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## Effects of Valproic Acid on Selected Kidney Function Indices in Rats

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### ABSTRACT

Valproic acid (VPA) has been demonstrated to exhibit anti-diabetic effect and attenuate hypertensive responses in animal models but its safety evaluation on the kidney has not been reported. This study investigated the effect of VPA on selected kidney function indices of rats. Twenty healthy Wistar rats were randomly grouped into 4 of 5 rats each. Rats in group 1 (control) were administered clean water only, while rats in groups 2, 3 and 4 were administered 100, 300 and 600 mg/kg body weight (bw) of VPA, respectively for 3 weeks. Serum concentrations of creatinine, urea, sodium, potassium, phosphate and chloride ions as well as the activities of alkaline phosphatase (ALP), acid phosphatase (ACP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in the kidney and serum of rats were determined. VPA at the 3 doses administered did not significantly affect ( $p > 0.05$ ) the kidney/body weight ratio, serum concentrations of creatinine, urea, sodium ion and phosphate ion when compared with control. VPA at 600 mg/kg bw alone and at both 300 and 600 mg/kg bw significantly increased ( $p < 0.05$ ) the serum levels of potassium and chloride ions, respectively. VPA did not significantly affect ( $p > 0.05$ ) the kidney ALP and ALT activities as well as the serum ALP, ACP, AST and ALT activities but significantly increased ( $p < 0.05$ ) the kidney ACP and AST activities at 300 mg/kg bw. These results imply that treatment with VPA at higher doses may adversely affect the reabsorption of chloride ion in the kidney which may alter the acid/base balance and impair nerve impulse transmission.

**Keywords:** Valproic acid, diabetes, hypertension, kidney.

### Introduction

Valproic acid (2-propylvaleric acid or 2-propylpentanoic acid), a derivative of valeric acid, is a branched short-chain fatty acid forming a clear liquid at room temperature with half-life of 9 to 16 hours (Soori *et al.*, 2015). Valproic acid (VPA) is quickly metabolized in the liver, and the various metabolites resulting from the metabolism are also pharmacologically active. Its bioavailability is ascribed to the high permeability of the cell membrane to this compound and the nearly

absence of first pass hepatic mechanism (Lauterbach *et al.*, 2010).

Valproic acid is generally used in clinics as an anti-epileptic drug and for the treatment of bipolar disorders and migraine prophylaxis (Kostrouchova *et al.*, 2007). It is extensively metabolized by microsomal glucuronide conjugation, mitochondrial beta-oxidation and cytochrome P450-dependent omega (omega-1 and omega-2) oxidation (Perucca, 2002). The pharmacological effects of VPA involve increased gamma-

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aminobutyric acid (GABA)-ergic transmission, reduced release and/or effects of excitatory amino acids, blockade of voltage gated sodium channels and modulation of dopaminergic and serotonergic transmission (Verrotti, 2010). VPA may regulate the expression of neuroprotective genes and protect against excitotoxicity (Bril *et al.*, 2011).

Studies have indicated that targeting histone deacetylases (HDACs) is a promising therapeutic strategy for some diseases including diabetes mellitus (Biddinger and Kahn, 2006), hypertension (Alam *et al.*, 2016) and cancers (Falkenberg and Johnstone, 2014). HDAC are enzymes that regulate the activities of histone acetyltransferases in chromatin remodeling and thereby play an essential role in gene transcription to regulate cell proliferation, migration and apoptosis, immune pathways and angiogenesis (Crosson *et al.*, 2010).

Valproic acid (VPA), an HDAC inhibitor, has been demonstrated to exhibit anti-diabetic effect and attenuate hypertensive responses in animal models (Lee *et al.*, 2013; Igunnu *et al.*, 2019). VPA has also shown pharmacological characteristics similar to those of other anti-cancer agents, exerting effect on cell growth, differentiation and apoptosis (Lauterbach *et al.*, 2010). However, its effect on a very vital organ, important for the body's homeostasis, the kidney, has not been reported. Therefore, this study evaluated the effect of VPA on selected kidney function indices of rats.

