Differential Hepatotoxic Effects of Sodium Valproate at Different Doses in Albino Rats

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ABSTRACT

Background

Liver plays an essential role for transforming and clearing chemicals that may cause harmful effects to it. Sodium Valproate, renowned to be a potent antiepileptic drug, when taken in overdose may cause toxic effects to liver and other organs as well. Liver damage can be assessed with histological changes and measurement of enzymes produced by it.

Objective

To investigate the histological changes induced by different doses of Sodium Valproate ranging from 100-500 mg/kg/day and observe its correlation with liver enzymes level in serum.

Method

Three-months old albino rats were divided into six groups, five in each. Control group was treated with normal saline and rest five groups with Sodium Valproate in different doses 100, 200, 300, 400 and 500 mg/kg/day respectively. Then, liver of those experimented rats were examined histologically under the light microscope. Furthermore, Liver enzymes; Alanine Transaminase and Aspartate transaminase were measured to assess the micro-anatomical changes in liver.

Result

Distorted hepatic lobular architecture with aggregations of nuclei at certain interval was observed in the groups of higher doses; 300 mg/kg/day and above. However, accumulation of adipocytes was observed in all the Sodium Valproate treated rats unlike the control group. When compared the enzyme levels among the groups, it was found to be significantly increased in dose dependent manner. Besides, it also showed skin lesions in all rats treated with the dose 400 mg/kg/day and above.

Conclusion

Higher doses of Sodium Valproate; 300 mg/kg/day and above induces hepatotoxicity and skin lesions in adult albino rats.

KEY WORDS

Hepatotoxicity, Liver enzymes, Sodium valproate

INTRODUCTION

Sodium Valproate (SV) is the sodium salt of Valproic acid which is an 8-carbon 2-chain fatty acid (by its official name 2-propylvaleric acid), with a chemical structure very similar to that of short chain fatty acids.[1] It is one of the mostly prescribed antiepileptic drug being effective in the treatment of many different types of partial and generalized epileptic seizures and also for other neuropsychiatric problems like bipolar disorders, schizoaffective disorder, social phobias, neuropathic pain as well as for prophylaxis of migraine headache.[1] Moreover, it has been more recently used as adjunctive therapy to benzodiazepines for treatment of alcohol and other sedative-hypnotic withdrawal syndromes and occasionally for chronic pain syndromes.[2]

Though it plays a huge role with such treatments, is associated with number of adverse effects too that may lead to life threatening condition of the patients if not taken care in time. A number of researchers have found Sodium Valproate causing adverse effects like pancreatitis, liver toxicity, teratogenicity and cutaneous reactions.[3-7] The postulated mechanism behind such devastating toxicity is the oxidative stress caused by the drug induced reactive oxidative metabolites and its insufficient detoxification.[8] As one of its consequences, the liver tissue gets damaged and releases their enzymes such as Aspartate Transaminase (AST) and Alanine Transaminase (ALT) into the systemic circulation, suggesting the fact that the degree of liver tissue damage can be predicted by the measurement of those enzymes in serum.[4,5]

Hence, the present study was purposed to observe the hepatotoxicity induced by different doses of Sodium Valproate and assessed with the help of morphological and microscopic study along with the analysis of biochemical markers; Aspartate Transaminase and Alanine Transaminase.

METHODS

Chemicals and reagents

The drug Sodium Valproate was received from Nepal Pharmaceuticals Pvt. Ltd. of Pulchowk. All the other reagents were obtained from Dhulikhel hospital, Kavre, Nepal. The Department of Anatomy, Kathmandu University School of Medical Sciences, Kavre, Nepal, approved the experimental protocol for this study.

Experimental design

Thirty virgin Wistar albino rats with initial body weight ranging between 150 – 250 g were used and kept in a well ventilated room maintaining the temperature (25 ± 5 °C) as well as natural light and dark cycle. All these rats were fed standard pellet diet (Bengal gram and cabbage) and tap water ad libitum.

Animals were divided into six groups having five in each. Control group (Group 1) was treated with 1 ml normal saline. For the rest five groups (Group 2 to 6) Sodium Valproate solution was administered in a dose of 100, 200, 300, 400 and 500 mg/kg/day respectively. All the injections were given via intraperitoneal route for eight consecutive days. Sodium Valproate solution was prepared by weighing the purified SV powder according to the body weight of the rats and then dissolved in normal saline to the final volume 1 ml.

