

## Appraisal of the Kidney Status of the Rat Model to Sub-acute Treatment with Piperazine Citrate

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**Abstract: Background:** We investigated the effects of sub-chronic treatment of albino rats with piperazine on the kidney morphology. The effects on the electrolytes, creatinine and urea were also evaluated as possible markers of the toxic states of the kidney. **Results:** The mean values for creatinine in the 30, 60 and 100 mg kg<sup>-1</sup> groups of rats and their p values compared to the control groups were 89.8±6.68 (p = 0.8868), 117.8±17.11 (p = 0.3378) and 148.2±27.37 mmol L<sup>-1</sup> (p = 0.1272), respectively. The average urea value in the 100 mg kg<sup>-1</sup> treated group was 6.16±0.31 mmol L<sup>-1</sup>. The difference when compared to controls was statistically significant (p = 0.0135). However, the values for the 30 and 60 mg kg<sup>-1</sup> groups, 5.16±0.13 and 4.98±0.60 mmol L<sup>-1</sup> were not statistically significant when compared to the control value (p = 0.1394 and p = 0.4193, respectively). In the case of Na<sup>+</sup> and K<sup>+</sup>, there was no statistical difference between the values of the 30 mg kg<sup>-1</sup> group and the control group except the 100 mg kg<sup>-1</sup> group, however, when the dose was increased to 60 and 100 mg kg<sup>-1</sup>, the difference between the ion levels in the treated groups and the control group became statistically significant (p = 0.0253 and 0.0012, respectively in respect of Na<sup>+</sup> and 0.0094 and 0.0005, respectively in respect of K<sup>+</sup>). In contrast none of the values for the anions, HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> showed a statistically significant difference following subchronic treatment with various doses of piperazine when compared with the control values. The kidney histology revealed extensively deranged morphology. **Conclusion:** It is concluded, therefore, that sub-chronic doses of piperazine would be inimical to the kidney and therefore, compromise the normal kidney function.

**Key words:** Electrolytes status, serum creatinine, serum urea, kidney morphology, rat

### INTRODUCTION

Piperazine is a useful and inexpensive anthelmintic agent active against *A. lumbricoides* and *E. vermicularis* (Tracy and Webster, 2001). Numerous pharmacodynamic studies have established piperazine as a potential clinical tool for the management of some disease conditions (Onuaguluchi and Ghasi, 2006; Ghasi and Onuaguluchi, 2007; Ghasi, 2008; Ghasi *et al.*, 2009). This is a welcome finding as piperazine is cheap and has minimal adverse effects.

Adverse events attributable to piperazine are rare with most of the recorded toxic effects being related to the neuromuscular system (Schuch *et al.*, 1966; Parsons, 1971; Bomb and Bedi, 1976). The relative safety of piperazine as a drug compared to many other drugs is understandable considering its high LD<sub>50</sub> (3.548 g kg<sup>-1</sup> i.p.) in mice (Onuaguluchi and Igbo, 1985). In the same

study, the LD<sub>50</sub> and Therapeutic Index (TI) of piperazine in the toad were given as 12.59 g kg<sup>-1</sup> and 8.5, respectively. Interestingly, the LD<sub>1</sub> and ED<sub>99</sub> for piperazine in the same animal model were 2.63 and 1.862 g kg<sup>-1</sup>, respectively (Onuaguluchi and Igbo, 1985), giving a Certain Safety Factor (CSF) of 1.4. In other words, increasing the dose expected to give about 100% therapeutic effect by just 40% could sometimes lead to emergence of serious toxic effects.