## Materials and Methods

### Chemicals and Reagents

Valproic acid was obtained from Sigma-Aldrich UK. Assay kits for the determination of alkaline phosphatase (ALP), acid phosphatase (ACP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were obtained from Randox Laboratories Limited. The assay kits used for the determination of serum levels of

electrolytes were obtained from Teco diagnostics. All other reagents used were of analytical grade and were prepared using distilled water.

### Experimental Animals

Twenty (20) healthy female albino rats (*Rattus norvegicus*) with an average weight of 170-200g used for this study were procured from the Animal House (Animal Breeding Unit) of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were housed in well ventilated plastic cages under standard conditions and they were acclimatized for one week before treatment during which they were fed normal diet and water. The research was conducted in accordance with internationally accepted principle for laboratory animal use and care (Clarke *et al.*, 1997; Garber *et al.*, 2011).

### Animal Grouping and Drug Administration

Twenty rats were randomly grouped into four (1 - 4) of five rats each. Each rat in the control group (group 1) was administered clean water orally for three weeks. Valproic acid was dissolved in clean water and doses of 100, 300 and 600 mg/kg body weight of valproic acid were administered orally to the rats in groups 2, 3 and 4 respectively for three weeks.

### Collection and Preparation of Samples

At the end of the treatment period, the rats were slightly anaesthetized with diethyl ether, the neck area was quickly cleared of fur and skin to expose the jugular vein from which blood was collected into sample bottles. The blood samples were allowed to stand for 10mins at room temperature for clot formation and subsequently centrifuged at 3000 rpm for 5 mins, after which the serum was pipetted out and stored in the freezer at -20°C till needed for analysis.

Also, the rats were quickly dissected and the organ of interest (kidney) was isolated, cleaned of blood, weighed, suspended in an ice-cold 0.25M sucrose

solution (1:5 w/v) and homogenized. The homogenates were frozen overnight to allow complete cell lysis and maximum release of enzymes (Akanji *et al.*, 1993).

### Biochemical Assays

Serum concentrations of urea and creatinine were determined using methods described by Amin *et al.* (2014). The serum levels of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{PO}_4^{2-}$  were determined using methods described in the Teco kit manuals. The activity of alkaline phosphatase was determined by the method of Wright *et al.* (1972). The activity of acid phosphatase was determined using the method described by Wright and Plumber (1974). The activities of alanine aminotransferase and aspartate aminotransferase were determined according to the method described by Reitman and Frankel (1957).

### Statistical Analysis

Statistical analysis was carried out by one-way analysis of variance followed by post hoc Tukey's comparison test using statistical package for the social science (SPSS 16.0) statistical software. Results were expressed as means  $\pm$  standard error of mean (SEM) for five rats in each group. A value of  $p < 0.05$  was considered to be statistically significant.

### Results

Valproic acid (VPA) at all the treatment doses (100, 300 and 600 mg/kg bw) administered did not significantly affect ( $p > 0.05$ ) the kidney/body weight ratio of rats when compared with control (Table 1).

VPA at the 3 doses administered did not significantly affect ( $p > 0.05$ ) serum concentrations of creatinine and urea when compared with control (Table 2). Also, treatment with VPA at the 3 doses

studied did not significantly affect ( $p > 0.05$ ) serum concentrations of sodium and phosphate ions but VPA at 600 mg/kg bw alone and at both 300 and 600 mg/kg bw significantly increased ( $p < 0.05$ ) the serum levels of potassium and chloride ions, respectively, when compared with control (Table 3).

VPA at the 3 doses administered did not significantly affect ( $p > 0.05$ ) ALP and ALT activities in the kidney when compared with control. Similarly, ACP and AST activities in the kidney were not significantly affected ( $p > 0.05$ ) at 100 and 600 mg/kg bw but were significantly increased ( $p < 0.05$ ) at 300 mg/kg bw when compared with control (Table 4). Furthermore, treatment with VPA at the 3 doses administered did not significantly affect ( $p > 0.05$ ) the serum ALP, ACP, AST and ALT activities (Table 5).

