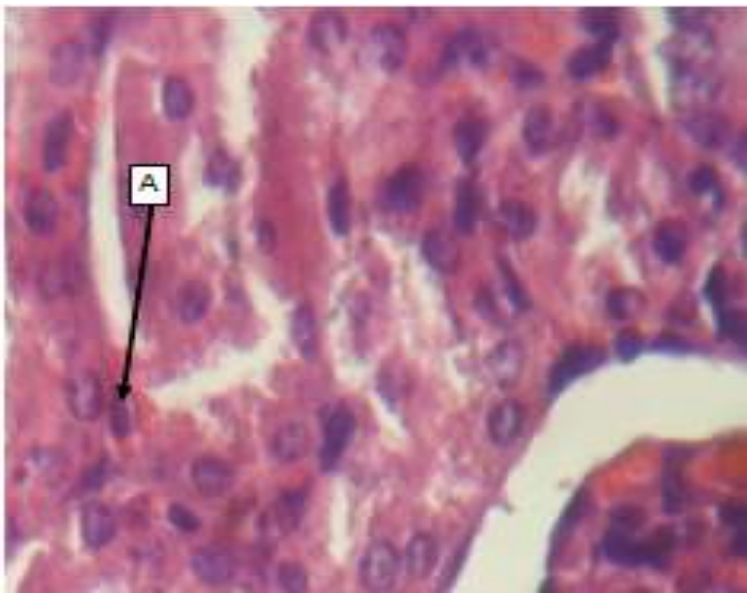


Plate 2: Kidney from mouse given extract only for 16 weeks (H & E, x400).

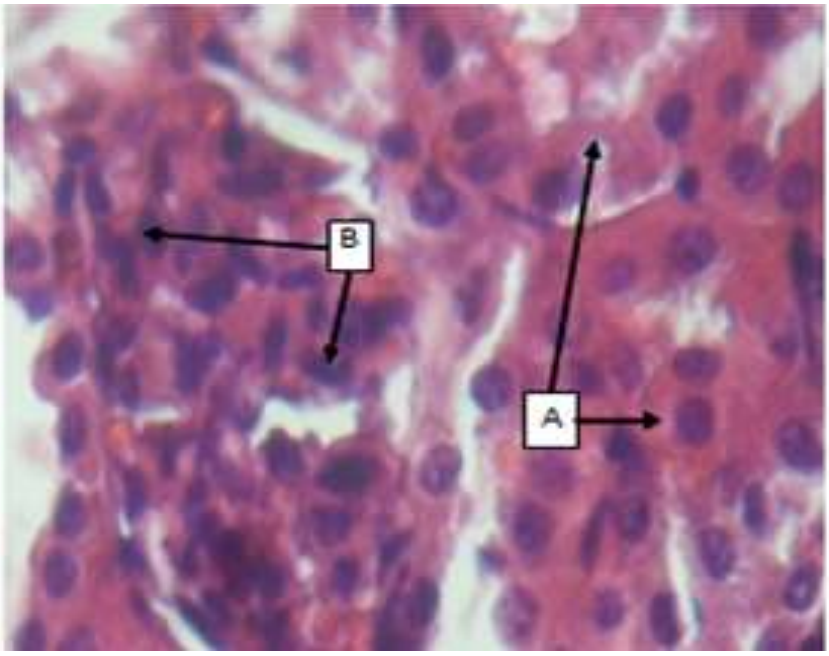


Plate 3: Kidney from mouse given paracetamol only for 16 weeks (H & E, x400).

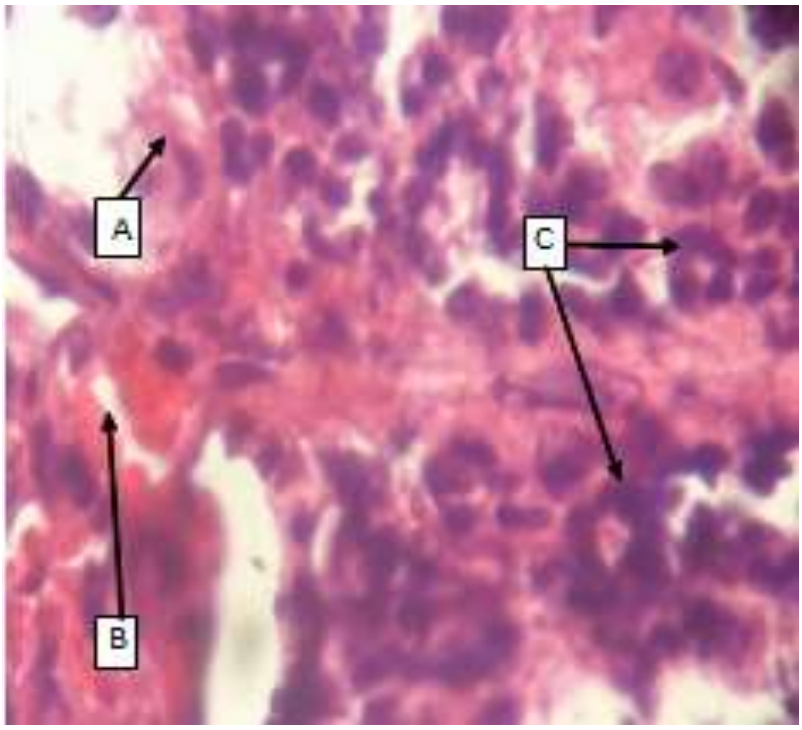


Plate 4: kidney from mouse given concurrent administration of paracetamol and extract for 16 weeks (H & E, x400).

