



Evaluation of the Lethal and Toxic Doses of Valproic Acid on the Liver and Kidney of Pregnant Mice

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ABSTRACT

Background: The main treatment for nervous system disorders requires patients to take sodium valproate as their primary medication for symptom management. The drug achieves its effectiveness through its ability to stop enzymes from working while it raises brain levels of gamma aminobutyric acid (GABA). However, using sodium valproate for prolonged periods can lead to side effects. The researchers investigated sodium valproate effects on pregnant mice while assessing folic acid as a protective substance.

Methods: The study involved thirty pregnant mice who received various treatments throughout six experimental groups. The first group functioned as the control group because they did not receive any treatment. The second group received 250 mg/kg of sodium valproate but the third group received 500 mg/kg of sodium valproate. The fourth group received 5 mg/kg of folic acid as their treatment.

Result: The fifth group received 500 mg/kg of sodium valproate together with 5 mg/kg of folic acid during the 18-day treatment period. The sixth group received 250 mg/kg of sodium valproate together with 5 mg/kg of folic acid throughout the entire 18-day study duration. The biochemical results from Valproate-treated groups showed major changes which included decreased GSH and SOD levels and elevated LPO and triglycerides and cholesterol and TNF- α levels. The histopathological examination revealed three main changes which consisted of hepatocyte death and inflammatory cell entry and glomerular tissue shrinkage. The combination of folic acid with other substances reduced oxidative and inflammatory damage but only when using 250 mg/kg of the substance. The study shows that sodium valproate causes major liver and kidney destruction in pregnant mice but folic acid proves to be a protective substance when valproate consumption remains low.

Key words: Antioxidants, Folic acid, Kidney, Liver, Mice, Valproic acid.

INTRODUCTION

Sodium valproate is commonly prescribed to treat conditions affecting the nervous system such, as epilepsy and bipolar disorder as well as for preventing migraines (Janković and Janković, 2020). The drug achieves its effectiveness through its ability to stop enzymes from working while it raises brain levels of gamma aminobutyric acid (GABA). However using sodium valproate in doses or for prolonged periods can lead to side effects, like liver and kidney damage that can be severe and even life threatening (Safdar and Ismail, 2023). The liver damaging property of sodium valproate stems from its impact, on liver cells by interrupting metabolic functions and raising stress within the cells which ultimately causes cell deterioration and demise (Ezhilarasan and Mani, 2022). When it comes to the kidneys health impact shows through issues, with how they work and higher levels of creatinine and urea in the blood which point to problems with kidney function being compromised during pregnancy when the liver and kidneys, under extra strain to help sustain the developing baby (Laville *et al.*, 2023; Lohiya *et al.*, 2017). Leading factors, in tissue and organ harm are stress and inflammatory responses. The biochemical responses of sodium valproate affect these results because it decreases glutathione (GSH) levels and increases lipid peroxidation (LPO) which indicates higher oxidative damage (Lohiya *et al.*, 2017). The inflammatory responses lead to the release of tumor necrosis factor alpha (TNF- α) which causes tissue damage to become

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worse (Gutiérrez-Cuevas *et al.*, 2021; Clower *et al.*, 2022). Super oxide dismutase (known as SOD for short) plays a role, in the bodys defense against harm; meanwhile increased levels, in triglycerides and cholesterol indicate how sodium valproate affects lipid processing and buildup in the bodys tissues (Gęgotek and Skrzydlewska, 2022). Folic acid is a vitamin that is involved in biological functions such, as DNA production and cell repair while also helping to reduce oxidative stress levels in the body. A common practice during pregnancy is to use folic acid to lower the risk of tube defects, in babies (Thakur *et al.*, 2020). Study shows that folic acid works as a

medication countermeasure because it contains antioxidants which activate cells and reduce inflammation (Sharma *et al.*, 2021). This study aims to investigate how varying amounts of sodium impact the liver and kidneys of mice-a model that offers insight, into drug toxicity amidst the body's changes during pregnancy's course of events. This study holds importance for addressing a pressing health issue and aiding in the formulation of treatment approaches to safeguard essential functions while reducing harmful impacts of toxic medications, in pregnancy. The study is focused on examining how amounts of sodium valproate affect the livers and kidneys of mice and also looks into the influence of sodium valproate, on glutathione (GSH) lipid peroxidation (LPO) tumor necrosis factor alpha (TNF- α) superoxide dismutase (SOD) activity levels in pregnant mice as well as their triglyceride and cholesterol levels while evaluating how effective folic acid is, in reducing these harmful effects.

