Original article

Evaluation of the Cytotoxicity Effect of Sodium Valproate on Wistar Rats

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Abstract

Sodium Valproate is an anticonvulsant drug, used in clinical medicine for the treatment of various nervous disorders especially as antiepileptic. Study aim to investigate the effects of various oral doses of sodium valproate, on the growth, biochemical, hematological and pathological characteristics of treated Wistar rats without epilepsy. The rats were allotted to three groups, each of eight rats. Group one received placebo as control. Groups 2 and 3 were given sodium valproate in daily oral doses of 200 and 400 mg/kg/day for 4 weeks. The weight gain, hematological and serobiochemical parameters were recorded in addition to pathological changes and certain behavioral changes were observed in rats during the experiment period. The doses (200 and 400 mg/kg/day selected were somewhat toxic but not lethal to Wistar rats and caused gradual reduction of reflexes, inability to move, inclination to recumbency and huddling together, hair loss, significant increase in weight, hepatotoxicity, nephrotoxicity and neurotoxicity and alteration in AST, ALT, cholesterol, urea concentration and granulocytosis. The damage to vital organs of rats given daily oral doses of 200 mg/kg sodium valproate was less marked. We concluded that one month administration of the various oral doses of sodium valproate at 200 and 400 mg/kg/day is toxic to Wistar rats.

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Introduction

Sodium valproate (2-propyl pentanoic acid, VPA) is the sodium salt of valproic acid is one of series of carboxylic acids that have antiseizure activity (Bertram, 2001). Sodium valproate is an anti-epileptic drug and anti-convulsant for most various seizure disorders used to calm or stabilize the electrical activity in the brain of patients with epilepsy. Epilepsy is defined as recurrent seizure provoked by any immediate unidentifiable cause curable. This drug has been found useful in the treatment of the various

seizure disorders in adults and children because of its wide therapeutic spectrum (Willmore, 1999). The drug is very effective in generalized tonic- clonic seizures, absence seizures, partial seizure and myoclonic seizure (Semah et al., 2004).Other uses of valproate include management of bipolar disorder (Lennkh and Simhandl, 2000) and migraine prophylaxis (Silberstein, 2002; Bertram 2001), Lennox-Gastaut syndrome, controls acute

episodes of mania and it may be useful in infantile spasms (Oka et al., 2004; Verity et al., 1995), as well as other psychiatric conditions requiring the administration of a mood stabilizer. Side effects can include gastrointestinal disturbances and reversible hair loss, tiredness, tremors, nausea and vomiting (Gelder et al., 2006). In pregnancy, valproate has the highest risk of birth defects. Epidemiologic studies of valproate suggest an increased incidence of spina bifida in the offspring of women who took the drug during pregnancy. In addition, an increased incidence of cardiovascular, orfacial and digital abnormalities has been reported (Bertram, 2001; Holmes et al., 2002). Acute pancreatitis occurs rarely (Roodhooft et al., 1990). Electrolyte abnormalities, including hypernatremia, hyperkalemia and hypocalcemia, have been rarely reported (Andreson and Ritland, 1995; Flomenbaum et al., 2006). The median lethal dose of sodium valproate in rodents varies between 1100 and 3900 mg/kg body weight (Walker et al., 1990).

Sodium valproate acts by blocking of sodium ion channels; it is also a weak inhibitor of enzymes that deactivate Gamma Amino Butyric Acid (GABA) such as GABA transaminase. It may also stimulate the synthesis of GABA. It works by restoring the balance of certain natural substances (neurotransmitters) in the brain (Tripathi, 2003; Brent et al., 2005). This study was conducted to estimate possible toxic effects of sodium valproate by investigating the effect of various oral doses (200 and 400 mg/kg/day) of sodium valproate on the growth, biochemical, hematological and pathological characteristics of Wistar rats dosed for four weeks.

Material and methods

Drug: Sodium valproate 200 mg gastro-resistant tablets used to epilepsy treatment was purchased from a pharmacy in Omdurman, Sudan in (August, 2013).

Animals: Twenty four-3-month old male Wistar rats, with average body weight ranged from 145-150 g were used in this study. They were housed in propylene cages and were provided bedding with sawdust. The rats were clinically healthy and housed within the premises of Faculty of cience and Technology-Al-Neelain University animal house under standardhusbandry conditions, (30±2°C, 60-70% relative humidity and 12h: 12h day-night cycle) and fed on the rat diet (flour 50.3%, meat 44%, edible oil 3.5%, sodium chloride 1.5% and vitamins and minerals 0.7) and drinking water provided ad libitum. Animals wereacclimatized to the experimental conditions for a period of one week prior to the commencement of the experiment. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee.

