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Original Research

β-Glucan Mitigates Valproic Acid-Induced Hepatorenal Toxicity in Rats

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Abstract

Valproic acid (VPA) is a common medication used to treat epilepsy, known for its broad-spectrum efficacy but also associated with hepatotoxicity and metabolic disturbances. β-glucan, a natural polysaccharide with hepatoprotective properties, may mitigate drug-induced liver damage. The study sought to assess hepatorenal toxicity of VPA and investigate the potential protective and therapeutic effects of β-glucan. Forty male rats were randomly distributed into four groups. The treated group received VPA (500 mg/kg), β-glucan (50 mg/kg), or combinations for 14–21 days via intraperitoneal injection. Serum biochemical parameters (ALT, AST, ALP, urea, creatinine, lipids) were measured. Histopathological evaluation of hepatic tissue was performed. VPA caused significant elevations in serum ALT and AST, as well as histological liver damage. Co-treatment with β-glucan significantly attenuated hepatic and renal dysfunction in VPA-treated groups, preserving tissue architecture. VPA exhibited both hepatotoxic and nephrotoxic potential. However, β-glucan co-administration provided a protective effect against both VPA-induced hepatic and renal toxicities.

Keywords: Valproic acid; hepatotoxicity; nephrotoxicity; β-glucan; oxidative stress

1. Introduction

Epilepsy is a long-term neurological disorder marked by repeated and spontaneous seizures caused by irregular electrical discharges in the brain. In Saudi Arabia, the prevalence of epilepsy is estimated at approximately 6.5 cases per 1,000 individuals, ranking it among the most common neurological disorders in the region [1]. Despite significant advances in antiepileptic drug (AED) development, many current therapies are associated with adverse effects, including hepatotoxicity and metabolic disturbances.

Valproic acid (VPA) is a broad-spectrum AED widely used for the management of generalized tonic-clonic, absence, and focal seizures [2]. Its mechanism of action primarily involves enhancing gamma-aminobutyric acid (GABA) neurotransmission, thereby stabilizing

neuronal excitability [3]. However, VPA is also associated with hepatotoxicity, which can range from mild, transient transaminase elevations to severe liver failure, especially in pediatric and polytherapy populations [4–5]. The mechanisms for VPA-induced liver injury include mitochondrial dysfunction, oxidative stress, altered DNA methylation, and carnitine depletion [6]. In earlier studies, VPA also increased urinary excretion of gamma-linolenic acid and induced vacuolization in hepatocytes [7].

β-Glucans are biologically active polysaccharides present in sources such as cereals, fungi, and yeast. They are distinguished structurally by β-(1→3) and β-(1→6) glycosidic bonds and recognized for their hepatoprotective, anti-inflammatory, and antioxidant qualities [8]. Recent evidence has highlighted their ability to modulate oxidative stress and immune responses, supporting their potential role as adjuncts in drug-induced hepatotoxicity.

While the hepatoprotective potential of β-glucans has been explored in various models of drug-induced injury, there is limited data regarding its efficacy in mitigating the specific hepatorenal syndrome-like dysfunction induced by high-dose valproic acid. This study aims to address this gap by evaluating whether β-glucan can serve as a protective and therapeutic adjunct to stabilize both liver and kidney functions during VPA therapy.

2. Materials and Methods

Study Location and Duration

This study was carried out in the Department of Pharmacology and Toxicology at the College of Pharmacy, Qassim University, Saudi Arabia. All experimental protocols received prior approval from the Ethics Committee of the College of Pharmacy, Qassim University (No. 25-24-10).

Animals

40 adult male albino rats, weighing 150 - 230 grams, were procured from National Research Facility, College of Pharmacy, Qassim University. Rats were maintained in polypropylene cages under standard laboratory conditions (12-hour light/dark cycle, temperature 22 ± 1°C) and provided unrestricted access to food and water. After a five-day acclimatization period, the rats were randomly divided into 4 groups, with ten animals in each group. All animal handling and procedures were conducted under ethical guidelines approved by the institution's Ethics Committee. The sample size (n=10 per group) was chosen based on previous similar toxicological studies in rats to ensure statistical power for biochemical and histological comparisons **.** A formal a priori power analysis was not performed.

Drugs and Chemicals

The study utilized sodium valproate (Depakine®, Sanofi SpA, 400 mg/4 mL), and beta-glucan (Vivamune™, JP Nutraceuticals, 250 mg). Biochemical markers included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, triglycerides, cholesterol, albumin & total protein levels were assessed using commercial kits provided by Crescent Diagnostics, Saudi Arabia. All drugs were freshly prepared in sterile normal saline and administered intraperitoneally (i.p.) in a volume appropriately adjusted to the animals' body weight. The dose of valproic acid (500 mg/kg) was selected based on its established ability to induce significant hepatotoxicity in rat models as reported in previous studies **[9 -10].** The dose of β-glucan (50 mg/kg) was chosen based on its reported efficacy in providing antioxidant protection without inducing toxicity **[11-12]**

