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Valproic acid ameliorates liver and kidney dysfunctions in type 2 diabetic rats

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Abstract

Type 2 diabetes has been reported to impair both liver and kidney functions but the effect of valproic acid (VPA), an anti-diabetic agent, on liver and kidney dysfunctions in type 2 diabetic models is still elusive. Therefore, this study investigated the effect of VPA on selected functional parameters of liver and kidney in type 2 diabetic rats. Type 2 diabetes was induced in female Wistar rats fed on high-fat diet for 2 weeks and then by intraperitoneal injection of 35 mg/kg body weight (bw) of streptozotocin. The nondiabetic Wistar rats were given water (normal control) while the diabetic groups were administered water (diabetic control), two doses of VPA (50 and 100 mg/kg bw) and 100 mg/kg bw metformin for 14 days. Then, the rats were sacrificed; liver, kidney and serum tissues were collected and analyzed. High-fat diet and streptozotocin significantly ($p < 0.05$) altered the liver to body weight ratio, kidney to body weight ratio, liver function indices, kidney function indices and enzymes' activities in the rat liver, kidney and serum but treatment with VPA and metformin ameliorated all the effects. In conclusion, results of this study indicate that VPA ameliorates liver and kidney dysfunctions in type 2 diabetic rats.

Keyword: Liver, kidney, dysfunction, diabetes, valproic acid

1. Introduction

Diabetes mellitus is a prolonged metabolic disease that is characterized by sustained high levels of blood glucose, which later lead to serious damage to some tissues including the heart, blood vessels, liver and kidney (Unai *et al*., 2020). Approximately 422 million people globally have diabetes, the majority living in low- and middle-income countries, and 1.5 million deaths are directly attributed to this disease yearly. Both the number of cases and the prevalence of diabetes have been increasing steadily over the past few decades. Type 2 diabetes mellitus (TDM2), the most common type of diabetes in adults, occurs when the body becomes resistant to insulin or does not make enough insulin. Type 1 diabetes mellitus (TDM1), also known as juvenile diabetes or insulin-dependent diabetes, is a condition in which the pancreas produces little or no insulin. For diabetic patients, access to affordable treatment, including insulin, is critical to their survival (Unai *et al.*, 2020).

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The liver performs a very important function in the regulation of glucose concentrations under normal physiologic and pathologic states such as diabetes mellitus (Yang *et al.,* 2018). Pertaining to TDM2, insulin resistance in the hepatocytes causes hyperglycemia, which then affects the regulation of glucose metabolism (Yang *et al.,* 2018). Diabetes is connected with some liver abnormalities, which include unusual glycogen deposition, fatty liver disease, liver fibrosis, liver cirrhosis, hepatocellular carcinomas (HCCs) and uncontrolled elevated hepatic enzymes (Roeb and Weiskirchen, 2021). In addition, the accumulation of a large quantity of fat in the liver may exacerbate insulin resistance and severely impair metabolic function (Roeb and Weiskirchen, 2021). Hyperglycemia and a fatty liver can destroy the hepatocytes and facilitate increased mortality among diabetic patients (Roeb and Weiskirchen, 2021). The kidney also plays an important role in carbohydrate metabolism regulation. The normal function of the kidney is essential for the maintenance of blood glucose levels and of a continued supply to organs that require glucose as energy source (Petrie *et al.,* 2020). There are several links between diabetes and the kidney: the kidney accounts for approximately 33% to 50% of the metabolic clearance of glucagon and insulin; glycosuria (the presence of glucose in the urine) is a defense mechanism against hyperglycemia regulated by a complex glucose sensing and glucose transport system; insulin resistance that is characteristic of type 2 diabetes affects the kidney; the kidney is capable of gluconeogenesis and contribute significantly to the total body glucose release (Petrie *et al.,* 2020).

Previous investigations have indicated that targeting histone deacetylases (HDACs) is a novel therapeutic strategy for several diseases, including diabetes mellitus (Christensen *et al.,* 2018). Valproic acid (VPA), a common antiepileptic drug, is an HDAC inhibitor that has been reported to have an anti-diabetic effect (Akindele *et al.,* 2015; Igunnu *et al.,* 2018) and enhance insulin expression in beta cells (Igunnu *et al.,* 2018). However, the effect of VPA on liver and kidney dysfunctions in type 2 diabetic models has not been adequately elucidated. Therefore, this study examined the effect of VPA on selected functional parameters of liver and kidney in type 2 diabetic rats.