Dose selection and drug preparation:

The dose of sodium valproate (200 mg/kg/day) was selected on the basis of gastro-resistant when used in epilepsy. From sodium valproate one tablet, 200 mg was weighted accurately and crashed into powder, then dissolved in5mL distilled water and used as stock drug.

Experimental design:

The rats were divided randomly to 3 groups, each of 8 rats. Group 1continued to be fed the normal diet and served as control. Groups 2 and 3 were given sodium valproate drug at 200 mg/kg/day and 400 mg/kg/day via the oral route through cathedral tube, respectively. All rats were dosed their designated experimental oral doses for 30 days. Body weights of rats were measured on day 0, 15th and day 30th of the treatment. All

animals were regularly observed for the signs of toxicity during the entire study.

At interval of 2 weeks, regularly, 4 rats from each group were anaesthetized with mild chloroform and dissected. Blood samples for biochemical parameters and tissue samples for histopathology were taken at the end of the experiment. At necropsy, all rats were examined to identify gross lesions and specimens of liver, kidneys, heart, spleen, intestines and brain were fixed in 10% neutral buffered formalin and processed for histopathology.

Haematological methods:

These techniques were performed according to an Automated Haematology Analyzer (Human GambH, max-planck-Ring 21, D- 5205 wiesbaden, Germany). Blood sample were collected in dry test tubes containing EDTA (Ethylene diamine tetra acetic acid). The parameters measured were Hemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBCs), platelets count, White Blood Cells (WBCs), differential WBCs counts and erythrocytes indices; Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Serobiochemical methods:

Blood samples were collected and allowed to clot and sera were separated by centrifugation at 3000 rpm for 5 min and stored at - 20°C until analyzed. Analysis of enzymes activity of control and test rats was performed according to the instructions in the manual of the Roche Diagnostic Hitachi 902 Analyzer (Germany, 1996). Here we measured Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and alkaline phosphatase (ALP) and concentrations of total protein, albumin, globulin, bilirubin cholesterol and urea.

Methods:

Necropsy was conducted to identify gross lesions, after anesthetizing, the rats were dissected. Specimens of the liver, kidneys, heart, spleen, intestines and brain were collected and immediately fixed in 10% neutral buffered formalin. The organs were embedded in paraffin wax, sectioned at 5µm diameter and stained routinely with haematoxylin and eosin (H&E) (Andrew et al., 2008).

Statistical analysis:

Statistical Package for Social Science (SPSS, version 18.2) was used for the analysis of the data. The values have been analyzed by one way Analysis of Variance (ANOVA) followed by Duncan's simple T-test. The significance of differences between means (mean ± Standard error (M± S.E)) was compared at each for all groups. $p<0.05$ was considered statistically significant.

Results

Effect on growth:

The effect on body weight of rats given daily oral doses of sodium valproate at 200 mg/kg/day (group2) and 400 mg/kg (group 3) for 4 weeks was shown in Table 1. All groups showed significant increase in weeks two and four ($p<0.05$) compared to control (group1). The rats in groups 2 and 3 huddled together became less active, lost hair and developed gradual loss of reflexes between days 7and 30 after commencement of sodium valproate treatment. No death among the rats occurred.

Hematological changes:

Hematological changes for rats given daily oral doses of sodium valproate at 200 mg/kg (group 2) and 400 mg/kg (group 3) for 4 weeks are presented in Table 2. Two weeks after treatment, the values of Hb, RBCs, PCV, WBCs and Neutrophils were lower ($p<0.05$) lymphocytes were higher ($p<0.05$) in all groups than control (group1). Four weeks after treatment the values of Hb and RBCs were lower ($p<0.05$) in all groups than control (group1). Lymphocytes were lower ($p<0.05$) and those of Neutrophils and WBCs were higher ($p<0.05$) in groups 2 and 3 than control (Group 1).

Serobiochemical changes:

Serobiochemical data are summarized in Table 3. The AST and ALT activity was lower ($p<0.05$) than control (group1) and the concentration of urea and cholesterol was higher ($p<0.05$) in groups 2 and 3compared to control (group1). Other serum parameters showed no significant differences between the control and test rats (groups 2 and 3), after 2 and 4 weeks of treatment.