Study Design

A total of forty rats were allocated randomly into four equal groups, with each group (n = 10). Group 1 (control) received intraperitoneal (i.p.) administration of normal saline. Group 2 was administered sodium valproate at 500 mg/kg/day i.p. for fourteen days to induce hepatotoxicity; animals were then subjected to sample collection and sacrificed for histopathological examination. Group 3 received β-glucan 50 mg/kg/day via intraperitoneal injection for a duration of 14 days, followed by sample collection, and histological assessment was performed. In Group 4, rats were given sodium valproate (500 mg/kg/day i.p.) for 21 days. On day fourteen, and β-glucan (50 mg/kg/day i.p.) was administered for more 7 days; final samples were collected, and hepatic as well as renal assessments were performed on day twenty-one. The study design was intended to evaluate both the induction phase (14 days for Group 2) and the therapeutic/protective phase (21 days for Group 4). Group 4 was specifically designed

to assess the effect of β-glucan intervention after the initial onset of VPA-induced damage, whereas Group 2 focused on the acute toxic phase

Sample Collection

Following the final analysis of treatment, animals were anesthetized with sodium thiopental (50 mg/kg, i.p.). Blood samples were then obtained through cardiac puncture and left 30 minutes at room temperature. Then samples were centrifuged at 1,500–2,000 × g for 10 minutes to separate serum, which was either analyzed immediately. Rats were euthanized by cervical dislocation, and livers were subsequently excised, rinsed with saline, gently blotted dry, weighed, and preserved for histological and immunofluorescence analyses.

Relative liver weights (RLW) was determined using the formula: RLW (%) = (liver weight ÷ body weight) × 100.

Measurement of AST, ALT, ALP, total protein, albumin, creatinine, urea, triglycerides, & cholesterol concentrations

ALT, AST, and ALP enzyme activities were determined using the kinetic UV method recommended by the International Federation of Clinical Chemistry (IFCC). Creatinine concentration was measured by a fixed-time kinetic assay based on the Jaffe reaction, wherein creatinine interacts with alkaline picrate producing a reddish-orange complex. Color intensity, measured at 490 nm, is directly proportional to the creatinine level in the samples. The blood urea, total protein, albumin, total cholesterol, and triglyceride levels were assayed by spectrophotometric method. The intensity of dye produced at 578 nm is proportional to the concentration of urea in the samples. Also, the absorbance of dye produced at 546 nm for total protein, albumin, total cholesterol, and triglycerides was proportional to their concentrations in the samples. The albumin-to-globulin (A/G) ratio was determined by calculating globulin concentration (difference between total protein & albumin), followed by dividing albumin by globulin. All assays were performed in duplicate, and quality control was maintained using internal standards and calibrators provided by the kit manufacturers. All the kits were provided by the Crescent Diagnostics Test, KSA.

Histopathological Analysis

Histological Examination of Liver Tissue Liver samples were fixed in 10% neutral formalin, followed by dehydration by a series of graded ethanol concentrations. The tissues then were embedded in paraffin and sliced into 5 µm sections. For histological evaluation & quantitative assessment of cellular abnormalities, the sections were stained with hematoxylin and eosin. For staining, two sections from each sample were deparaffinized in xylene and rehydrated by descending ethanol series (100%, 95%, 80%, and 70% ethanol), ending with distilled water. Sections were stained with hematoxylin for 10 minutes, rinsed in running tap water, and differentiated in 1% lithium carbonate with 10 dips. Afterward, sections were washed again in running tap water and 95% ethanol for 1 minute, counterstained with eosin for 20 seconds, and rinsed under running tap water. Subsequently, the sections dehydrated through increasing concentrations of ethanol (70%, 80%, 95%, and 100%) were cleared using xylene. After staining, the sections were mounted with a permanent medium. All slides were observed under a light microscope at magnifications ranging from 200X to 400X [13].

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Data were assessed for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test prior to conducting one-way analysis of variance (ANOVA). All variables satisfied the assumptions required for parametric testing. One-way ANOVA was followed by the Tukey–Kramer post hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics version 21 (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Effect of Valproic Acid on Body Weight, Liver Weight & Relative Liver Weight in Rats and Role of Beta-Glucan (Table 1):

The control group demonstrated the highest body weight (195.6 ± 10.8), which was significantly elevated than that of VPA group (175.7 ± 14.1). Beta-glucan alone contributed to maintenance of body weight (194.8 ± 10.1), whereas the VPA + beta-glucan group showed further reduced body weight (155 ± 11.4).

Table 1. Effect of Valproic Acid (500mg/kg) on Body Weight, Liver Weight, and Relative Liver Weight in Rats and Role of Beta-Glucan (50mg/kg).

Parameters	CONTROL (mg/kg)	VPA (mg/kg)	Beta-GLUCAN (mg/kg)	VPA+beta-GLUCAN (mg/kg)	p-value (ANOVA)
Mean body weight (g)	195.6 ± 10.8 ^{A}	175.7 ± 14.1^{B}	194.8 ± 10.1^{A}	155 ± 11.4 ^{C}	*P=0.001*
Mean liver weight (g)	7.28 ± 0.42^{A}	7.03±0.56^{B}	7.33 ± 0.46^{A}	6.22±0.45^{B}	*P=0.022*
Relative liver weight (%)	3.72 ± 0.21^{A}	4.00 ± 0.45^{B}	3.76 ± 0.22^{A}	4.01 ± 0.41^{B}	*P=0.512*

Regarding liver weight, the VPA group (7.03 ± 0.56) experienced a reduction compared to the control group (7.28 ± 0.42). However, the VPA + β-glucan group exhibited an even greater reduction in liver weight (6.22 ± 0.45). Relative liver weight was elevated in VPA group (4.00 ± 0.45) compared to the control (3.72 ± 0.21), and β-glucan treatment did not significantly influence this parameter in the treated groups (3.76 ± 0.22 for β-glucan alone, and 4.01 ± 0.41 for VPA + β-glucan).