This observation was found to be true during our investigation of the electrocardiographic patterns of piperazine in the rat (Ghasi and Onuaguluchi, 2007). It was found that piperazine 100 mg kg<sup>-1</sup> given intravenously via the external jugular vein sometimes caused serious aberrations in some of the animals. This finding necessitated further studies on the possible toxic effects of piperazine especially at cellular level. Elevation of the serum enzymes could point to some disease

conditions. Recently, a piperazine derivative, M-chlorophenylpiperazine was shown to affect some brain hormones (Hatzidimitriou *et al.*, 2002). This may arise as a consequence of histological effect of the drug as some piperazine derivatives have been demonstrated to cause lesions (De-Deurwaerdere and Chesselet, 2000; Eilon *et al.*, 2000; Van-Bogaert *et al.*, 2001). Since some piperazine derivatives have equally been found to have protective effects (Merz *et al.*, 1998; Minato *et al.*, 1996, 1997; Williams *et al.*, 1996), it is suggested that sub-chronic study of pathologic effects of piperazine be undertaken in which case effects of piperazine are evaluated to identify any possible pathological changes. This is especially important since the morphological evidence of a pathological process is the most consistent of the changes that can be identified as the result of a toxic process.

The kidney is the primary organ for clearance and excretion of xenobiotics including drugs and drug-products from the body and many of them cause various types of damage to the kidney in the process. In the present study, we investigated the effects of sub-chronic treatment of albino rats with piperazine on the kidney morphology. The effects on the electrolytes, creatinine and urea were also evaluated as possible markers of the toxic states of the kidney.

## MATERIALS AND METHODS

Forty albino wistar rats weighing originally between 175 and 250 g were divided into 4 groups of ten sex-matched rats each. Each group of rats received orally one dose of piperazine, 30, 60, or 100 mg kg<sup>-1</sup> given two times daily along with standard diet. The fifth group of rats served as the control and was fed orally with the standard diet only. The total period of treatment was 16 weeks.

At the end of treatment, the rats were anaesthetized with thiopentone sodium (50 mg kg<sup>-1</sup> i.p.) and blood samples collected by cardiac puncture for biochemical studies. The results were expressed as Mean±SEM and the significance of difference between treated and control determined using unpaired Student's 't' test. Differences between the various treated groups were then evaluated using one-way ANOVA and when found statistically significant, Bonferroni's Multiple Comparison Test was done to determine patterns of significance on group-to-group basis.

Furthermore, some of the animals were sacrificed under chloroform anaesthesia. The kidney was harvested and fixed in 10% formalin for 24 h. The tissues were processed using an automatic tissue processor, embedded in paraffin wax and thin sections (about 4-5 mm

thick) cut using a rotary microtome. The sections were stained by haematoxylin and eosin (H and E) method, examined and photographed using a light microscope.

**Serum urea estimation:** A direct method of urea estimation according to Natelson *et al.* (1951) was used.

Urea reacts with diacetylmonoxime under strongly acidic condition to give a yellow condensation product. The reaction can be intensified by the presence of ferric ions and thiosemicarbazide. The red coloured complex so formed is more linear with urea concentration than the yellow one. The reaction product is measured at 480 nm.

### Estimation of electrolytes

**Sodium and potassium ion:** The Na<sup>+</sup> and K<sup>+</sup> were estimated using flame emission photometry in the Department of Chemical Pathology of the University of Nigeria Teaching Hospital, Enugu, as described by Tietz (1970).

Dilute solution of the test sample is passed through a burner where the solution evaporates and the salt dissociates to give neutral atoms. Some of these atoms being excited more into a higher energy state. Being unstable, they fall back to the ground state and emit light of characteristic wavelength. The amount of light emitted which is directly proportional to the concentration of sodium or potassium in the test sample is detected by the galvanometer reading.

**Serum bicarbonate ion estimation:** The modified Van Slyke (1922) method, which employs the titrimetric method for HCO<sub>3</sub><sup>-</sup> estimation was used.

Carbon dioxide is released from plasma bicarbonate with dilute hydrochloric acid. Excess HCl is then titrated against NaOH using phenol red as indicator. Stock phenol red solution was prepared by dissolving 0.4 g powder in 100 mL of deionised water (CO<sub>2</sub> free). Working phenol red solution was prepared by diluting stock 1 in 10 with deionised water and stored in the refrigerator.