**DISCUSSION**

Hepatotoxicity is more common than nephrotoxicity in paracetamol overdose. However, damage to the renal tubules may occur even if hepatotoxicity is not present (Jones and Vale, 1993). Higher than normal serum concentrations of creatinine and/or urea with or without alterations in serum electrolytes usually indicate nephrotoxicity (Saka *et al.*, 2012). In this study, paracetamol administration (Table 1) brought about a significant increase ($P<0.05$) in urea and creatinine, relative to control. This agrees with the study by Roy *et al*., (2015) who reported a significant rise in urea and creatinine of rats exposed to paracetamol. However concurrent administration of paracetamol with HSCE led to a decrease in the values of both urea and creatinine indicating a possible reduction in the toxic effect of paracetamol on the kidneys. Increased serum urea may indicate reduced reabsorption within the renal epithelium, while high levels of creatinine may indicate deficiency in the kidneys' glomerular filtration rate (Farrar, 2018). Studies have also shown that increased urea and serum creatinine levels indicate progressive renal damage. Paracetamol administration caused alterations in the electrolyte levels (Table 1). There was significant decrease ($P < 0.05$) in bicarbonate and sodium, and increase in chloride and potassium, relative to control. Our work agrees with that of Pakravan *et al.*, (2015), who stated that electrolyte levels of rats were altered following exposure to paracetamol at harmful levels. Concurrent administration of extract and paracetamol helped to reverse the drug induced changes observed in the electrolyte results. Serum concentration of potassium increases (hyperkalaemia) in acute or chronic renal failure due to decreased excretion. Decreased sodium concentration (hyponatraemia) can occur due to an increased extracellular fluid volume, resulting from the inability of the kidneys to excrete water (Puri, 2011). Serum chloride is very useful to assess electrolyte, acid-base and water balance. Serum chloride is increased in metabolic acidosis associated with prolonged diarrhoea, renal tubular diseases, respiratory alkalosis, some cases of hyperparathyroidism, diabetes insipidus, dehydration, and in conditions causing decreased renal blood flow, that is, congestive heart failure (Maheshwari, 2008).

Oxidative stress is the presence of reactive oxygen species (ROS) in excess of the available antioxidant-buffering capacity, that is, an imbalance between pro-oxidant (free radical species) and antioxidant. ROS cause damage to molecular target; proteins, lipids, and DNA, thus altering the structure and function of the cell (Yoshikawa and Naito, 2002). Oxidative stress is assessed by determining malondialdehyde (MDA), which is the product of lipid peroxidation. A rise in MDA level is associated with concomitant decline in one or more antioxidants (such as superoxide dismutase, catalase and reduced glutathione); this is suggestive of toxicity (Kuete, 2014). In this study, administration of paracetamol led to a significant increase ($P<0.05$) in MDA levels and a decrease in the antioxidant levels (Table 2). This also agrees with work by Roy *et al.*, 2015 who reported a significant increase in MDA levels and decrease in antioxidant enzymes SOD, catalase and

GSH level in rats administered paracetamol. Concurrent administration of paracetamol with the extract reversed the observed changes for MDA, SOD and glutathione to values close to control. This correlates with several studies both *in vitro* and *in vivo* that have shown that the extracts of the plant have a potent antioxidant effect (Farombi & Fakoya, 2005; Hirunpanich *et al*., 2005; Mohd-Esa, *et al.*, 2010). The bioactive agents in the extract which are known antioxidants (anthocyanins, ascorbic acid, quercetin and protocatechuic acid) (Hirunpanich, *et al*., 2005), most likely enhanced cellular level of GSH, by inducing its biosynthesis. The antioxidant activity of the extract is due to its strong scavenging effect on reactive oxygen species and free radicals (Farombi & Fakoya, 2005). An *in vivo* study also revealed that the nephroprotective effect of the extract is as a result of the protection of the kidney from oxidative stress (Mossalam, *et al.*, 2011).

Results from histopathology of kidney sections also support our position. Chronic administration of paracetamol was found to induce patchy necrotizing pyelitis, whereas concurrent administration of extract and drug afforded some measure of protection.

In conclusion, this study has proven that taking paracetamol at high levels and for a prolonged period is harmful to the kidneys of mice. Aqueous extract of *Hibiscus sabdariffa* Linn calyx was able to offer protection when concurrently administered with the drug. The antioxidant component of the extract probably played a role in protecting the kidney from the reactive oxygen species and free radicals produced by paracetamol.

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