Abbreviations: *VPA*, Valproic acid; Values are expressed as means ± SD (n=10). Values with common superscript ^{A} are insignificantly different. (P< 0.05).

3.2 Impact of Valproate on Liver Functions in rats and The Role of Beta-Glucan (Table 2, Figure 1):

The results demonstrated that the average serum level of ALT was markedly elevated in the VPA group (873.34±29.52B) in comparison to the control group (87.25±24.18A). The levels in the β-glucan group (85.25± 22.08) were insignificantly different in relation to the control group. The VPA+β-glucan (56.27±8.59C) also showed significantly lower levels compared to VPA.

For AST, the VPA group (965.42±274.29B) showed significantly higher levels than the control group (55.84±16.11A). The AST levels in the β-glucan group (64.84±17.11A), The VPA+β-glucan group (181.47±64.68C) exhibited significantly higher results compared to control group; however, no notable differences were observed among groups treated with β-glucan.

Regarding ALP, the VPA group (61.34±45.04B) showed significantly higher levels compared to the control group (23.43±1.59A), the β-glucan group (25.33±1.48A), and the combined treatment group (VPA+β-glucan: 27.91±12.94A). No significant differences observed among the control group, β-glucan group, and the VPA+ β-Glucan group. The results for Albumin, Globulin, A/G ratio, and Total Protein showed no significant differences among studied groups.

TABLE 2. Impact of Valproate (500mg/kg) on Liver Function and The Role of β-Glucan (50mg/kg).

Parameters	CONTROL (mg/kg)	VPA (mg/kg)	Beta-GLUCAN (mg/kg)	VPA+beta-GLUCAN (mg/kg)	p-value (ANOVA)
ALT (U/L)	87.25± 24.18^{A}	873.34 ± 29.52^{B}	85.25± 22.08^{A}	56.27 ± 8.59^{C}	*p<0.001*
AST(U/L)	55.84± 16.11^{A}	965.42 ± 274.29^{B}	64.84± 17.11^{A}	181.47 ± 64.68^{C}	*p<0.001*
ALP(U/L)	23.43 ± 1.59^{A}	61.34 ± 45.04^{B}	25.33 ± 1.48^{A}	27.91 ± 12.94^{A}	*p = 0.055*
ALBUMIN (g/dL)	2.91 ± 0.02^{A}	2.97 ± 0.52^{A}	2.88 ± 0.42^{A}	2.26 ± 0.53^{A}	*p<0.001*
GLOBULIN (g/dL)	2.01± 0.57^{A}	1.74±0.47^{A}	1.46 ± 0.51^{A}	1.84±0.28^{A}	*P = 0.379*
A/G ratio	1.45± 0.04^{A}	1.70± 1.11^{A}	1.97 ± 0.82^{A}	1.23± 1.89^{A}	*P = 0.019*
TOTAL PROTEIN (g/dL)	4.92 ± 0.59^{A}	4.71 ± 0.99^{A}	4.34± 0.93^{A}	4.10± 0.81^{A}	*p<0.001*

Abbreviations: VPA, Valproic acid; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransaminase; ALP, Alkaline phosphatase; A/G, Albumin/Globulin ratio. Values are expressed as means ± SD (n=10). Values with non-common superscript capital letters are significantly different in relation to each other's (P< 0.05).