On 9th day, all the experimented rats were anaesthetized to collect blood sample by direct cardiac puncture in order to assess the biochemical parameters. Then, those rats were sacrificed giving deep anesthesia and took out the liver for further microscopic examination.

For microscopic and biochemical analysis

Histological slides of the liver were prepared with Hematoxylin and Eosin staining procedure and observed under the light microscope. The activity of biomarkers responsible for hepatotoxicity was assessed with the measurement of AST and ALT level of the serum collected from the experimented rats. The estimation of those enzymes was accomplished by kinetic method.[9-11] The laboratory works were carried out in the Department of Pathology and Biochemistry, Dhulikhel Hospital, Kavre, Nepal.

The data were entered in Minitab Version 15 Computer Program. Then, one-way ANOVA test was used to find if the differences in enzyme levels were significant. Two-sample T-test was performed to find the p value.

RESULTS

Effect on the liver histology

When the histological sections of liver of experimental rats were observed under light microscope, following observations were made: In the control group (Group 1), hepatic lobular architecture was intact. The laminae, sinusoids, portal canals of the rat liver were very distinct (fig. 1).

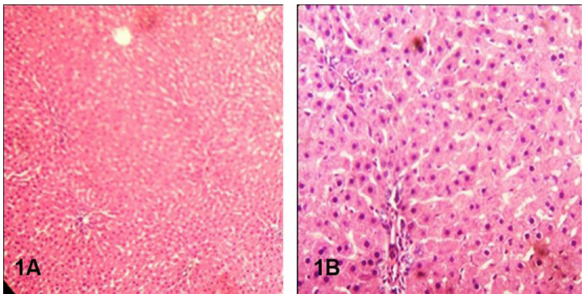

Figure 1. Histological observations of liver showing the intact hepatic lobular architecture. Photomicrographs were taken under the light microscope with the magnifications; 10x (A) and 40x (B).

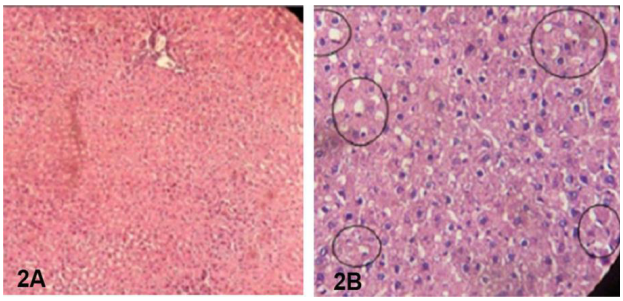

Figure 2. Histological observations of SV-treated liver showing the accumulation of adipocytes (shown with black circles) among the hepatic cords. Magnifications; 10x (A) and 40x (B).

Those structures were found to be well preserved in the rats of Group 2 and 3 i.e. treated with Sodium Valproate in the doses of 100 mg/kg/day and 200 mg/kg/day. However, adipocytes (fig. 2 shown with circles) were observed, arranged in diffused form among the hepatic cords. From Group 4 onwards i.e. the rats treated with dose of 300 mg/Kg/day and above, the lobular architecture was found to be distorted (fig. 3-5) as compared to the control group and other groups treated with lower doses of SV. Besides, another important observation in those groups was the nuclei aggregations at certain intervals which might have occurred due to the damage of hepatocytes (fig. 3-5 shown with arrow head).

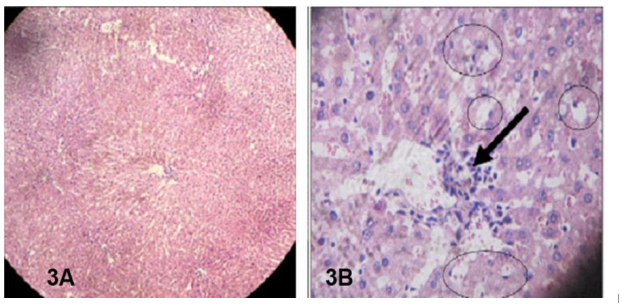

Figure 3. Histological observations of SV-treated liver showing the aggregation of nuclei reflecting the tissue damage (shown with black arrow). Magnifications; 10x (A) and 40x (B).