MATERIALS AND METHODS

Animals

In this study project Swiss albino mice weighing, between 25 and 30 grams and aged 8 to 2 weeks were utilized for the experiment to determine pregnancy by observing the presence of a plug on the day following mating. The mice were kept in laboratory conditions with regard, to temperature humidity and diet. The experiment was conducted at the Department of Basic Nursing Sciences, College of Nursing, Nineveh University, Mosul, Iraq. The experiment was carried out between March and June 2023.

Experimental design

A total of 30 pregnant mice were divided into six groups (5 mice per group):

- **G1 (Control group):** No treatment.
- **G2:** Sodium valproate 250 mg/kg.
- **G3:** Sodium valproate 500 mg/kg.
- **G4:** Folic acid 5 mg/kg.
- **G5:** Sodium valproate 500 mg/kg + folic acid 5 mg/kg.
- **G6:** Sodium valproate 250 mg/kg + folic acid 5 mg/kg.

Dose administration

Doses were administered orally using a specialized syringe daily for 18 days. At the end of the treatment period, the mice were anesthetized with ether for blood collection via orbital puncture. The mice were then dissected to obtain the liver and kidneys for histopathological examination.

Sample collection

- **Blood samples:** Collected on day 18 to separate serum for biochemical analysis.
- **Tissue samples:** Liver and kidney tissues were preserved in 10% formalin for histopathological analysis.

Histological study

Liver and kidney samples were fixed in 10% formalin to preserve the tissues. Standard histological techniques were

employed to prepare thin tissue sections. These sections were stained with hematoxylin and eosin (H and E) to highlight cellular and tissue details, allowing for the evaluation of pathological and structural changes caused by the studied factors.

Biochemical tests

The following parameters were measured:

- **Glutathione (GSH):** Measured using reagents from Biolabo, France, following the manufacturer's instructions, as previously described in studies evaluating valproic acid-induced oxidative stress in experimental models (Jiang *et al.*, 2022).
- **Lipid peroxidation (LPO):** Measured using Biolabo reagents.
- **Tumor necrosis factor-alpha (TNF- α):** Measured using Biolabo reagents Kits.
- **Superoxide dismutase (SOD):** Measured using Biolabo kits.
- **Triglycerides:** Measured using Biolabo kits.
- **Cholesterol:** Measured using Biolabo kits.

Data analysis

The findings were analyzed through a setup employing a completely random design method. Duncan's multiple range test was applied to detect variations, among the groups. Significance was attributed to results with a likelihood level of ($p \leq 0.05$). To compute the standard error values statistical software (SAS).

RESULTS AND DISCUSSION

The histological results demonstrate that valproic acid induces liver and kidney damage which increases with dose in pregnant mice. The liver tissue from animals that received 250 and 500 mg/kg doses showed three main pathological changes which included coagulative necrosis and sinusoidal dilation and inflammatory cell infiltration (Fig 1). The kidney tissue from these animals showed three main pathological changes which included glomerular atrophy and Bowman's space expansion and tubular degeneration and hyaline cast formation. The lesions indicate that maternal tissues become susceptible to xenobiotic-induced oxidative stress because their metabolic and filtration systems fail to function properly (Fig 2).

The biochemical results demonstrated that GSH and SOD levels decreased while LPO and TNF- α levels increased which confirmed the histological results by demonstrating that oxidative damage and inflammation are essential factors in valproate toxicity. The body shows dysregulated metabolism because it cannot control lipids effectively after liver cell damage based on the elevated triglyceride and cholesterol levels.

The study demonstrated that folic acid supplements protected tissues from damage while they increased antioxidant levels when patients took valproate at any of the two prescribed doses. The protective abilities of this compound proved unable to stop the harmful effects which occurred when valproic acid was given at high doses thus

demonstrating the need for controlled medication doses and pregnancy antioxidants to minimize organ damage.

Biochemical analysis results (Table 1)

The experimental groups were compared based on the measured values of biochemical variables (GSH, LPO, TNF- α , SOD, triglycerides and cholesterol) using a completely randomized design (CRD) with Duncan's test to identify significant differences between groups.