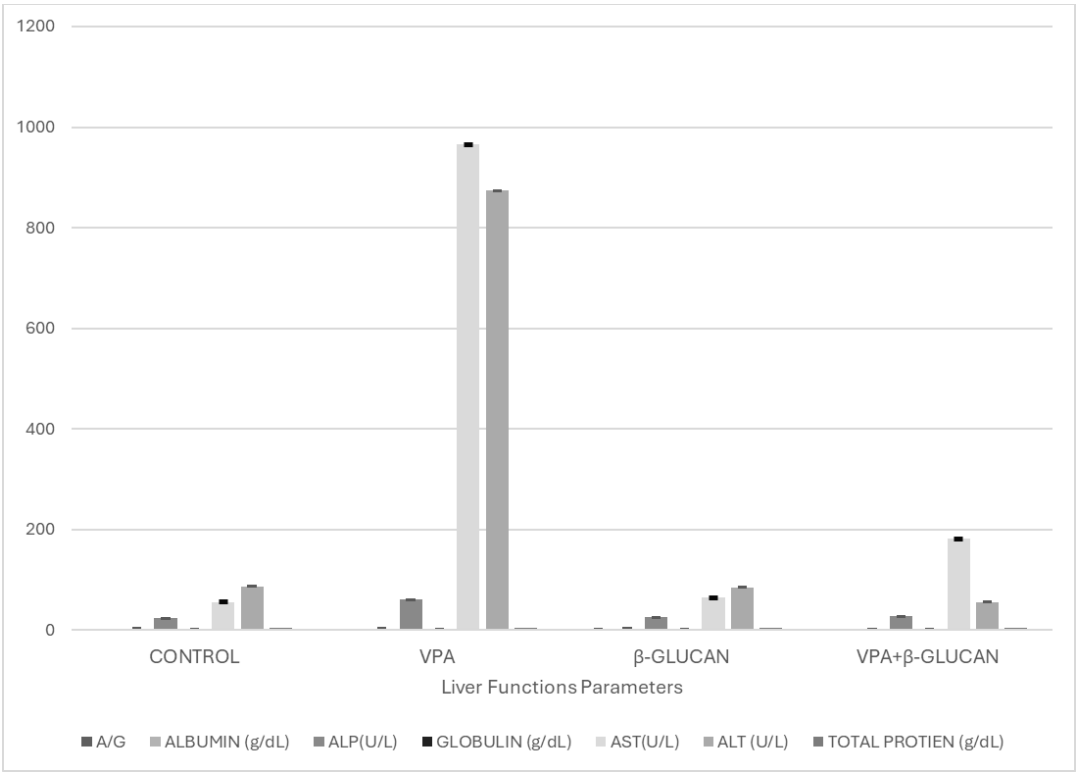

Figure 1. Impact of Valproate (500mg/kg) on Liver Functions in rats and The Role of β-Glucan (50mg/kg).

3.3 Impact of Valproate on Kidney Function in rats and The Role of Beta-Glucan (Table 3, Figure 2):

The findings of this study showed that mean serum creatinine level was significantly elevated in the VPA group (4.12 ± 1.14B) compared to the control group (0.70 ± 0.11A). In contrast, the β-glucan group (0.55±0.02A) and the combination treatment group (VPA+β-GLUCAN: 1.05±0.31C) exhibited significantly lower levels in relation to VPA group.

The mean level of urea in the VPA group (68.49±6.4A) was non significantly different from the control group (66.86± 2.11). On the other hand, β-glucan group (40.66±2.01B) and the combination treatment group (VPA+β-GLUCAN: 37.37±9.31B) showed significantly lower urea levels than the control, and VPA groups.

BUN levels in the VPA group (32.00 ± 3.02A) was non significantly different in relation to the control group (31.22±0.98A). β-glucan group (22.32±0.88B) and the combination treatment group (VPA+β-GLUCAN: 20.99±24.02B) showed significantly lower BUN levels.

3.4 Impact of Valproate on Lipid Profile in rats and The Role of Beta-Glucan (Table 4, Figure 3):

The study results demonstrated that total cholesterol levels in the β-glucan group were measured at (40.09 ± 4.01A), the VPA group (44.63±10.15A) and the VPA+β-Glucan (45.81 ± 11.03A) were insignificantly different from control group.

VPA group (226.07±31.70B) exhibited significantly higher triglycerides levels compared to control group. In contrast, the β-glucan group (145.09±55.33C) showed significantly lower triglycerides levels compared to the control group. The combination treatment group (VPA+β-GLUCAN: 170.00±66.95D) also exhibited significantly lower triglycerides levels compared to both monotherapy groups.

3.5 Histopathological Evaluation of Hepatic Tissue Damage in Rats Treated with VAP: Impact of Co-Treatment with beta glucan (figure 4).

Descriptive pictures of hepatic tissues of rats with various treatments labeled in Figure 4. Normal architecture was seen in the control and β-Glucan rat tissues. VAP-treated group displayed pericentral necrotic areas (blue arrows), apoptotic nucleus (red arrows), disturbed sinusoidal area (green arrows) and hemorrhage (yellow arrow) interstitial immune cells

TABLE 3. Impact of Valproate (500mg/kg) on Kidney Function in rats and The Role of Beta-Glucan (50mg/kg).

Parameters	CONTROL (mg/kg)	VPA (mg/kg)	β-GLUCAN (mg/kg)	VPA+β-GLUCAN (mg/kg)	p-value (ANOVA)
Creatinine (mg/dL)	0.70 ± 0.11^{A}	4.12 ± 1.14^{B}	0.55 ± 0.02^{A}	1.05 ± 0.31^{C}	*p<0.001*
UREA (mg/dL)	66.86 ± 2.11^{A}	68.49 ± 6.4^{A}	40.66 ± 2.01^{B}	37.37 ± 9.31^{B}	*p<0.001*
BUN (mg/dL)	31.22 ± 0.98^{A}	32.00 ± 3.02^{A}	22.32 ± 0.88^{B}	20.99 ± 24.02^{B}	*P = 0.356*

- BUN, blood urea nitrogen.
- Values are expressed as means ± SD (n=10)
- Values with non-common superscript capital letters are significantly different in relation to each other's ($P< 0.05$).