In the Group 5 and 6 i.e. the rats treated with Sodium Valproate in the doses of 400 mg/Kg/day and 500 mg/Kg/day respectively, alopecia and skin lesions were observed towards the abdominal side (fig. 5A-B). However, the intensity of the hair loss and the severity of the lesions were found to be variable when compared among the individual rats of the same group.

Effect on the liver enzymes

The serum level of two enzymes namely Alanine Transaminase (ALT) and Aspartate Transaminase (AST) were estimated to assess the damage caused by different doses of Sodium Valproate in the liver of rats, which are supposed to be released in the circulation from the damaged hepatocytes. Both of the liver enzymes were found to be increased significantly (p value < 0.05) in the Sodium Valproate treated groups than in the control group (fig. 6-7).

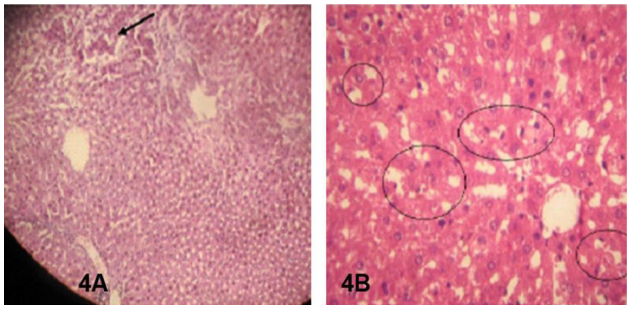

Figure 4. Histological observations of SV-treated liver showing the distorted hepatic lobules (shown with black circles). Magnifications; 10x (A) and 40x (B).

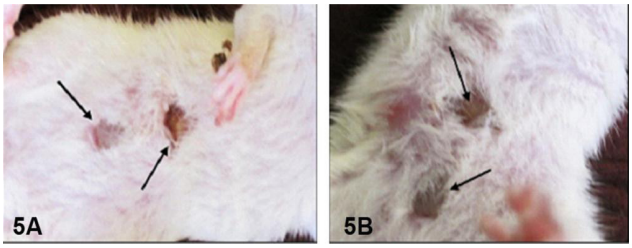

Figure 5. Hair loss and skin lesion observed on SV-treated rats (shown with black arrow head). A) SV at the dose of 400 mg/kg/ml. B) SV at the dose of 500 mg/kg/ml.

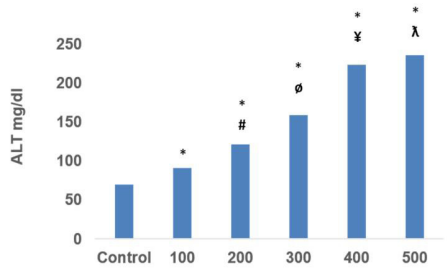

Figure 6. Effect of different doses of SV at ALT level in the serum. (n=5, *p<0.05 Vs Control, #p<0.05 Vs 100 mg/kg/day,øp<0.05 Vs 200 mg/kg/day, ¥p<0.05 Vs 300 mg/kg/day and ƛp<0.05 Vs 400 mg/kg/day)

Similarly, even in the Sodium Valproate treated groups, the mean value of enzyme level of ALT was found to be increased significantly (p value < 0.05) in dose dependent manner (fig. 6). But, in case of AST, the rise in mean values was found to be increased significantly only up to the dose of 300 mg/Kg/day (Group 4) and above it was non-significant i.e. p values were 0.7 and 0.9 when compared between the groups 4 and 5, groups 5 and 6 respectively (fig. 7).

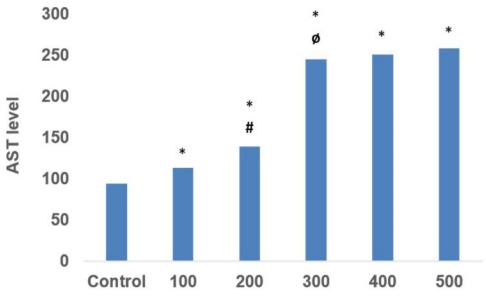

Figure 7. Effect of different doses of SV at AST level in the serum. (n=5, *p<0.05 Vs Control, #p<0.05 Vs 100 mg/kg/day and øp<0.05 Vs 200 mg/kg/day)

DISCUSSION

Sodium Valproate acts as a broad spectrum within a certain therapeutic range normally up to the maximum dose 60 mg/kg/day. However, in complicated and emergency cases, the dose has to be increased to some extent in order to control the given situations of the brain, though it could be toxic for other organs of our body. One of the most feared and serious reaction of SV is hepatotoxicity as the drug has to be metabolized in liver.[12-13]

The present study had taken SV ranging from 100 mg/kg/day to 500 mg/kg/day and treated rats with parenteral route (intraperitoneal) to ensure that animals would receive full dose of Sodium Valproate.