**Serum chloride estimation:** The serum chloride content was determined by the method of Schales and Schales (1941).

**Histological studies:** The kidney was placed in 10% formalin for a period of 5 days for proper fixation after which the organ was cut open and smaller portions of it were selected and processed for light microscopy. The organ was dehydrated by ascending grades of isopropyl alcohol by immersing in 80% isopropanol overnight and 100% isopropyl alcohol for 1 h. The dehydrated organs were cleared in two changes of xylene 1 h each.

The cleared organs transferred to 2 changes of liquid paraffin wax for 3 h each using histokinette tissue processor II (Miles, USA). The wax-impregnated tissues were embedded in paraffin blocks using the same grades wax. The paraffin blocks were sectioned with Hertz Rotary microtome at 3-micron thickness. The sections were floated on a tissue flotation bath at 40°C and taken on glass slides and smeared with equal parts of egg albumin and glycerol. The sections were then melted in an incubator at 60°C for 5 min and allowed to cool.

The sections were deparafinised by immersing in xylene for 10 min in a horizontal staining jar. The deparafinised sections were washed in 100% isopropyl alcohol and stained in Ehrlich's heamatoxylin for 8 min in a horizontal staining jar. The sections were washed in tap water and dipped in acid alcohol to remove excess stain. The sections were then placed in running tap water or 10 mins for bluing (slow alkalanization). The section were counter stained in 1% aqueous Eosin for 1 min and excess stain was washed in tap water and the sections were allowed to dry. Placing the sections in the incubator at 60°C for 5 min ensured complete dehydration of stained sections. When the sections were cooled, they were mouted in DPX mount having the optical index of glass (The sections were wetter in Xylene and inverted on to the mount and placed on the cover slip). The sections were examined using swift® binocular microscope with in-built lighting systems. The sections were then photomicrographed using 35 min films with an Olympus-Photomicroscope.

## RESULTS

**Serum creatinine and serum urea:** None of the rats in all the groups apart from the group that was treated with piperazine 100 mg kg<sup>-1</sup> had creatinine or urea level outside the normal range. Even within this group, only one rat showed a creatinine level (242 µmol L<sup>-1</sup>) that exceeded the upper limit of the normal range (44.2 -194.5 µmol L<sup>-1</sup>) while in respect of the urea values, the upper limit of the normal range (2.5 - 6.5 mmol L<sup>-1</sup>) was exceeded in two rats (6.7 and 6.9 mmol L<sup>-1</sup>, respectively).

The mean values for creatinine and urea in control animals were 92.6±17.83 and 4.32±0.50 mmol L<sup>-1</sup>, respectively. Compared to these values, creatinine levels of 89.8±6.68 (p = 0.8868), 117.8±17.11 (p = 0.3378) and 148.2±27.37 mmol L<sup>-1</sup> (p = 0.1272) in the 30, 60 and 100 mg kg<sup>-1</sup> groups respectively were not statistically significant. The average urea value in the 100 mg kg<sup>-1</sup> treated group was 6.16±0.31 mmol L<sup>-1</sup>. The difference when compared to controls was statistically significant (p = 0.0135). However, the values for the 30 and

Table 1: Creatinine and urea as well as Na<sup>+</sup> and K<sup>+</sup> values in both the control group and groups treated with various doses of piperazine

Parameter	Dose (mg kg <sup>-1</sup> )	Mean±SEM	p-value
Creatinine (µmol L <sup>-1</sup> )	Control	92.60±17.83	-
	30	84.80±6.68	0.8868
	60	117.80±17.11	0.3378
	100	148.20±27.37	0.1272
ANOVA			0.1371
Urea (mmol L <sup>-1</sup> )	Control	4.32±0.50	-
	30	5.16±0.13	0.1394
	60	4.98±0.60	0.4193
	100	6.16±0.31	0.0135
ANOVA			0.1155
Sodium ion (mmol L <sup>-1</sup> )	Control	137.40±0.81	-
	30	139.40±1.44	0.2598
	60	142.40±1.63	0.0253
	100	142.60±0.68	0.0012
ANOVA			0.1977
Potassium ion (mmol L <sup>-1</sup> )	Control	3.96±0.17	-
	30	4.10±0.13	0.5305
	60	4.54±0.02	0.0094
	100	4.92±0.04	0.0005
ANOVA			0.0001