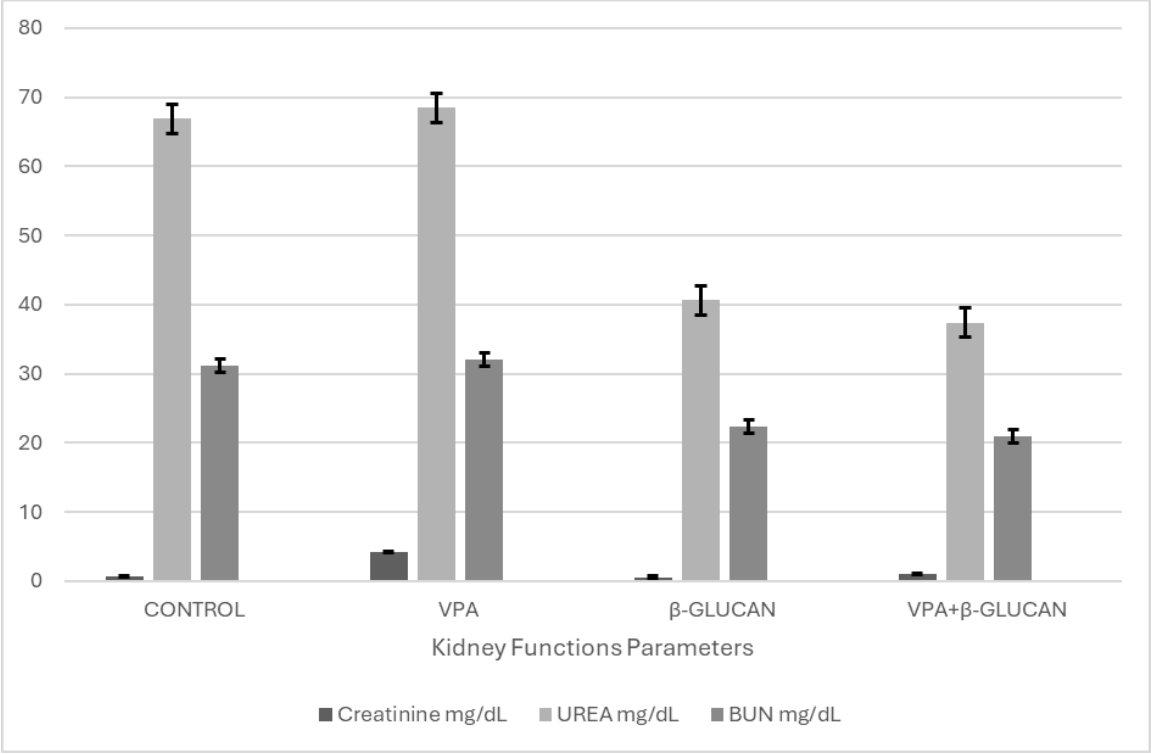

Figure 2. Impact of Valproate (500mg/kg) on Kidney Functions in rats and the Role of Beta-Glucan (50mg/kg).

TABLE 4. Impact of Valproate (500mg/kg) on Lipid Profile in rats and The Role of Beta-Glucan (50mg/kg).

Parameters	CONTROL (mg/kg)	VPA (mg/kg)	β-GLUCAN (mg/kg)	VPA+β-GLUCAN (mg/kg)	p-value (ANOVA)
CHOLESTROL (mg/dL)	39.14 ± 3.78^{A}	44.63 ± 10.15^{A}	40.09 ± 4.01^{A}	45.81 ± 11.03^{A}	*P = 0.048*
TRIGLYCERIDE (mg/dL)	195.07 ± 60.03^{A}	226.07 ± 31.70^{B}	145.09 ± 55.33^{C}	170.00 ± 66.95^{D}	*P = 0.003*

- Values are expressed as means ± SD (n=10)
- Values with non-common super script capital letters are significantly different in relation to each other's. Values with common super script capital letters are insignificantly different ($P< 0.05$).

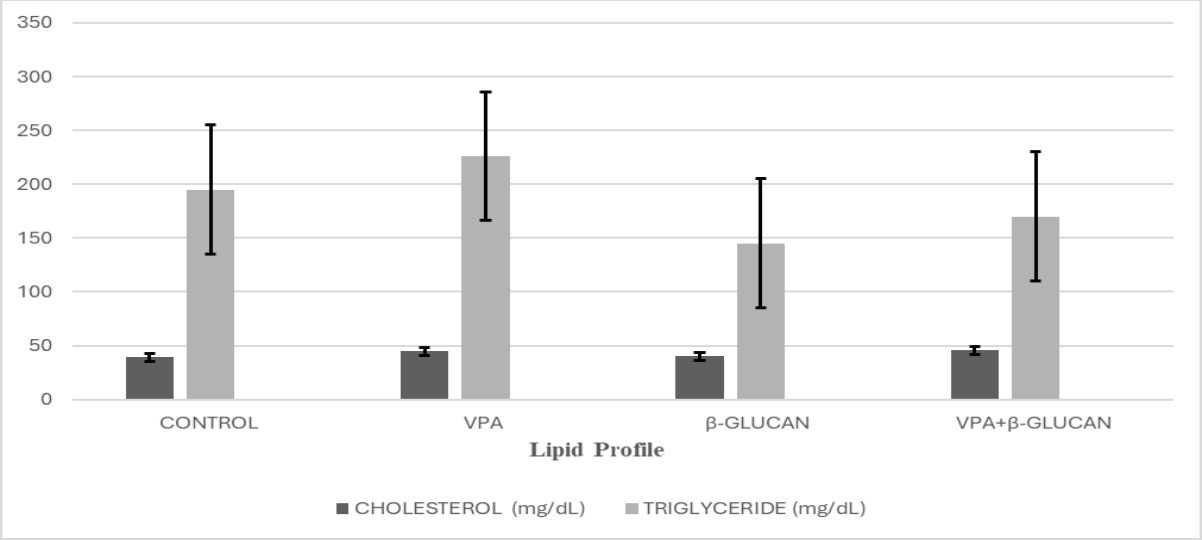

Figure 3: Impact of Valproate (500mg/kg) on Lipid Profile in rats and the Role of Beta-Glucan (50mg/kg).