GSH analysis (Glutathione)

- The highest GSH level was observed in the control group (5.187 ± 0.037), indicating higher glutathione levels in untreated mice.
- Groups treated with folic acid alongside valproate showed a relative improvement in GSH levels (4.70 ± 0.03 and 4.23 ± 0.02) but did not reach the control group levels.
- Groups treated with valproate alone (2.851 ± 0.02 and 3.251 ± 0.089) exhibited a significant decrease in GSH levels, reflecting the toxic effect of valproate.

LPO analysis (Lipid peroxidation)

The group that received folic acid alone demonstrated the highest LPO levels which reached 1.05 ± 0.35 . The study results demonstrate that folic acid functions as a key factor which reduces oxidative stress.

The LPO levels in valproate-treated groups at 250 and 500 mg/kg showed an increase which demonstrated that oxidative stress occurred because of toxic effects.

The study results showed that patients who took folic acid supplements while taking valproate medication had lower LPO levels than patients taking valproate alone in the 3.76 ± 0.85 and 3.08 ± 0.89 groups.

TNF- α analysis (Tumor necrosis factor- α)

The folic acid-only group achieved the highest value of 600 ± 1.03 which shows that folic acid supplements cause a minimal rise in inflammatory markers.

The TNF- α levels in Valproate-treated groups (250 and 500 mg/kg) were moderate but the control group showed the lowest value at 230 ± 0.198 .

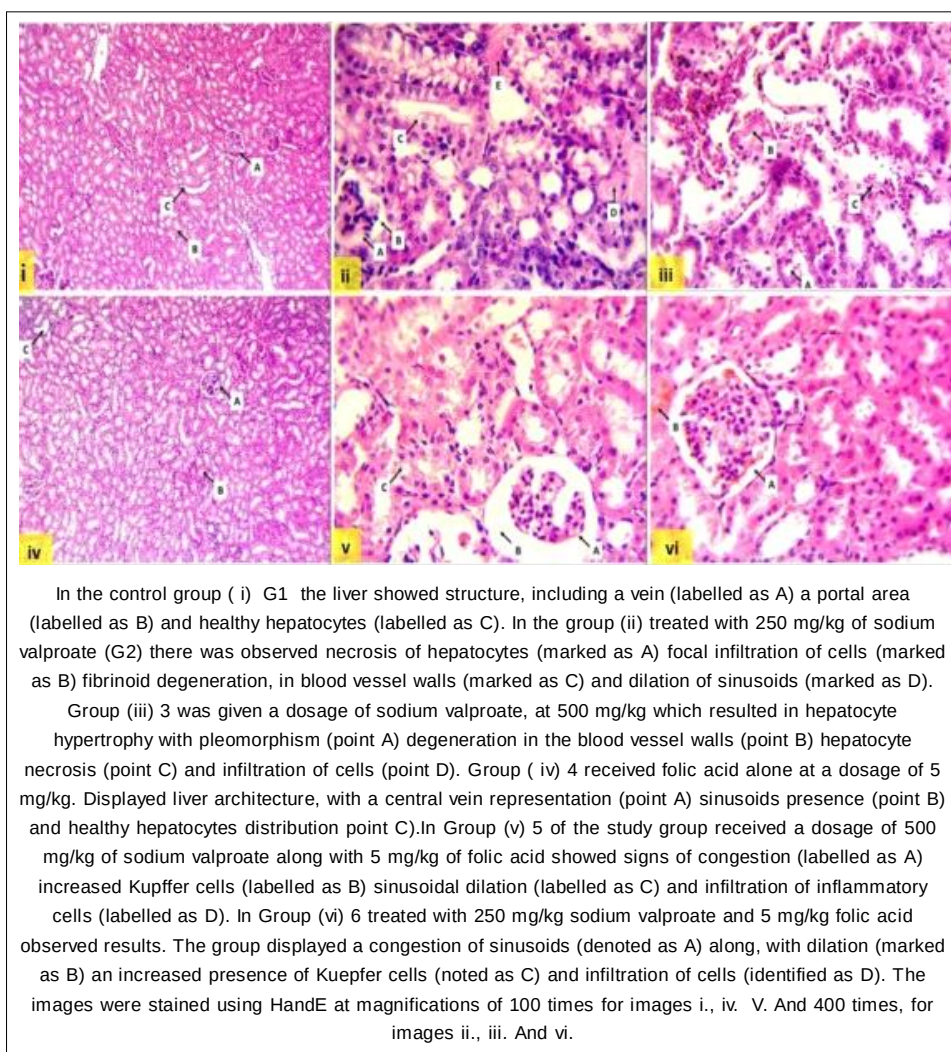


Fig 1: Histological samples of mouse livers were examined for the study.