2. Materials and Methods

Chemicals

Valproic acid sodium salt and streptozotocin were obtained from Sigma Aldrich, UK, and metformin from Wells Biosciences Pvt. Ltd., India. Analytical grade chemicals were used for all other aspects of the study.

Formulation of feed

The normal and high-fat diets were prepared by modifying the method outlined by Srinivasan *et al*. (2005) and their compositions were previously reported by Igunnu *et al*. (2018).

Experimental animals and induction of type 2 diabetes mellitus

This study used 20 female Wistar rats weighing 180-200 g. The rats were obtained from the Animal Holding of the Biochemistry Department, University of Ilorin, Nigeria, and were treated in accordance with approved animal care guidelines. After acclimatization, the rats were fed a normal diet and grouped into separate cages. To induce type 2 diabetes, the experimental rats received a high-fat diet for two weeks followed by an intraperitoneal injection of streptozotocin (STZ) at a dose of 35 mg/kg bw. Hyperglycemia was established by measuring fasting blood glucose (FBG) 48 hours after STZ infusion, with rats having FBG above 200 mg/dl considered diabetic.

Animal grouping and drug administration

Four rats were in Group I, the normal control, and were fed a normal diet throughout the study. The diabetic rats were grouped into II-V, with four animals in each group. Group II, the diabetic control, received a high-fat diet (HFD). Group III received HFD and 50 mg/kg bw VPA, Group IV received HFD and 100 mg/kg bw VPA, and Group V received HFD and 100 mg/kg bw metformin. The treatment period lasted for 14 days, and fasting blood glucose (FBG) levels were measured on days 0, 7, and 14 using blood from the rats' tail veins. After the treatment period, the rats were fasted overnight and then sacrificed, and samples of liver, kidney and blood were collected for analysis.

Preparation of samples

The serum was gotten by centrifugation of the blood samples at 4000 rpm for 5 minutes and the supernatant pipetted. The liver and kidney were put in 0.25 M sucrose solution (1: 5 w / v) that was ice-cold and then homogenized. Serum and homogenates were frozen until when needed.

Biochemical assays

The glucose meter was used to determine fasting blood glucose (FBS) levels. Liver/kidney-body weight ratios were calculated by dividing liver weight by the animals' body weight. Serum albumin concentration was determined using the method described by Doumas *et al*. (1971), while bilirubin concentration was evaluated based on the method of Jendrassik and Grof (1938). Total protein concentration in the liver, kidney, and serum was analyzed using the method described by Gornall *et al*. (1949). Activities of alanine aminotransferase and aspartate aminotransferase were determined using the procedure of Reitman and Frankel (1957), while the activities of alkaline phosphatase and acid phosphatase were determined using the method of Wright *et al*. (1972). Gamma-glutamyltransferase activity was determined using the procedure of Szasz (1969). Sodium and potassium ions concentrations in the serum were determined using the procedure described by Burtis and Ashwood (1996). The TECO kits were used to determine Chloride ion, Creatinine, and Urea concentration in the serum.