**Table 1:** Kidney/body weight ratios of rats administered valproic acid (VPA)

Group	Kidney/body weight ratio
Control	0.0056 $\pm$ 0.0002 <sup>b</sup>
100 mg/kg bw VPA	0.0050 $\pm$ 0.0004 <sup>ab</sup>
300 mg/kg bw VPA	0.0051 $\pm$ 0.0001 <sup>ab</sup>
600 mg/kg bw VPA	0.0052 $\pm$ 0.0003 <sup>ab</sup>

All data are presented as mean of five replicates  $\pm$  standard error of mean (S.E.M.). Values along the same column with different superscripts are significantly different ( $p < 0.05$ ).

**Table 2:** Creatinine and urea concentrations in serum of rats administered valproic acid (VPA)

Group	Creatinine ( $\mu\text{mol/L}$ )	Urea ( $\mu\text{mol/L}$ )
Control	15.5705 $\pm$ 0.909 <sup>a</sup>	10.426 $\pm$ 2.160 <sup>a</sup>
100 mg/kg bw VPA	43.827 $\pm$ 9.275 <sup>a</sup>	14.085 $\pm$ 1.509 <sup>ab</sup>
300 mg/kg bw VPA	46.133 $\pm$ 17.592 <sup>a</sup>	15.096 $\pm$ 1.704 <sup>ab</sup>
600 mg/kg bw VPA	53.053 $\pm$ 15.729 <sup>a</sup>	14.209 $\pm$ 0.663 <sup>ab</sup>

All data are presented as mean of five replicates  $\pm$  standard error of mean (S.E.M.). Values along the same column with different superscripts are significantly different ( $p < 0.05$ ).

**Table 3:** Concentrations of serum electrolytes in rats administered valproic acid (VPA)

Group	K <sup>+</sup> (mEq/L)	Na <sup>+</sup> (mEq/L)	Cl <sup>-</sup> (mEq/L)	PO <sub>4</sub> <sup>2-</sup> (mg/dL)
Control	3.675 $\pm$ 0.621 <sup>a</sup>	76.704 $\pm$ 12.688 <sup>ab</sup>	75.943 $\pm$ 8.724 <sup>a</sup>	15.000 $\pm$ 4.213 <sup>a</sup>
100 mg/kg bw VPA	4.372 $\pm$ 0.387 <sup>a</sup>	53.687 $\pm$ 9.063 <sup>a</sup>	118.710 $\pm$ 13.142 <sup>abc</sup>	11.423 $\pm$ 1.537 <sup>a</sup>
300 mg/kg bw VPA	4.667 $\pm$ 0.230 <sup>a</sup>	72.514 $\pm$ 14.284 <sup>ab</sup>	147.710 $\pm$ 15.668 <sup>c</sup>	20.037 $\pm$ 4.155 <sup>a</sup>
600 mg/kg bw VPA	8.558 $\pm$ 0.230 <sup>b</sup>	75.643 $\pm$ 4.015 <sup>ab</sup>	131.570 $\pm$ 25.266 <sup>bc</sup>	16.715 $\pm$ 1.488 <sup>a</sup>

All data are presented as mean of five replicates  $\pm$  standard error of mean (S.E.M.). Values along the same column with different superscripts are significantly different ( $p < 0.05$ ).

**Table 4:** Activities of selected enzymes in kidney of rats administered valproic acid (VPA)

Group	Specific Enzyme Activity (nmol/mg protein/min)		Specific Enzyme Activity (U/mg protein)	
	ALP	ACP	AST	ALT
Control	2559.3 $\pm$ 646.911 <sup>a</sup>	840.15 $\pm$ 149.307 <sup>a</sup>	1162.30 $\pm$ 555.254 <sup>a</sup>	617.94 $\pm$ 275.157 <sup>ab</sup>
100 mg/kg bw VPA	941.33 $\pm$ 186.466 <sup>a</sup>	1252.40 $\pm$ 108.368 <sup>ab</sup>	1074.20 $\pm$ 123.702 <sup>a</sup>	235.25 $\pm$ 71.908 <sup>a</sup>
300 mg/kg bw VPA	2060.20 $\pm$ 535.858 <sup>a</sup>	1857.30 $\pm$ 327.763 <sup>b</sup>	3396.40 $\pm$ 836.901 <sup>b</sup>	304.87 $\pm$ 49.019 <sup>a</sup>
600 mg/kg bw VPA	5888.40 $\pm$ 4315.68 <sup>a</sup>	961.46 $\pm$ 175.420 <sup>a</sup>	2260.10 $\pm$ 88.543 <sup>ab</sup>	783.84 $\pm$ 270.737 <sup>ab</sup>