400X (A) 200X (B)

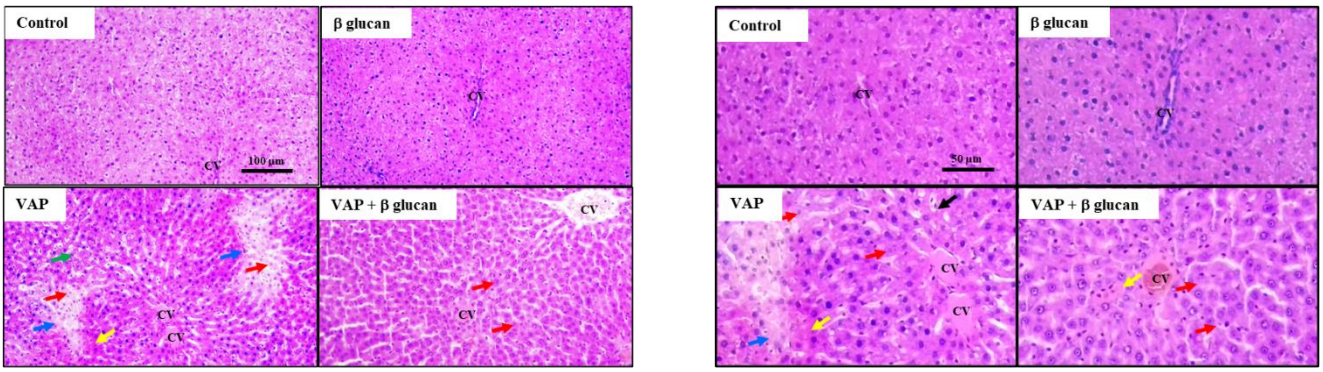

Figure 4. Histopathological assessment of hepatic tissues in rats treated with beta glucan VAP in the presence or absence of beta glucan. Descriptive pictures of hepatic tissues of rats with various treatments labeled in Figure 4. Normal architecture was seen in the control and β-Glucan rat tissues. VAP-treated group displayed pericentral necrotic areas (blue arrows), apoptotic nucleus (red arrows), disturbed sinusoidal area (green arrows) and hemorrhage (yellow arrow) interstitial immune cells migration (black arrows). Rats treated with β-Glucan represent an improvement of the pathological architectures induced by VAP. Magnification power is 400 X for (A) and 200 for (B)

migration (black arrows). Rats treated with β-Glucan represent an improvement of the pathological architectures induced by VAP. Magnification power is 400 X for (A) and 200 for (B).

4. Discussion

Valproic acid is a commonly prescribed antiepileptic medication recognized for its broad-spectrum effectiveness in treating different types of seizures, such as absence, myoclonic, and generalized tonic-clonic seizures. Its mechanism of action includes elevating gamma-aminobutyric acid (GABA) levels in the brain through inhibition of GABA transaminase, along with modulation of voltage-gated sodium and calcium channels. This multifaceted pharmacological profile contributes to its effectiveness in epilepsy management and mood stabilization. Despite its therapeutic benefits, valproic acid is associated with several adverse effects, including hepatotoxicity, teratogenicity, and weight gain, necessitating careful monitoring during long-term use [13,14].

The study evaluated the protective and therapeutic actions of beta-glucan against valproic acid-induced hepatotoxicity in rats. This work assessed liver functions through an evaluation of serum biochemical markers, including ALT, AST, ALP, albumin, globulin, A/G ratio, and total protein. Histopathological analysis was conducted to evaluate tissue integrity, inflammatory responses.

The study demonstrated that VPA administration significantly reduced body and liver weights, with the VPA + β-glucan group showing the most pronounced decline. β-glucan alone maintained body and liver weights near control levels. However, β-glucan did not significantly alter the VPA-induced increase in relative liver weight.

In this study, the VPA-treated group exhibited significantly elevated ALT levels relative to the control group. Giving β-glucan alone did not cause a noticeable change in ALT but combining β-glucan with VPA helped lower ALT levels. For AST levels, the VPA group showed a strong increase compared to control. Regarding ALP, the highest levels were observed in the VPA group, while the control, β-glucan, and combination group showed similarly low values. Finally, no significant differences were observed among groups in the levels of albumin, globulin, total protein, or the albumin/globulin (A/G) ratio.

This is consistent with reports by Wormley & Kraus, who found that VPA causes ALT and AST elevation, particularly in children or polytherapy [15]. The results are in accordance with those reported by Hussein et al., who demonstrated that 62.5% of the patients treated with Valproic Acid had an increase in Alkaline Phosphatase (ALP) activity [16]. Another study conducted by Mcpherson & Jani [17] documented cases where patients on sodium valproate therapy exhibited isolated increases in serum ALP levels, suggesting a direct effect of VPA on ALP activity. Similarly, Rawat et al. reported that increased levels of ALP in patients with epilepsy could be associated with the condition itself, rather than just resulting from the impact of antiepileptic drug treatment [18]. The findings also align with Vafaee-Shahi et al. who stated that VPA significantly increased the ALT level and suggested measuring liver enzymes prior to, during, and six months following therapy in order to avoid irreversible, long-term negative effects [19].

On the other hand, Adedapo et al. found a negative correlation between VPA dosage and ALP levels, suggesting that higher VPA doses might be associated with lower ALP levels [20]. Because the liver serves as the main pathway for the elimination of many antiepileptic drugs (AEDs), and some AEDs have been linked to increased liver enzyme levels indicative of hepatocellular damage, it is essential to evaluate and understand the risk of liver toxicity associated with these medications. While both AST and ALT are often regarded as liver enzymes due to their high levels in hepatocytes, only ALT is significantly specific to liver function. ALT is exclusively produced in hepatocytes, which makes it more indicative of hepatic function compared to AST, which is found in various tissues, including the liver, muscle, and red blood cells. As a result, conditions such as muscle injury or trauma (including intramuscular injections and severe restraint during handling) and hemolysis can cause notable increases in AST levels [21]. Moreover, AST has a relatively short half-life of about 12 hours, while ALT remains in circulation longer, with a half-life of approximately 60 hours. Nevertheless, the serum concentrations of AST and ALT tend to rise and fall concurrently.