Statistical analysis

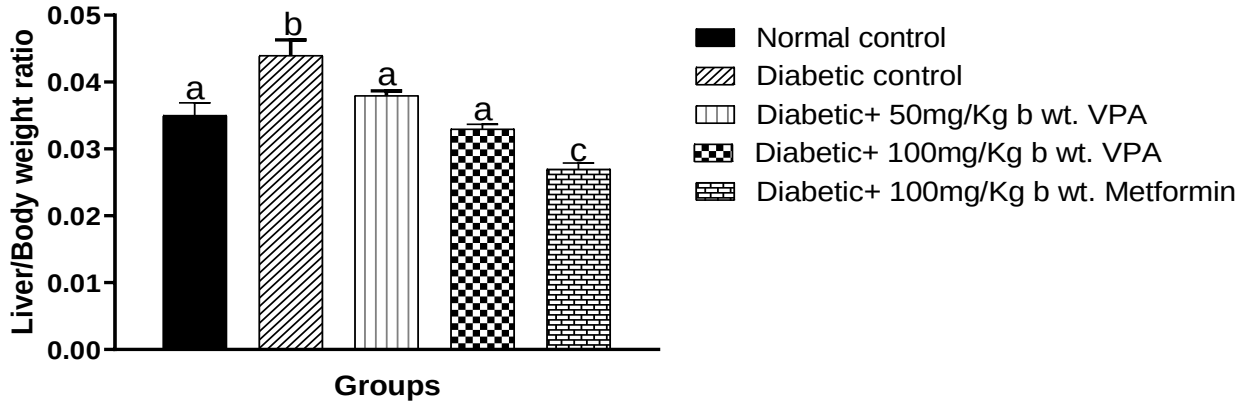
The data are shown as means of four replicates with standard error of the mean (SEM). Statistical analysis used one-way analysis of variance (ANOVA) followed by Turkey's comparison test (SPSS 20.0, SPSS Inc., Chicago, IL). Differences were significant at $p < 0.05$ compared to control.

3. Result and Discussion

Liver-to-body weight and kidney-to-body weight ratios of diabetic rats induced by a high-fat diet and streptozotocin and treated with valproic acid

The liver/body weight and kidney/body weight ratios of rats induced with diabetes through high-fat diet (HFD) and streptozotocin (STZ) were significantly increased ($p < 0.05$). However, treatment with valproic acid (VPA) at doses of 50 and 100 mg/kg bw significantly ($p < 0.05$) reduced and normalized these ratios in diabetic rats. Likewise, diabetic rats treated with 100 mg/kg bw metformin also showed a significant ($p < 0.05$) decrease in these ratios (Figure 1 a and b).

(a)



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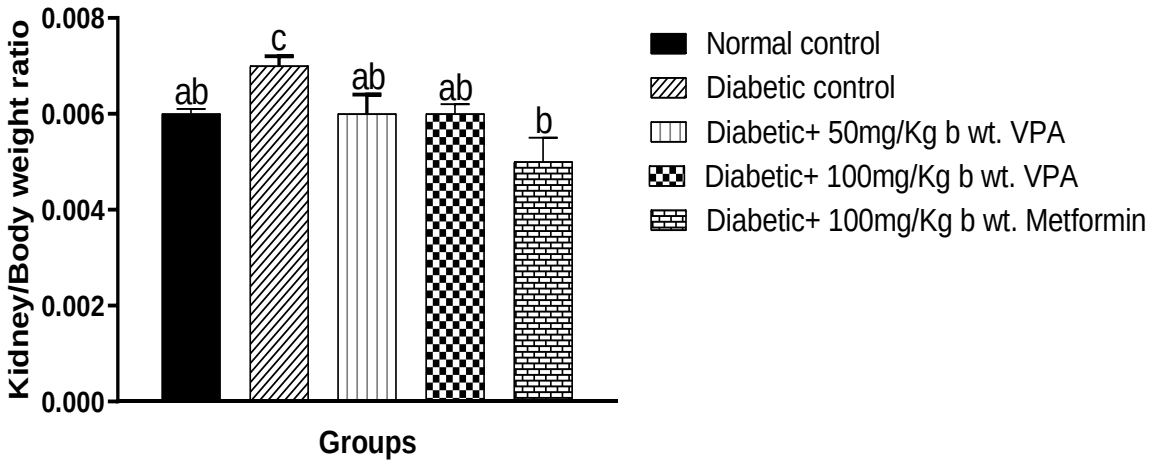


Figure 1: The impact of valproic acid (VPA) on liver-to-body weight (a) and kidney-to-body weight ratios (b) in rats with high-fat diet and streptozotocin-induced diabetes. The mean value of each data point is based on 4 measurements. Bars labeled with different superscripts indicate a significant difference at a level of $p < 0.05$.