Table.1. Body weight and Body weight gain in rats given sodium valproate orally for four weeks

Treated groups	Body weight gain (g)		
	Day 0	2 weeks	4 weeks
1.Control (normal diet)	145.8±4.9	15.2±4.6	19.7±4.9
2. 200 mg/kg/day	145.0±4.6	19.3±2.8^{NS}	28.0±3.5*
3. 400 mg/kg/day	145.0±0.5	20.3±4.6*	31.4±2.1*

Values are expressed as mean ±S.E; NS = not significant; *Significant = ($p<0.05$)

Table.2. Hematological changes in rats given sodium valproate orally for four weeks

Parameters	1. Control (normal diet)	2.Sodium valproate (200 mg/kg/day)	3. Sodium valproate (400 mg/kg/day)
2 weeks			
Hb (g/dl)	11.0±0.8	9.1±0.1	8.4±0.3*
RBC (x10^6mm^3)	6.0±0.5	4.0±0.1*	4.8±0.1*
PCV (%)	34.1±2.8	27.5±0.5*	26.1±0.7*
MCV (m^3)	56.7±0.5	55.4±0.4^{NS}	53.9±0.6*
MCH (pg)	18.2±0.4	18.1±0.3^{NS}	17.3±0.1^{NS}
MCHC (g/L)	32.1±0.4	32.5±0.5^{NS}	32.1±0.1^{NS}
WBC (X10^6mm^3)	3.6±0.7	2.3±0.1*	2.2±0.3*
Lymphocytes (%)	34.2±2.1	29.6±1.4*	26.2±1.2*
Neutrophils (%)	65.8±2.1	70.4±1.4*	73.8±1.2*
4 weeks			
Hb (g/dl)	10.2±0.7	8.8±0.3*	9.0±0.4*
RBC (x10^6mm^3)	5.5±0.4	4.2±0.0*	4.3±0.3*
PCV (%)	33.1±2.1	31.9±0.3^{NS}	31.3±2.1^{NS}
MCV (m^3)	60.0±0.6	60.9±0.9^{NS}	58.7±0.7^{NS}
MCH (pg)	18.4±0.0	18.7±0.6^{NS}	18.8±0.6^{NS}
MCHC (g/L)	30.7±0.3	30.7±0.6^{NS}	32.1±1.1^{NS}
WBC (X10^6mm^3)	4.7±0.7	6.8±0.6*	6.9±2.1*
Lymphocytes (%)	31.2±3.4	42.6± 3.5*	47.5±3.4*
Neutrophils (%)	68.8±3.4	57.6±3.7*	52.5±3.4*

Values are expressed as mean±SE; NS = not sinificant; *significant = ($p<0.05$)

Table.3. Changes in serum constituents of rats given sodium valproate orally for four weeks

Parameters	1. Control (normal diet)	2.Sodium valproate (200 mg/kg/day)	3. Sodium valproate (400 mg/kg/day)
2 weeks			
AST (IU)	186.0±1.0	168.5±7.5*	165.7±3.5*
ALT (IU)	39.50±0.5	26.5±3.5*	31.70±3.6^{NS}
Total protein (g/dl)	6.300±0.1	6.60±0.1^{NS}	6.100±0.2^{NS}
Albumin(g/dl)	4.300±0.3	4.40±0.2^{NS}	4.300±0.2^{NS}
Globulin(g/dl)	2.000±0.1	2.20±0.1^{NS}	1.800±0.1^{NS}
Bilirubin (mg/dl)	0.100±0.0	0.10±0.0^{NS}	0.100±0.0^{NS}
Cholesterol (mg/dl))	70.50±0.5	88.5±5.5*	85.00±2.9*
Urea(mg/dl)	36.50±0.5	64.5±3.5*	66.40±4.3*
4 weeks			
AST (IU)	201.0±1.0	182.5±2.5*	193.0±27.5*
ALT (IU)	50.50±0.5	44.0±3.0*	48.7±04.2*
Total protein (g/dl)	5.700±0.1	6.4±0.0^{NS}	6.2±00.2^{NS}
Albumin(g/dl)	4.000±0.1	4.3±0.2^{NS}	4.0±00.2^{NS}
Globulin(g/dl)	1.900±0.1	2.2±0.2^{NS}	2.2±00.2^{NS}
Bilirubin (mg/dl)	0.100±0.0	0.1±0.0^{NS}	0.1±00.0^{NS}
Cholesterol (mg/dl))	75.0±3.0	94.5±2.5*	101.0±04.4*

Values are expressed as mean±S.E; significant: *significantly = ($p<0.05$)

Pathological changes:

After four weeks of treatment with daily oral doses of sodium valproate at 200 mg/kg/day (group 2) and 400 mg/kg/day (group 3) for four weeks showed consistent lesions that included epithelial cell degeneration or necrosis of the renal convoluted tubules, shrinkage of the glomeruli and aggregates of lymphocytes in the renal cortex (Fig. 1a to e). Cytoplasmic fatty vacuolation of centrilobular hepatocytes and isolated cell necrosis in liver (Fig. 2a to d), infiltration of lymphocytes in the intestinal lamina propria (Fig. 3a and b) and vacuolation of the cerebral neurons in brain were detected in rats given 400 mg/kg/day of sodium valproateorally (Fig. 4). These changes were less marked in group 2. No significant lesions were observed in the control rats (Group1) or in the spleen and heart of any of the sodium valproatedosed rats throughout the four weeks in vital organs.

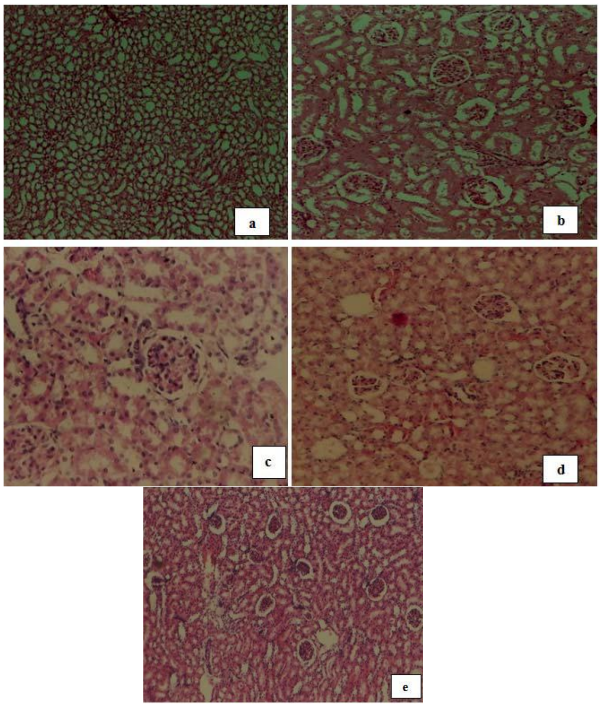

Fig. 1: Kidneys of rats received daily oral doses of sodium valproate at 400 mg/kg for two weeks showing (a) Cytoplasmic fatty change in medulla, (b) Glomerular alteration, packing, segmentation and dilatation, (c and d) Necrosis and fatty vacuolation of kidney tubules. And after two weeks at dose of 200 mg/kg orally showing: (e) egeneration, shrinkage, dilation and necrosis of renal tubules in cortex. (H&E)×200

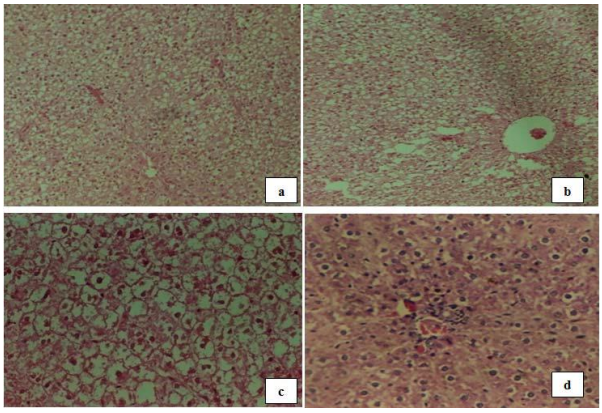

Fig. 2: Liver of rats received daily oral doses of sodium valproate at 400 mg/kg for four weeks showing (a & b) Fatty Cytoplasmic vacuolation of entrilobularhepatocytes, (c(X250) &d) Cytoplasmic fatty vacuolation and necrosis of the centrilobular hepatocytes (H&E)×100

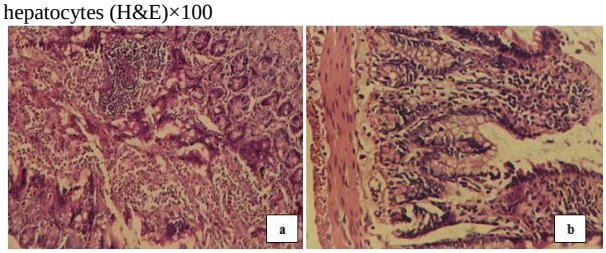

Fig. 3: Intestine of a rats received oral doses of sodium valproate at 200 mg/kg/day for four weeks (a) and 400 mg/kg/day for 2 weeks (b), showing Catarrhal enteritis and lymphocytic infiltration in intestinal lamina Propria H&E X20