60 mg kg<sup>-1</sup> groups, 5.16±0.13 mmol L<sup>-1</sup> and 4.98±0.60 mmol L<sup>-1</sup> were not statistically significant when compared to the control value (p = 0.1394 and p = 0.4193, respectively).

**Serum electrolytes:** After 4 months of treatment with piperazine, the control and the test serum electrolytes levels were within the normal range for any particular biochemical parameter. There was however a tendency towards increase in values with increasing doses of piperazine although generally not statistically significant.

The mean Na<sup>+</sup> and K<sup>+</sup> values in the group of rats that received piperazine 30 mg kg<sup>-1</sup> (139.4±1.45 and 4.1±0.13 mmol L<sup>-1</sup>, respectively) were not statistically significant over the control values of 137.4±0.81 and 3.96±0.17 mmol L<sup>-1</sup> (p = 0.2598 and 0.5305, respectively). However, when the dose was increased to 60 and 100 mg kg<sup>-1</sup>, the difference between the ion levels in the treated groups and the control group became statistically significant (p = 0.0253 and 0.0012, respectively in respect of Na<sup>+</sup> and 0.0094 and 0.0005, respectively in respect of K<sup>+</sup>). The mean Na<sup>+</sup> and K<sup>+</sup> values in the two groups were 142.4±1.63 and 4.54±0.02 mmol L<sup>-1</sup> for the 60 mg kg<sup>-1</sup> group and 142.6±0.68 and 4.92±0.037 mmol L<sup>-1</sup> for the group that received piperazine 100 mg kg<sup>-1</sup>. Table 1 shows the creatinine and urea as well as Na<sup>+</sup> and K<sup>+</sup> values in both the control group and groups treated with various doses of piperazine.

With respect to the anions, HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup>, the values following chronic treatment with various doses of piperazine compared to the control values were not statistically significant. Compared to the control value of 24.6±0.4 mmol L<sup>-1</sup>, the bicarbonate values in the treated groups which received 30, 60 and 100 mg kg<sup>-1</sup> body

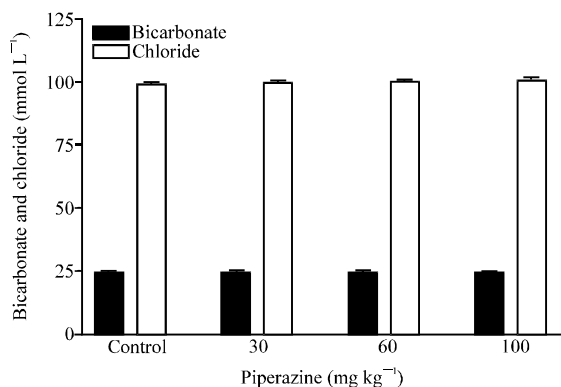


Fig. 1: Effects of various doses of piperazine on the serum bicarbonate and chloride ions of the Wistar rat. Differences between the control rats and drug treated rats were not statistically significant

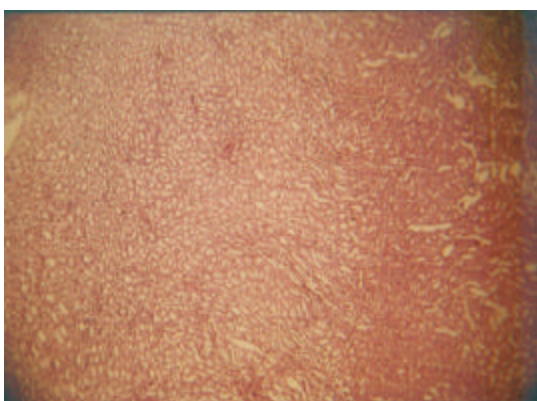


Fig. 2: Kidney section of control rat showing normal architecture. H and E Stain X100