Histopathological analyses in this study revealed marked liver damage following treatment with VAP, compared to the preserved architecture observed in the control group. VAP exposure led to severe pathological changes, including pericentral necrosis, apoptotic nuclei, disruption of sinusoidal, hemorrhage, and immune cell infiltration, indicating substantial hepatocellular injury [22].

The co-administration of the beta glucan showed a protective role against the hepatic injuries induced by VAP. Histological evaluations demonstrated that beta glucan markedly alleviated structural disorganization, reduced apoptotic events, and limited immune cell infiltration. This improvement highlights the potential of beta glucan as an adjunct therapy capable of mitigating liver inflammation and preserving tissue architecture in drug-induced liver injury.

The histopathological observations align with findings of Silva et al., who reported that valproic acid administration in rats induces hepatocellular degeneration, sinusoidal dilation, and inflammatory cell infiltration [23]. Similar liver injuries were described by Tong et al., who observed hepatic necrosis

and steatosis following chronic VPA exposure [24]. The protective effect of beta glucan observed in our study is in agreement with Liu et al. [25], who demonstrated that Salecan, a natural β-glucan exerts significant hepatoprotective effects by modulating inflammatory responses and enhancing antioxidant defenses.

VPA has been previously associated with alterations in metabolism, characterized by elevated levels of total cholesterol, triglycerides, and low-density lipoprotein (LDL), accompanied by decreased high-density lipoprotein (HDL) concentrations [26–28]. However, literature findings are inconsistent, with some studies reporting decreases in total cholesterol and LDL levels.

In the present study, the VPA-treated group showed a marked increase in triglyceride levels relative to the control group, whereas the increase in total cholesterol levels was not statistically significant. This partially supports findings of Li et al., who concluded that valproate use may affect lipid metabolism, potentially through endocrine mechanisms [29]. Additionally, Płonka-Półtorak et al. highlighted that while not always statistically significant, VPA treatment is associated with trends toward higher serum lipid levels and increased cardiovascular risk, particularly among overweight individuals [30].

The results are also in accordance with those reported by Grünig et al., who observed that valproate increases hepatocellular triglyceride accumulation through mechanisms such as reduced very-low-density lipoprotein (VLDL) production and diminished expression of fatty acid-binding protein 1 (FABP1) and microsomal triglyceride transfer protein (MTTP) [31]. This may contribute to lipid dysregulation and increased hepatic fat content. Furthermore, Sidhu et al. proposed that VPA-induced hypoadiponectinemia correlates with insulin resistance and may underlie weight gain and metabolic disturbances, particularly in obese female patients [32]. Similarly, Abaci et al., found that long-term VPA treatment led to increased total cholesterol and LDL levels [33]. However, Manimekala et al. observed only a weak correlation between the duration of antiepileptic drug use and lipid profile changes, suggesting that other factors, such as individual susceptibility and metabolic status, play critical roles [34].

Conversely, some studies have reported findings contrary to ours. Guo et al. [26b] indicated that VPA therapy can lead to a decrease in total cholesterol and LDL cholesterol. Likewise, Jaeri [35] and others found no significant long-term effects of VPA treatment on lipid profiles. These discrepancies highlight the influence of variables such as treatment duration, baseline metabolic status, seizure control, and study design.

Concerning the impact of drugs on renal function, the present study showed Grünig et al. reported that VPA treatment significantly increased serum creatinine levels compared to controls. However, they found no significant changes in blood urea nitrogen (BUN) or urea levels between the VPA and control groups [36].

These results align with previous studies indicating that antiepileptic medications, particularly valproic acid, are commonly associated with long-term therapy-related adverse effects, including renal injury [36,37]. These results are further corroborated by Asghar et al. [37] who reported that VPA significantly elevates oxidative stress markers, which leads to dysfunction in the liver, neurons, kidneys, and compromises overall cellular integrity.

Similarly, Heidari et al. [38] indicated that mitochondrial dysfunction and oxidative stress are major mechanisms underlying VPA-induced Fanconi syndrome. The present results are also consistent with observations of Anguissola et al. [39], who stated that prolonged administration of VPA for seven months or longer can infrequently lead to proximal tubular injury, manifesting as de Toni-Debré-Fanconi syndrome. Several investigations, including Watanabe et al. [36], have demonstrated that VPA therapy induces hypertrophy of the proximal convoluted tubules and the appearance of lipid vacuoles in these renal structures. In addition, the current findings agree with those of Koga et al. [40], who demonstrated that bedridden patients receiving VPA are prone to hypocarnitinemia, a condition that predisposes to proximal tubular dysfunction and may culminate in Fanconi syndrome. Therefore, it is recommended that urinary β2-microglobulin levels be regularly monitored in patients on VPA therapy to assess renal tubular function.