When examined under light microscopy, the hepatic lobular architecture was found to be distorted in the rats treated with the doses of 300 mg/kg/day and above. Similar results have been found in one of the research done by Khan et al. who has also observed the distorted lobular architecture of liver at the same doses.[14] Other several studies have found liver steatosis and necrosis induced by Sodium Valproate.[14-18] However, the present study did not observe any such specific pathological lesion which supports the study made by James W. Keterson et al. that also didn't show such effects even at nearly lethal doses of 700 mg/Kg/day. The drug did however, induced hepatic lipid accumulation in mature rats and in young rats dosed concomitantly with phenobarbital.[18] Similar observations were there in our study as well, for all SV treated groups.

Furthermore, aggregations of nuclei at certain intervals were also observed in the groups treated with higher doses of SV i.e. above 300 mg/kg/day. The aggregation of nuclei could be a process leading towards necrosis, the specific pathological lesion that most of the scientists reported in their studies.

Besides, alopecia and skin lesions were also observed in the groups treated with higher doses 400 and 500 mg/kg/day as shown in (fig. 6). One of the studies of Roy S et al. had observed a patient under the Sodium Valproate therapy 500 mg/kg/day who developed hair fall with white scaly lesions on scalp, trunk, chest and limbs. Similar skin rashes were also observed by the other study made by Ghaffarpour et al. induced by Sodium Valproate and other antiepileptic drugs.[7,19]

The other parameters that we measured to assess liver injury caused by Sodium Valproate was the estimation of liver enzymes AST and ALT. The activities of these enzymes are often used for evaluating the hepatotoxicity induced by chemicals or toxicants as plasma transaminases are sensitive indicators of liver cell damage.[20, 21] Our result was concurrent with one of the studies made by Qiaoli et al. who also observed the activities of both of the enzymes to be increased significantly in dose dependent manner.[22] But unlike to that study, we found increased AST level not significant above the dose of 300 mg/Kg/day. It may be because, ALT is more specific to the liver than AST with limited concentration in other organs. This enzyme has a longer half-life (37-57 h) than AST (12-24 h). As a result, elevation of ALT persists longer after hepatic damage has ceased. However, levels of AST rise in response to hepatic damage but clear quickly once damage ceases.[23] According to Giannikou et al. mild and reversible hepatic involvement during Sodium Valproate treatment is dose related and generally consists only of abnormalities in liver enzymes without clinical symptoms.[24] The present study also supports the same observations.[24]

Limited sample size and lack of electron microscopic observations were the limitations of our study as we couldn't observe any probable ultra-structural changes like altered mitochondria, dilated Golgi cisternae containing lipoprotein particles etc. as found in one of the studies made by Jezequel et al.[25]

CONCLUSION

Sodium Valproate of different toxic doses (100-500 mg/kg/day), distinct histological changes have been found only at the doses of 300 mg/kg/day and above. Moreover, the enzyme levels have also increased accordingly. Hence, it can be concluded that structural changes/damage don't occur even with the higher doses up to 200mg/kg/day when examined under the light microscopy.