The analysis of organ-body weight ratio is a crucial parameter for detecting harmful chemicals in the body. According to Asrani *et al*. (2019), the increase in the liver/body weight ratio of rats after being induced with diabetes via a high-fat diet and streptozotocin could be an indication of liver inflammation. This could also be ascribed to an increase in the accumulation of triglycerides leading to increased liver, which may be a consequence of the increased influx of fatty acids into the liver caused by hypo-insulinemia (Asrani *et al.,* 2019). Multiple low-dose streptozotocin injections have also been demonstrated to cause gradual attenuation of insulin secretion, which is comparable to the natural progression of TDM2 and low secretion of lipoprotein secreted by lipoproteins apolipoprotein B (Kaneko *et al.*,2019). The increase in kidney/body weight ratio observed in diabetic rats supports the proposition made by Sawinski and Poggio (2021) that during the early stage of diabetes, the glomerular filtration rate (GFR) is elevated and kidney size is increased due to heightened single-nephron GFR and expanded nephron size. This may result from several primary abnormalities in vascular control leading to renal vasodilation causing early hyperfiltration. In the present study, administration of VPA at both 50 and 100 mg/kg bw reversed the increased liver-body weight ratio in diabetic rats, suggesting that VPA can counteract inflammation caused by triglyceride accumulation and fatty acid influx to the liver.

Liver function indices of high-fat diet and streptozotocin-induced diabetic rats treated with valproic acid

The serum concentrations of total bilirubin and conjugated bilirubin were significantly increased by high-fat diet and STZ ($p < 0.05$), while albumin and total protein concentrations were significantly decreased ($p < 0.05$) in rats. VPA treatment at both doses significantly reduced and normalized the concentrations of total bilirubin and conjugated bilirubin ($p < 0.05$), and increased the concentrations of albumin and total protein in diabetic rats ($p < 0.05$), according to Table 1.

Table 1: Effects of valproic acid on liver function indices in high-fat diet and streptozotocin-induced diabetic rats

Treatment Group	Total bilirubin (mg/dl)	Conjugated bilirubin (mg/dl)	Albumin (mg/dl)	Total protein concentration (g/dl)
Normal Control	0.79 ± 0.040^{a}	1.09 ± 0.083^{a}	3.87 ± 0.099^{a}	58.13 ± 1.210^{a}
Diabetic Control	1.03 ± 0.018^{c}	1.71 ± 0.104^{bc}	2.34 ± 0.077^{c}	49.68 ± 1.320^{b}
Diabetic + 50 mg/kg bw VPA	0.87 ± 0.034^{a}	1.15 ± 0.133^{a}	2.85 ± 0.269^{bc}	50.29 ± 3.110^{b}
Diabetic + 100 mg/kg bw VPA	0.82 ± 0.054^{a}	1.36 ± 0.354^{ab}	3.42 ± 0.167^{a}	59.79 ± 0.330^{a}
Diabetic + 100 mg/kg bw Metformin	0.95 ± 0.068^{bc}	1.88 ± 0.053^{c}	3.32 ± 0.201^{b}	56.94 ± 2.870^{a}

The mean of four replicates ± S.E.M was used to present all data, and one-way ANOVA followed by a post hoc Tukey's comparison test was used for data analysis. Significantly different values at $p < 0.05$ were denoted by different superscripts in the same column.

Serum bilirubin concentrations can be employed to detect different types of liver abnormalities (Guerra Ruiz *et al.,* 2021). Bilirubin is a product of hemoglobin catabolism with an important diagnostic value. The main causes of hyperbilirubinemia are intra-hepatic cholestasis and extra-hepatic biliary obstruction; the latter prevents bilirubin from crossing into the intestines. Diseases and autoimmune disorders are the most prevalent causes of hyperbilirubinemia (Guerra Ruiz *et al.,* 2021). In this study, the increase in both serum concentrations of total and conjugated bilirubin in the diabetic control and the decrease in their concentrations on the administration of VPA which compared well with the control (Table 2) suggest that VPA was able to ameliorate and restore defect in liver function which might have been caused by hemolysis and attenuation of secreted bilirubin conjugate in the liver of diabetic rats. Thus, VPA aided bilirubin clearance.