The discrepancy observed between creatinine and urea levels may be explained by their distinct physiological regulation. Serum creatinine is a more specific and

sensitive indicator of glomerular filtration rate (GFR), whereas urea concentration is influenced by multiple non-renal factors, including hepatic urea synthesis, protein metabolism, and hydration status**.** In early intrinsic (tubular) renal injury, serum creatinine may increase due to reduced filtration efficiency, while urea levels may remain relatively unchanged [55]. Therefore, parallel elevations of both parameters are not always expected in the early stages of acute tubular dysfunction induced by Valproic acid.

Beta-glucan is a non-starch polysaccharide made up of beta-D-glucose monomers linked through β-(1→3), β-(1→4), and/or β-(1→6) bonds. It is derived from various sources, including cereals, microorganisms, mushrooms, lichens, and seaweed [41,42]. Despite low bioavailability (0.5%–4.9%), beta-glucans exert antioxidant, anti-inflammatory, anticarcinogenic, and cardioprotective effects [43,44]. In the present study, beta-glucan (50 mg/kg/day) significantly lowered serum ALT, AST, urea, BUN, creatinine, and triglycerides in VPA treated rats, supporting its hepatoprotective and renoprotective roles. This is consistent with Erkol et al. [45], who demonstrated beta-glucan's antioxidative protection against liver injury. Additionally, beta-glucans have been reported to regulate hepatocyte apoptosis and proliferation and to confer renal protection in ischemia/reperfusion models [46–49]. The protective effects observed in the present study may be mechanistically linked to the antioxidant and anti-inflammatory properties of β-glucan reported in previous investigations. β-Glucan has been shown to enhance endogenous antioxidant defense systems, including superoxide dismutase, catalase, and glutathione peroxidase, while reducing lipid peroxidation markers such as malondialdehyde. In addition, β-glucan has been reported to suppress pro-inflammatory cytokines, including tumor necrosis factor-α and interleukin-6, and to modulate apoptotic signaling pathways through regulation of Bax/Bcl-2 balance and caspase-3 activation. These mechanisms may collectively contribute to attenuation of oxidative stress, inflammation, and cellular apoptosis associated with VPA-induced hepatic and renal injury.In the present experimental model, β-glucan demonstrated protective effects against VPA-induced biochemical and histopathological alterations. These findings indicate a potential protective role in preclinical settings. However, whether such effects translate into clinically meaningful benefits in patients receiving valproic acid remains to be determined.

It is important to emphasize that these findings are derived from an experimental animal model. Although the results provide mechanistic insight into the protective effects of β-glucan against VPA-induced toxicity, direct clinical extrapolation is premature. Future clinical studies are necessary to confirm translational relevance in human populations.

Limitations:

The limitations of The present study include. First, the absence of a dedicated 21-day VPA-only control group limits full interpretation of the time-dependent progression of VPA-induced hepatorenal toxicity. Although Group 2 (14-day VPA exposure) served as a defined acute toxicity baseline, inclusion of a longer-term VPA-only group would have allowed clearer differentiation between natural progression of injury and therapeutic reversal. Future investigations should incorporate such a group to better evaluate the temporal dynamics of toxicity and to further delineate the therapeutic window of β-glucan. Second, direct molecular assessments of oxidative stress, inflammatory mediators, and apoptosis-related markers were not performed. While the biochemical and histopathological findings support a protective role of β-glucan, incorporation of mechanistic biomarkers such as superoxide dismutase, catalase, glutathione peroxidase, malondialdehyde, tumor necrosis factor-α , interleukin-6, caspase-3, and Bax/Bcl-2 expression would provide deeper insight into the underlying pathways. Finally, a formal a priori power analysis was not conducted for sample size determination. Although the number of animals per group was consistent with comparable experimental studies, future research should include statistical power calculations to enhance methodological rigor and ensure adherence to ethical principles of animal reduction.

5. Conclusions

This experimental study evaluated the protective role of β-glucan against valproic acid (VPA)-induced hepatic and renal toxicity in male rats. VPA administration resulted in significant elevations of liver enzymes (ALT, AST, ALP), creatinine, and triglycerides, along with histopathological signs of liver damage such as necrosis and inflammation. These findings confirmed the hepatotoxic and nephrotoxic

potential of VPA. In contrast, co-treatment with β-glucan significantly mitigated biochemical and histological alterations, restoring enzyme levels and preserving hepatic and renal architecture. β-glucan also reduced triglyceride levels elevated by VPA, indicating an improvement in lipid metabolism.

These results suggest that β-glucan may have hepatoprotective and renoprotective effects in animal models. Valproic acid induces marked hepatotoxicity and nephrotoxicity in rats. Co-administration of β-glucan mitigated these toxic effects, indicating a potential protective role; however, further studies are needed before extrapolating these findings to human.

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AI Declaration Statement: The authors declare that artificial intelligence tools were used only for language editing and did not contribute to study design, data analysis, or scientific interpretation.

Data Availability Statement: The datasets generated during the current study are available from the corresponding author upon reasonable request.

Author Contributions Section: Hossam Abdelkader Elsisi contributed to conceptualization, supervision, project administration, data curation, formal analysis, manuscript review and editing, and overall study oversight. Maha Mohammad Almutairi contributed to conceptualization, methodology, investigation, data curation, formal analysis, and writing of the original draft. Hussein Mohammad Ali contributed to methodology and biochemical investigations. Mohamed Abdelbakky participated in methodology and histopathological examination. Ahmad Hamad Alhowail contributed to conceptualization and resources. Maha Aldubayan contributed to conceptualization and resources, and Razan Alawaji contributed to histopathological examinations. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

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