REFERENCES

1. Johannessen CU, Johannessen SI. Valproate: past, present, and future. *CNS Drug Rev.* 2003;9:199-216.
2. Loscher W. Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. *CNS Drugs*. 2002;16: 669-94.
3. Powell-Jackson PR, Tredger JM and Williams R. Hepatotoxicity to Sodium Valproate a review. *Gut*. 1984;25:673-81.
4. Lewis JH, Zimmerman HJ, Garrett CT, Rosenberg E. Valproate-induced hepatic steatogenesis in rats. *Hepatology.* 1982;2:870-3.
5. Lee MH, Hong I, Kim M, Lee BH, Kim JH, Kang KS, Kim HL, et al. Gene expression profiles of murine fatty liver induced by the administration of valproic acid. *Toxicology and Applied Pharmacology.* 2007;220:45–59.
6. Zoran D.L., Boeckh A., Boothe D.M. Hyperactivity and alopecia associated with ingestion of valproic acid in a cat. *J Am Vet Med Assoc.* 2001;218:1587-9.
7. Ghaffarpour M, Hejazie SS, Harirchian MH and Pourmahmoodian H. Phenytoin, Carbamazepine, Sodium Valproate and Lamotrigine induced cutaneous reactions. *Acta Medica Iranica.* 2005;43(1);37-42.
8. Schlienger RG, Shear NH. Antiepileptic drug hypersensitivity syndrome. *Epilepsia*. 1998;39Supl7:S3-7
9. Bergmeyer HU, Scheibe P, and Wahlefeld AW. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clinical Chemistry.* 1978;24:58-73.
10. Bermeyer HU. IFCC methods for the measurement of catalytic concentrations of enzymes: Part3, IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC2.6.1.2) *Clin Chem Acta.* 1980;105(1):147-54.

11. Thefald Wetal, Hoffmister H, Busch EW, Koller PU, Vollmar J. Reference values for the determination of GOT, GPT and alkaline phosphatase in serum with optimal standard methods. *Dtsch Med Wochenschr.* 1974;99(8):343-4.

12. Murugesan V., Subramanian P. Enhancement of circulatory antioxidant by alpha-ketoglutarate during Sodium valproate treatment in Wistar rat. *Pol J Pharmacol*. 2003;55:31-36.

13. Ubeda N, Alono AE, Varela MG. Acute valprovate dministration impairs methionine metabolism in rats. *Nut.* 2002;132:2737-42.

14. Khan SK, Shakoor KA, Jan MA, Khattak AM, Shah SH. Study of the Histopathologic changes in the Liver of the Albino rats, induced by toxic doses of Valproic acid. *Gomal Journal of medical sciences.* 2005;3(1):15-8.

15. Sobaniec-Lotowska ME. Effects of long term administration of the antiepileptic drugs–sodium valprovate upon the ultra structure of hepatocytes in rat. *Exp toxicol pathol*. 1997;49:225-32.

16. Ware M, Sadler GH. Acute liver disease associated with sodium valprovate. *Lancet.* 1998;2:1110-13.

17. Sugimoto T, Woo M, Nishida N, Tachiochi T, Sakane Y, Kobayashi Y. Hepatotoxicity in rat following administration of valproic acid. *Epilepsia.* 1987;28:142-6.

18. Kesterson JW, Granneman GR, Machinist JM. The hepatotoxicity of valproic acid and its metabolites in rats. I. Toxicologic,biochemical and histopathologic studies. *Hepatology.* 1984(4):1143–52.

19. Roy S, Goel D. Unusual cutaneous reaction with Sodium Valproate. Neurol India 2009; Roy S, Goel D. Unusual cutaneous reaction with Sodium Valproate. *Neurol India.* 2009;57(2):216-7.

20. Dosson WE, Tasch V. Pharmacology of Valproic acid in children with severe epilepsy; clearance and hepatotoxicity. *Neurology.* 1981;31:1047-50.

21. Burton BS. On the propyl derivatives and decomposition products of ethylacetoacetate. *Am Chem Journal.* 1882;3:385–395.

22. Qiaoli Ji, Xiaolian Shi , Rong Lin , Youhua Mao , Xiaogang Zhai , Qinqin Lin et.al., *Toxicology in Vitro.* 2010;24:1086–1091.

23. Friedman L. Liver, Biliary Tract, & Pancreas. In: McPhee S., Papadakis M., Tierney L. (eds), Current Medical Diagnosis and Treatment, 46e. McGraw-Hill 2007;667.

24. Roma-Giannikou E., Syriopoulou V., Kairis M., Pangali A., Sarafidou J., and Constantopoulos A. In vivo effect of sodium valporate on mouse liver. *Cell. Mol. Life Sci*. 1999;56:363-369.

25. Jezequel A. M., Bonazzi P., Novelli G., Veturini C. and Orlandi F. Early structural and functional changes in liver of rats treated with a single dose of Valproic acid. *Hepatology.* 1984;4(6):1159-66.