Evaluation of serum albumin and total protein concentrations could be used to ascertain the secretory and synthetic functions of the liver (Yakubu *et al.,* 2003). In diabetic conditions, synthesis and secretion of albumin are reduced because of insulin deficiency and increased protein glycation (Adebayo *et al.,* 2013). Diabetes is characterized by elevated plasma glucose levels, which successively modify blood proteins with a non-enzymatic reaction called glycation. The glycation of protein can lead to the formation of toxic molecules known as advanced glycation end products (AGEs). The accumulation of AGEs is quickened in diabetes and implicated in the pathogenesis of diabetic complications (Cole and Florez, 2020). In this study, diabetic induction decreased serum albumin concentration in rats while VPA ameliorated its effect in a dose-dependent manner (Table 2). This suggests that VPA at 100 mg/kg body weight enhances the restoration of liver secretory function in diabetic rats which may be a result of improved secretion of insulin and reduced production of AGEs. The study also found that diabetic induction led to a decrease in the total protein level in the serum, which could be attributed to increased protein catabolism and the movement of amino acids into the liver, feeding gluconeogenesis, as suggested by Cole and Florez (2020). In uncontrolled diabetes, the impaired glucagon-mediated regulation of cyclic AMP formation in insulin deficiency leads to increased proteolysis. This could explain the observed decrease in the total protein concentration in diabetic rats. However, treatment with VPA significantly increased the serum protein levels to normal.

Kidney function indices of high-fat diet and streptozotocin-induced diabetic rats treated with valproic acid

Diabetic induction caused a significant increase ($p < 0.05$) in serum creatinine, sodium ion, and chloride ion concentrations in rats, but did not significantly affect serum urea and potassium ion concentration. However, treatment with VPA (at both doses of 50 mg/kg bw and 100 mg/kg bw) and 100 mg/kg bw metformin significantly decreased ($p < 0.05$) and normalized serum creatinine and Na^+ concentration in the diabetic rats. The serum Cl^- concentration in the diabetic rats treated with VPA at both doses and 100 mg/kg bw metformin was not significantly different ($p > 0.05$) from the diabetic control, but was significantly higher ($p < 0.05$) than the normal control (Table 2).

Table 2: Effects of valproic acid on kidney function indices in high-fat diet and streptozotocin-induced diabetic rats

Treatment Group	Creatinine (mg/dl)	Urea (mg/dl)	$[K^+]$ (mEq/l)	$[Na^+]$ (mEq/l)	$[Cl^-]$ (mEq/l)
Normal Control	0.84 ± 0.063^a	25.26 ± 3.185^a	1.32 ± 0.081^a	6.21 ± 2.725^a	7.10 ± 1.473^a
Diabetic Control	2.07 ± 0.566^b	22.29 ± 2.217^a	1.51 ± 0.186^a	20.06 ± 7.012^b	24.78 ± 2.271^b
Diabetic + 50 mg/kg bw VPA	0.83 ± 0.232^a	29.00 ± 2.421^a	1.34 ± 0.173^a	9.56 ± 2.959^a	22.19 ± 9.044^b
Diabetic + 100 mg/kg bw VPA	0.62 ± 0.107^a	26.91 ± 0.587^a	1.91 ± 0.050^a	12.29 ± 2.962^a	17.03 ± 3.237^{ab}
Diabetic + 100 mg/kg bw Met	0.34 ± 0.009^a	22.67 ± 3.991^a	1.54 ± 0.650^a	16.73 ± 2.321^a	17.31 ± 6.238^{ab}

The statistical analysis involved a one-way ANOVA followed by a post hoc Tukey's comparison test. The mean of four replicates ± S.E.M. was used to present the data. Significant differences between values in the same column were indicated by different superscripts, with a significance level of $p < 0.05$.

Urea and creatinine concentrations are important indicators of renal efficiency. Elevated serum levels of these molecules are typically associated with impaired renal function, as explained by Pérez-Burillo *et al*. (2019). On the other hand, a decrease in urea levels may result from impaired urea cycle function, leading to reduced urea production, as noted by Cole and Florez (2020). However, in this study, treatment with VPA at all doses did not significantly alter the serum urea concentration. This suggests that the administration of VPA did not adversely affect the urea cycle or protein catabolism. Nevertheless, VPA reduced serum creatinine concentration at all doses administered (Table 2). These findings support reports from previous studies that VPA administration has anti-renal dysfunction and anti-inflammatory roles (Singh *et al.*, 2021). Electrolytes are essential for various bodily functions, including fluid regulation, pH balance, nerve impulse conduction, muscle movement, and blood clotting. Potassium, sodium, and chloride are crucial for maintaining proper electrolyte balance. Individuals with diabetes mellitus may experience an imbalance in their water and electrolyte levels due to insulin deficiency, hyperglycemia, and hyperketonemia, as described by Singh *et al*. (2021). In this study, the experimental analysis carried out on serum electrolytes showed that VPA had no significant effect on $[K^+]$ and $[Cl^-]$, but at all doses administered to the diabetic rats, VPA reduced and normalized $[Na^+]$ when compared to the Normal Control (Table 2). The lack of significant difference in Potassium and Chloride ion concentration showed that VPA may not induce electrolyte imbalance which might result in further complications such as kidney failure, dehydration, fever, and vomiting.