weight were:  $24.8 \pm 0.37$  ( $p = 0.7245$ );  $24.6 \pm 0.4$  ( $p = 1.000$ ) and  $24.4 \pm 0.51$  ( $p = 0.7655$ ), respectively. For the chloride ion, the values were  $99.4 \pm 0.93$ ,  $100.0 \pm 0.71$  and  $100.8 \pm 1.02$   $\text{mmol L}^{-1}$  for the groups treated with piperazine 30, 60 and 100  $\text{mg kg}^{-1}$ . When compared to the control value of  $99.0 \pm 0.71$  the difference was not statistically significant ( $p = 0.7404$ ,  $0.3466$  and  $0.1850$ , respectively). Figure 1 is a histogram comparing the values of  $\text{HCO}_3^-$  and  $\text{Cl}^-$  in both the control group and the groups that received piperazine.

**Kidney morphology:** The kidney showed piperazine induced derangement of the Kidney architecture that appeared to be dose-dependent in character (Fig. 2, 6). Changes in kidney morphology included aglomerular nephrons, glomerular loss due to necrosis, condensed

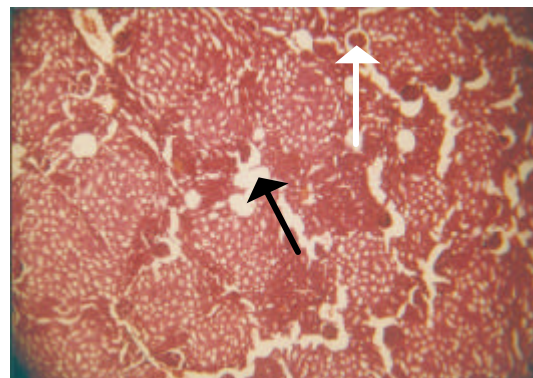


Fig. 3: Kidney section of rat chronically treated with piperazine 30  $\text{mg kg}^{-1}$  showing aglomerular nephrons, glomerular loss due to necrosis (black arrow) and condensed shrunken glomeruli (white arrow). H and E Stained X100



Fig. 4: Kidney section of rat chronically treated with piperazine 60  $\text{mg kg}^{-1}$  showing vascular congestion, shrunken pyknotic (white arrow) and degenerate glomeruli (black arrow). H and E Stain X100

shrunken glomeruli, vascular congestion, shrunken pyknotic and degenerate glomeruli, hyalinization, tubular cell necrosis and tubular hyaline droplets

## DISCUSSION

Urea, creatinine and electrolyte studies were undertaken to determine the status of the kidney after sub-chronic treatment with piperazine. Although high doses of piperazine led to significant elevation of serum  $\text{Na}^+$ ,  $\text{K}^+$  and urea over the control level, the concentrations

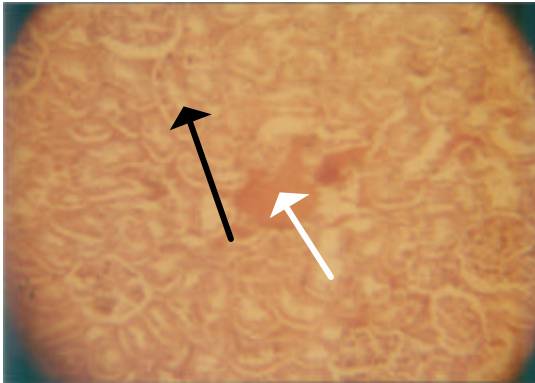


Fig. 5: Kidney section of rat chronically treated with piperazine 60 mg kg<sup>-1</sup> showing hyalinization (white arrow), tubular cell necrosis and degeneration (black arrow). H and E Stain X400