The combination of valproate with folic acid produced superior TNF- α results than valproate alone which shows that folic acid contains anti-inflammatory compounds.

SOD analysis (Superoxide dismutase)

The folic acid treatment produced the highest SOD levels which reached 12.9 ± 0.04 in the study.

The SOD levels in valproate-only groups decreased significantly which proved that valproate compromises antioxidant defense systems.

The combination of valproate with folic acid resulted in better SOD levels but these values remained below the normal control group values.

Triglycerides (TG) analysis

The 500 mg/kg valproate group showed the highest triglyceride levels which reached 4.81 ± 180.12 , showing evidence of lipid metabolism problems.

The control group maintained typical triglyceride values which differed from the other study groups. The treatment

of folic acid as a single agent or in combination with valproate resulted in better triglyceride levels for all groups.

Cholesterol analysis

The cholesterol levels in Valproate-treated groups (250 and 500 mg/kg) showed a substantial rise because valproate treatment causes damage to lipid metabolism. The folic acid-only group showed moderate levels, supporting the role of folic acid in reducing metabolic disturbances. Combining folic acid with valproate improved cholesterol levels but did not reach the control

This study showcased the impact of administering acid (VPA) at varying doses of 250 and 500 mg/kg on pregnant mice by emphasizing its negative effects, on the liver and kidneys through histological and biochemical analysis levels (Adewole *et al.*, 2023). Additionally, it also illustrated the effect of folic acid (FA) especially when used alongside valproic acid. The use of acid led to changes, in the liver and kidneys on a cellular level suggestive of a strong negative impact particularly, with increased doses