Selected liver, kidney and serum enzymes of high-fat diet and streptozotocin-induced diabetic rats treated with valproic acid

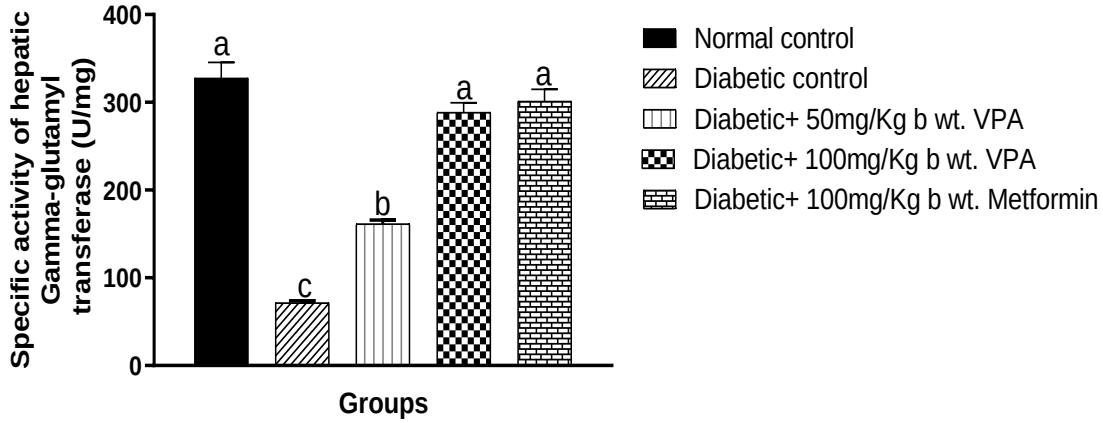
The use of high-fat diet and STZ to induce diabetes in rats significantly ($p < 0.05$) decreased the specific activity of liver gamma-glutamyl transferase (GGT), while also significantly

increasing the specific activity of serum GGT. However, treatment with VPA at 50 and 100 mg/kg bw dose-dependently increased GGT activity in the liver of diabetic rats, with similar effects observed for 100 mg/kg bw metformin treatment. The diabetic rats also showed a significant decrease ($p < 0.05$) in the specific activity of kidney GGT, which was significantly increased ($p < 0.05$) by treatment with 50 mg/kg bw VPA and 100 mg/kg bw metformin. Treatment with 100 mg/kg bw VPA significantly increased and normalized the specific activity of kidney GGT in the diabetic rats. In addition, treatment with VPA significantly ($p < 0.05$) decreased the specific activity of serum GGT in a dose-dependent manner, with similar effects observed for 100 mg/kg bw metformin treatment.

Enzymes are typically found in higher concentrations within cells compared to serum. They may also be specific to certain tissues and localized in various cellular compartments, such as the nucleus, lysosome, or cytoplasm. The natural level of enzymes in the serum is influenced by the balance between their synthesis and release into the serum during cell turnover, as well as their clearance from circulation (Khan *et al.*,2020). Measurement of marker enzyme activities in the body tissues and fluids plays an important role in disease diagnosis and the evaluation of the drug or herbal extract for safety/toxicity risk (Khan *et al.*,2020). Gamma-glutamyl transferase (GGT) is mainly utilized as a diagnostic marker for liver abnormalities. Increased serum GGT activity has been observed in diseases of the liver, pancreas, and biliary system (Cho *et al.,* 2023). In this study, the decreased activity of GGT in the liver and kidney of diabetic rats (Figure 2a and 1b) and its corresponding elevation in serum (Figure 2c) indicate that their functions in diabetic rats have been compromised. VPA administered at both 50 and 100 mg/kg bw increased GGT activity in the liver/Kidney and decreased its activity in the serum of diabetic rats, respectively. This suggests that VPA ameliorates liver and kidney dysfunction in type 2 diabetes and VPA does not pose any risk of causing kidney diseases, diseases of the biliary system and pancreatic diseases since it was able to improve GGT-specific activity.