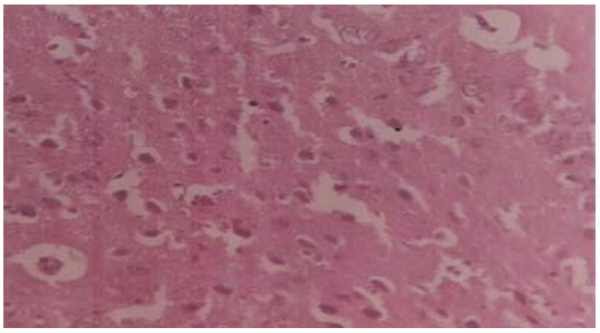

Fig. 4: Brain of a rat received daily oral doses of sodium valproate at 400 mg/kg for two weeks showing cerebral neuronal vacuolation H&E X200

Discussion

Although sodium valproate is used for the treatment of epilepsy in humans and animals, the results of the present study indicated that, sodium valproate is toxic but not fatal to Wistar rats dosed 200 and 400 mg/kg/day for one month orally. This observed that pathological, hematological and biochemical change were indicative of sodium valproate affecting the intestines, liver, kidneys and Brain and damage of these organs could explain the development of gradual loss of reflexes, inability to move, hair loss and inclination to recumbency.

Treating rats with sodium valproate induced a noticeable decrease in Hb, RBCs, PCV, WBCs and increase in total cholesterol, urea concentration and lymphocytes, these data was supported by a research conducted by Heldenberg *et al.* (1983) who have also found an increased cholesterol levels and urea in epileptic children treated with sodium valproate. Tripathi *et al.* (2003) and Acharya and Bussel (2000) stated that, Sodium valproate can cause bone marrow suppression leading to aplastic anaemia or peripheral cytopenia, thrombocytopenia and neutropenia, this findings might be an evidence for what we has been found regarding hematological changes, in which there was a decrease in RBCs, Hb and PCV values with no change in MCV, MCH or MCHC.

The acute changes in the liver and kidneys were probably contributed to the decreased serum AST, ALT activity and total protein. Increased urea, cholesterol concentration and significant increase in the body weight in comparison with control rats.

In another study undertaken by Shaat *et al*. (2006) and Bertram(2001) showed a decreased liver enzymes activity and increased appetite, weight gain and hair loss, this found was obvious in the present study in which enzymes of liver were also decreases as well as the other changes mentioned by Shaat. Tong *et al*. (2005) has also stated that, plasma transaminases are sensitive indicators of liver cell injury.

The liver depicted fatty vacuolation of the hepatocytes and isolated cell necrosis, kidneys had altered glomeruli and degeneration of convoluted tubules, cerebrum showed vacuolar neurons in brain and aggregates lymphocytes in intestinal lamina propria observed in this study, these findings confirmed by a previous studies conducted by Raza *et al*. (2000) and khan *et al.* (2005) reported ahistopathological changes in liver of albino rat induced by toxic dose of valproic acid. Yosry and Seham (2014) and Ibrahim (2012) reported that sodium valproate induces toxic effects on liver tissue of albino rat or mice.

The development of locomotor disturbances in rats given 400 mg/kg/day sodium valproate for 4 weeks might be due to hepatotoxicity because of the absence of significant changes in the brain these data has been supported by Bertram (2001) and Holmes *et al*. (2002). The organ damage resulted from treatment with 200 mg/kg/day of sodium valproate for 4 weeks was less intense. RBCs indices have not been seriously altered and the granulocytes seem to be decreased probably due to infiltration in the vital organs.

Although the sodium valproate (VPA) is a drug widely used to treat epilepsy, but it has serious adverse effects including hepatotoxicity (Khan and Jena, 2013), teratogenicity (Kalter, 2003) Hematotoxicity (Acharya and Bussel, 2000) and genotoxicity (Laxminarayana *et al.*, 2010) in both animals and humans. The experimental results indicated a need for caution in its use.

Conclusions

The data of the present study concludes that, the daily oral doses of 200 and 400 mg/kg/day of sodium valproate for 4 weeks were toxic but not fatal to Wistar rats and caused hepatonephro-toxicity, haematological, serobiochemical changes and neurotoxicity, which may be resulted from consistent damage to liver, kidneys, Intestine and brain. The mechanism whereby the drug injured body tissues cannot be derived from the present study. Investigations into mechanism of action of sodium valproate in animals are suggested.

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