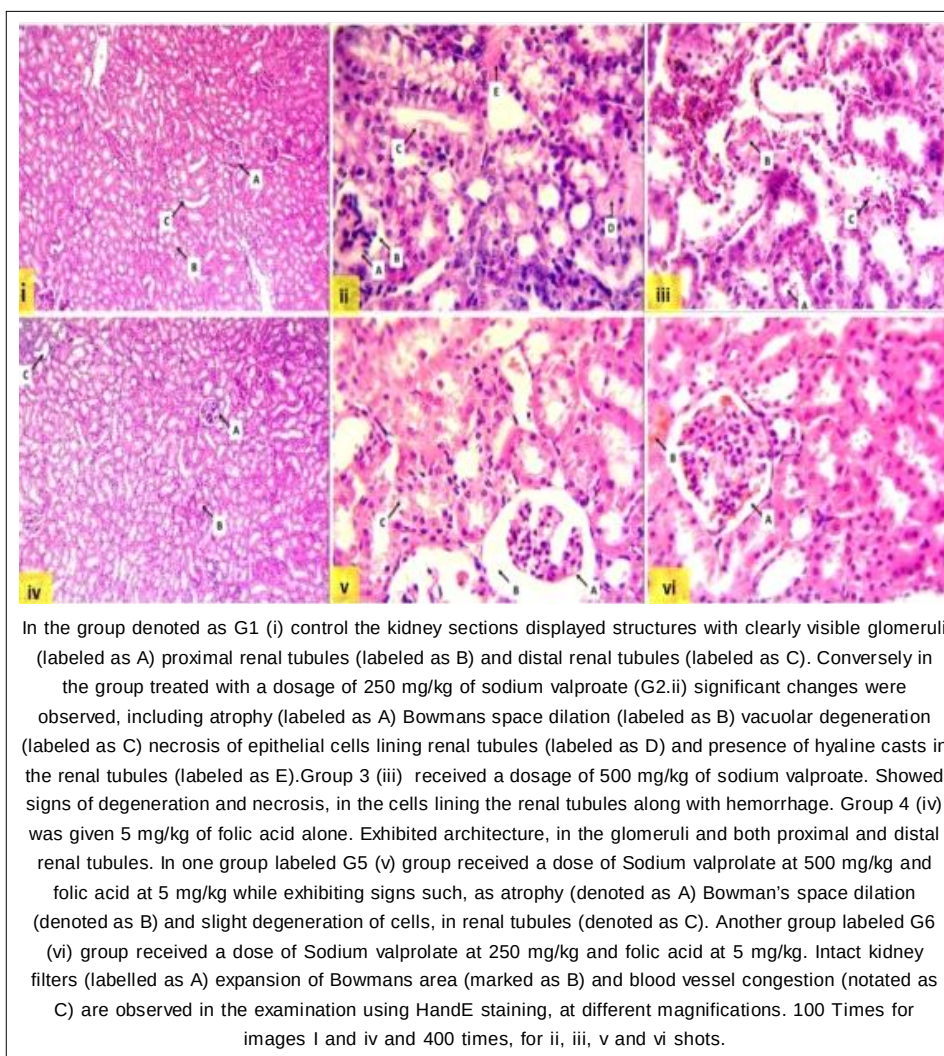


Fig 2: Histological slices of mouse kidneys captured features, in the study groups.

Table 1: Selected biochemical changes following the administration of valproic acid and folic acid.

Group standard	Control	Valpr. acid 250 mg/kg	Valpr.acid 500 mg/kg	Folic acid 5 mg/kg	Valpr. acid 250 + folic acid (5) mg/kg	Valproic acid 500 + folic acid (5) mg/kg
GSH	5.187±0.037a	3.251±0.089c	2.851±0.02b	4.67±0.11b	4.23±0.02b	4.70±0.03b
LPO	2.85±1.9c	4.06±1.97a	4.87±0.85a	1.05±0.35c	3.08±0.89b	3.76±0.85b
TNF-α	230±0.198d	410±0.29c	486±0.24c	600±1.03a	525±0.042b	530±0.035b
SODng/L	8.07±0.21b	6.98±1.02c	6.52±0.73c	12.9±0.04a	11.7±0.145a	12.2±0.327a
Total TGμmol /L	3.91±104.62b	4.81±180.12c	4.75±171.32c	1.76±124.52a	2.23±172.63b	2.93±163.25b
Cholesterol	2.91±117.11b	3.64±137.13a	3.53±124.25a	2.98±125.83a	1.86±142.23c	1.89±34.82c

or prolonged usage. These histopathological alterations in hepatic and renal tissues are further demonstrated in Fig 1 and 2.

The liver showed alterations such, as cell death necrosis and inflammation infiltration alongside degeneration and sinusoidal dilation (Ali *et al.*, 2021). The liver tissue showed severe damage because valproic acid caused inflammatory cells to enter the tissue while it triggered oxidative stress which led to hepatocyte death (Gai *et al.*, 2020). The study showed that folic acid supplements protected cells from these changes because they possess antioxidant properties which help cells defend against valproate toxicity. The study results showed that folic acid protection depends on the dosage amount because patients with lower valproic acid exposure achieved better results but higher doses of the medication exceeded the protective effects of folic acid (Rajesh *et al.*, 2023). The kidney showed identical pathological changes because of failed renal filtration which scientists link to oxidative imbalance and inflammatory cascades which caused glomerular shrinkage and tubular degeneration (Magaji *et al.*, 2024). The study shows that higher valproic acid doses lead to more severe liver and kidney damage which makes it difficult to protect patients from toxicity even when using antioxidant treatments (Lenders *et al.*, 2023; Clower *et al.*, 2022). The biochemical markers confirm this pattern because GSH and SOD levels decreased to show decreased antioxidant protection but folic acid treatment improved these markers to fight oxidative stress.

The study indicates that TNF-α and LPO levels increased at the same time to show both inflammatory reactions and damage to cell membrane lipids; folic acid therapy restored these values to their typical range which proves its anti-inflammatory properties and membrane protection abilities (Sachdev *et al.*, 2021; Dama *et al.*, 2024; Oktay *et al.*, 2024; Seyidoglu *et al.*, 2020; Sengupta *et al.*, 2024; Ammari *et al.*, 2024). The increase in triglycerides and cholesterol levels shows that valproate exposure causes metabolic problems which increase the chances of developing hepatic steatosis and lipid accumulation (Ezhilarasan and Mani, 2022; Mishra *et al.*, 2021).

CONCLUSION

The study showed that valproic acid causes histological harm which underscores its importance, in clinical settings and the need for preventive actions to be taken in advance

to prevent such effects from occurring later on down the line. Folic acid was found to have a function by lessening oxidative stress levels and inflammation while also enhancing lipid metabolism capabilities; this indicates that it could serve as a beneficial supplement, alongside valproic acid treatment. The study suggests additional studies to better understand how folic acid protects and how it can be used in healthcare to improve the safety of treatments based on acid.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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