All data are presented as mean of five replicates  $\pm$  standard error of mean (S.E.M.). Values along the same column with different superscripts are significantly different ( $p < 0.05$ ).

**Table 5:** Activities of selected enzymes in serum of rats administered valproic acid (VPA)

Group	Specific Enzyme Activity (nmol/mg protein/min)		Specific Enzyme Activity (U/mg protein)	
	ALP	ACP	AST	ALT
Control	11.7201 ± 0.461 <sup>abc</sup>	4.49 ± 0.278 <sup>a</sup>	8.19 ± 32.434 <sup>a</sup>	54.75 ± 10.790 <sup>ab</sup>
100 mg/kg bw VPA	9.6665 ± 0.819 <sup>abc</sup>	3.34 ± 0.105 <sup>a</sup>	76.26 ± 29.137 <sup>a</sup>	50.48 ± 8.356 <sup>a</sup>
300 mg/kg bw VPA	11.2417 ± 1.321 <sup>bc</sup>	4.29 ± 0.399 <sup>a</sup>	42.49 ± 7.022 <sup>a</sup>	85.34 ± 8.393 <sup>b</sup>
600 mg/kg bw VPA	6.6239 ± 0.601 <sup>a</sup>	2.76 ± 0.226 <sup>a</sup>	37.72 ± 21.683 <sup>a</sup>	65.77 ± 7.586 <sup>ab</sup>

All data are presented as mean of five replicates ± standard error of mean (S.E.M.). Values along the same column with different superscripts are significantly different ( $p < 0.05$ ).

## Discussion

This study demonstrated that treatment with valproic acid (VPA) may adversely affect the reabsorption of chloride ion in the kidney at higher doses.

The analysis of organ/body weight ratio is an essential parameter for identifying potentially harmful effects of chemicals in the body. The kidney/body weight ratio is an important index for assessing renal function (Kumar *et al.*, 2005). In this study, administration of VPA did not affect the kidney/body weight ratio in rats (Table 1) suggesting that VPA does not induce renal hypertrophy.

Serum concentrations of urea and creatinine are used to evaluate the functionality of the kidney (Whelton *et al.*, 1994; Oyewole *et al.*, 2012). Higher levels of these biomolecules in the serum, which could be a consequence of reduced glomerular filtration rate, suggest deficiency in renal function (Whelton *et al.*, 1994). Valproic acid, at various doses administered to rats in this study, caused no significant alteration ( $p > 0.05$ ) in serum urea and creatinine concentrations (Table

2) which suggests that the normal functioning of the kidney was not compromised.

Electrolytes play essential roles in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction, blood clotting and muscle contraction (Bhave and Neilson, 2011). Electrolyte levels in the serum are being governed by factors such as diet, renal excretion and pathological conditions and could serve as parameters of kidney function (Guyton and Hall, 2000). Potassium, sodium, chloride and phosphate ions are all important for proper electrolyte balance maintained by the kidney. Derangement of water and electrolyte balance may occur in subjects with consumption of xenobiotics (Kitabchi *et al.*, 2006). Potassium ion is essential in muscle contraction and the transmission of nerve impulses in animals through action potentials and it also influences osmotic balance between cells and the interstitial fluid (Agarwal and Udipi, 2014). Healthy kidneys remove excess potassium ions in the urine to help maintain normal levels in the blood (Terker *et al.*, 2015). The observed significant increase ( $p < 0.05$ ) in serum potassium ion levels in rats administered 600 mg/kg body weight of VPA in

this study could be as a result of its reduced excretion by the kidney or its leakage into the serum from damaged cells and tissues.