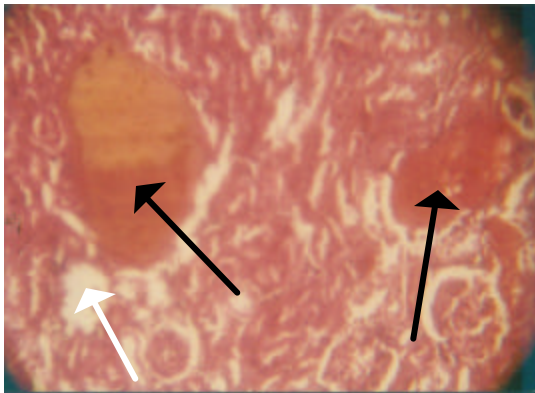


Fig. 6: Kidney section of rat chronically treated with piperazine 100 mg kg<sup>-1</sup> showing two prominent areas of hyalinisation (black arrow), glomerular loss due to severe necrosis (white arrow), tubular hyaline droplets and necrosis. H and E Stain X400

were however within the normal ranges. HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> in contrast remain largely unaffected. With respect to creatinine it was found that the serum concentrations showed dose dependent increases that did not differ significantly from the control values and which remained within the established normal ranges.

Urea, creatinine and electrolytes (Na<sup>+</sup>, K<sup>+</sup>, HCO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>) have been universally accepted as the most sensitive biochemical markers in the determination of renal damage

because these are excreted in the kidney. The electrolytes enter the renal tubule by glomerular filtration. Potassium may also be actively secreted into the proximal convoluted tubules. In certain segments of the tubule some of the electrolytes are reabsorbed. It would seem that in disorders that decrease the function of the kidney the most affected is glomerular filtration. Therefore, cellular damage will cause the retention of urea, creatinine and the electrolytes in the blood as the ability of the kidney to excrete becomes compromised (Jackson, 2001; Nwanjo *et al.*, 2004).

Increases in serum creatinine and urea are usually indications of functional damage to the kidney because they are excreted by the kidney. Creatinine is a waste product made by the muscles and is usually a more accurate marker of kidney function than urea. Urea is a waste product formed from the breakdown of proteins (Panda, 1999). Increases in creatinine and urea values were noted for all the doses studied compared to the control values but these values were within the normal limits. It was only in the group that received 100 mg kg<sup>-1</sup> piperazine that statistically significant difference over the control was seen for the urea estimation. Increases in serum urea may arise from many factors including drugs, diet and dehydration, but kidney damage is the only significant factor that increases serum creatinine level (Cheesbrough, 1998). Since even at high doses piperazine failed to cause statistically significant increase in serum creatinine, the increase seen with serum urea may or may not be entirely due to nephrotoxicity.

There are numerous causes of hypernatraemia and hyperkalaemia; these may include kidney disease and dehydration. Hyperkalaemia is a medical emergency because of the risk of life threatening cardiac arrhythmias (Fry and Farrington, 2006). Interestingly, piperazine appeared to have more profound effect on serum potassium as compared to serum sodium. Although both electrolytes increased significantly at all doses with respect to the control values, however, analysis of variance to determine if effect seen was dose dependent gave statistical significant p-value for potassium but insignificant value in the case of sodium. The serum concentrations of the anions namely HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> of the treated groups compared to the control remained virtually unchanged.

The kidney histology showed that piperazine caused derangement of the Kidney architecture that appeared to be dose-dependent in character. Changes in kidney morphology included aglomerular nephrons, glomerular loss due to necrosis, condensed shrunken glomeruli, vascular congestion, shrunken pyknotic and degenerate glomeruli, hyalinization, tubular cell necrosis and tubular

hyaline droplets. These alterations in the structure of the kidney of the animals showed that piperazine may be nephrotoxic. It is known that the morphological architecture of an organ has to be extensively deranged before any significant aberration is seen in the biochemical parameter. Therefore, it is understandable that the values for the biochemical markers remained within the normal limit for all doses administered.

In conclusion, piperazine is inimical to the kidney and one must be wary of administering high doses for a prolonged period of time.

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