The specific activity of liver alkaline phosphatase (ALP) was significantly ($p < 0.05$) decreased by a high-fat diet and STZ, but treatment with VPA at doses of 50 and 100 mg/kg bw significantly ($p < 0.05$) increased and restored liver ALP activity in diabetic rats. However, when treated with 100 mg/kg bw metformin, the ALP activity of diabetic rats was significantly ($p < 0.05$) higher than that of the normal control (Figure 3a). In addition, the specific activity of ALP in the rat kidney was significantly ($p < 0.05$) increased by diabetic induction with HFD and STZ. Treatment with 50 mg/kg body weight VPA significantly ($p < 0.05$) reduced the specific activity of ALP in the kidney of diabetic rats. When compared with the normal control and diabetic control, treatment with both 100 mg/kg body weight VPA and metformin significantly ($p < 0.05$) reduced and normalized the specific activity of ALP in the rat kidney of diabetic rats (Figure 3b). Furthermore, the serum ALP activity of rats was significantly ($p < 0.05$) increased by HFD and STZ, but treatment with VPA at both doses significantly ($p < 0.05$) reduced and normalized the serum ALP activity of diabetic rats. Treatment with 100 mg/kg bw metformin significantly ($p < 0.05$) reduced the serum ALP activity of diabetic rats (Figure 3c).

(a)



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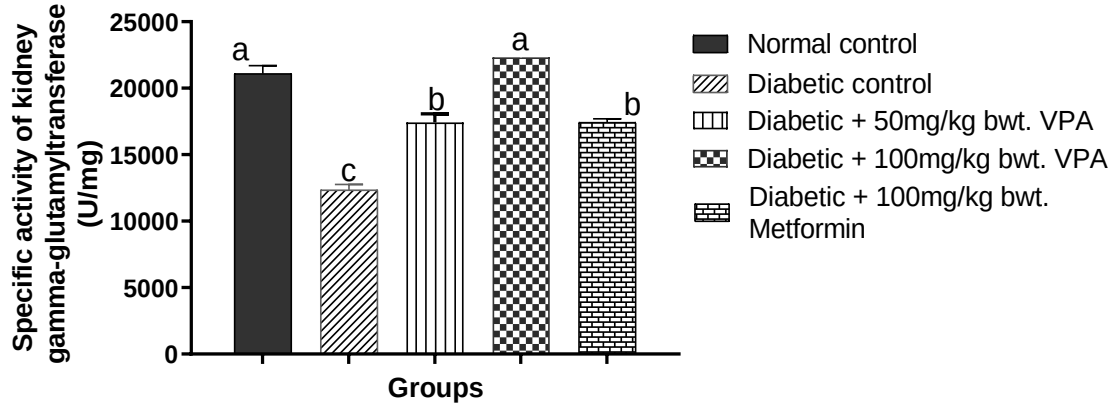