Sodium ion plays important roles in maintaining the salt/water balance in the body (Jackson *et al.*, 2014) while phosphate ion, in conjunction with calcium ion help to build and repair bones and teeth and to some extent support muscles and nerves (Jackson *et al.*, 2014). The various doses of VPA administered to rats in this study did not cause any significant alteration ( $p > 0.05$ ) in the serum concentration of sodium and phosphate ions suggesting that VPA protects the body against excessive fluid loss and neuronal dysfunction (Flanigan, 2000). Chloride ion is an important electrolyte located in all body fluids, responsible for maintaining acid/base balance, transmitting nerve impulses and regulating fluid across cell or intracellular membranes (Burtis and Ashwood, 1994). The observed significant increase ( $p < 0.05$ ) in serum level of chloride ion of rats administered 300 and 600 mg/kg bw of valproic acid could be due to loss of body fluids (dehydration) or a kidney disease. It could also be due to hyper-activity of the parathyroid glands. This can lead to depression of myocardial function and reduce cardiac output, renal and intestinal perfusion (Waters *et al.*, 2001). All these suggest that VPA at higher doses may adversely affect the reabsorption of chloride ion in the kidney which may alter the acid/base balance and impair nerve impulse transmission.

Most enzymes occur in cells at much higher concentrations than in serum. Some occur primarily in cells of certain tissues, where they may be located in different cellular compartments such as the cytoplasm or the mitochondria. 'Normal' serum enzyme levels reflect the balance between the rate of synthesis and release into plasma during cell turnover, and the rate of clearance from the circulation (Sulaiman and Ekanem, 2009). The assessment of the activities of the marker enzymes in tissues and body fluid

play a significant and important role in disease diagnosis and in the assessment of drug or plant extract for safety/ toxicity risk (Sulaiman and Ekanem, 2009). Alkaline phosphatase (ALP) is a marker enzyme for plasma membrane integrity (Wright and Plummer, 1974; Akanji *et al.*, 1993), such that any alteration in the activity of the enzyme in tissue, caused by interaction of membrane with xenobiotics (Molina *et al.*, 2005), would indicate likely damages to the plasma membrane of the cells (Yakubu *et al.*, 2006). In this study, VPA did not cause any significant change ( $p > 0.05$ ) in the activity of ALP in both the kidney and serum of rats suggesting that the integrity of the renal membrane is not compromised.

Acid phosphatase (ACP) is a marker enzyme for the lysosome. Different isozymes of ACP are found in different organs and their serum levels are used to diagnose various types of diseases such as prostate cancer (Holtzman, 1989). A significant increase ( $p < 0.05$ ) in the activity of ACP was observed in the kidney of animals administered 300 mg/Kg body weight valproic acid but there was no significant change ( $p > 0.05$ ) in its activity in the serum (Tables 4 and 5). This could be as a result of the increased macromolecule digestion and waste removal activities of the lysosomes in the kidney (Holtzman, 1989). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are two closely related enzymes involved in amino acid metabolism and well-known biomarkers for predicting possible toxicity to body tissues especially the liver and the kidney (Kechrid and Kenouz, 2003; Sulaiman and Ekanem, 2009). The observed significant increase ( $p < 0.05$ ) in the activity of AST in the kidney of rats administered 300 mg/kg bw valproic acid but no significant change ( $p > 0.05$ ) in its activity in the serum suggest that the increase in activity was due to an increase in amino acid metabolism in the kidney rather than a damage to the kidney cells. Also, the insignificant effect ( $p > 0.05$ ) of VPA on the

activity of ALT in both the rat kidney and serum further supports that VPA did not cause renal damage.

### Conclusions

The findings from this study imply that treatment with VPA at higher doses may adversely affect the reabsorption of chloride ion in the kidney which may alter the acid/base balance and impair nerve impulse transmission.

### Conflict of Interest:

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