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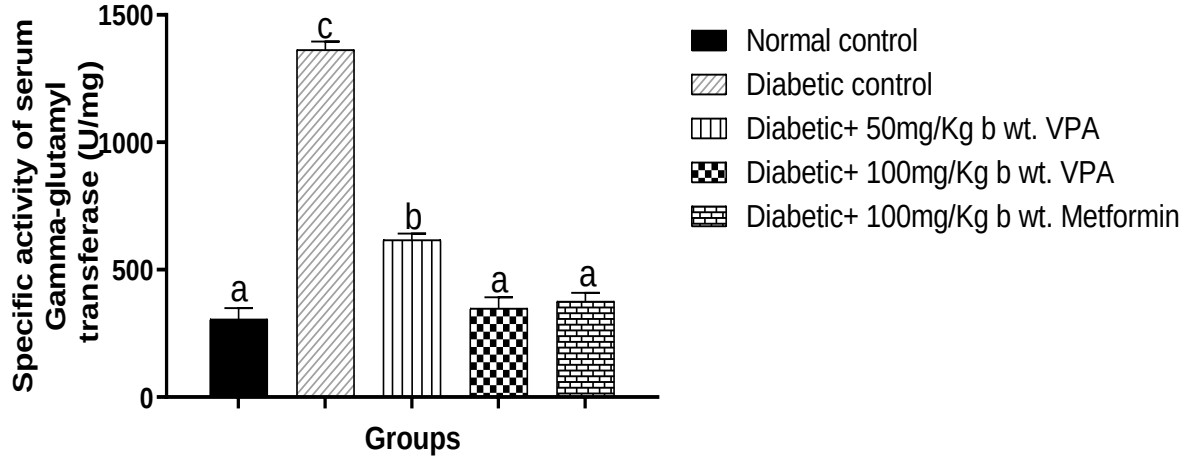
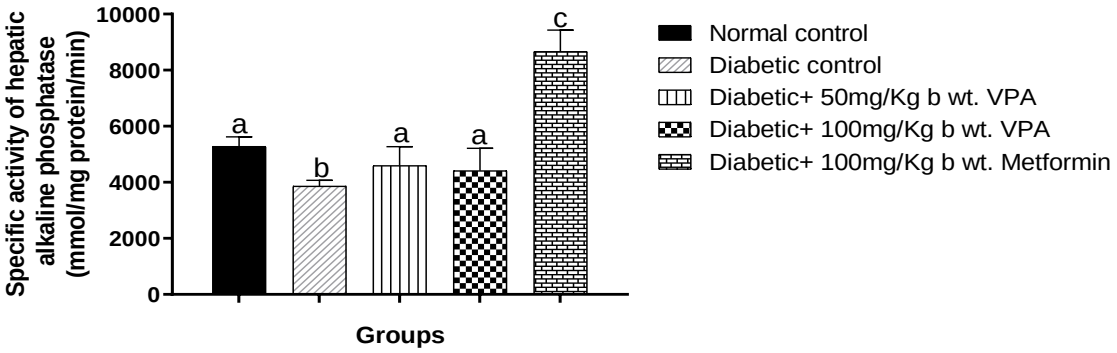


Figure 2: Specific activity of gamma-glutamyltransferase in the liver (a), kidney (b) and serum (c) of high-fat diet and streptozotocin-induced diabetic rats treated with valproic acid. The average of four measurements represents each value. Bars marked with dissimilar superscripts exhibit a significant difference ($p < 0.05$).

(a)



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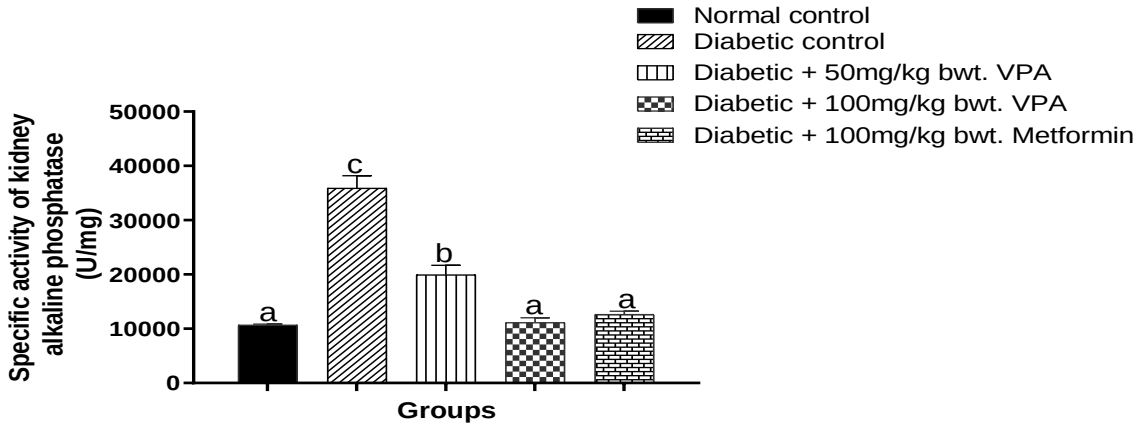

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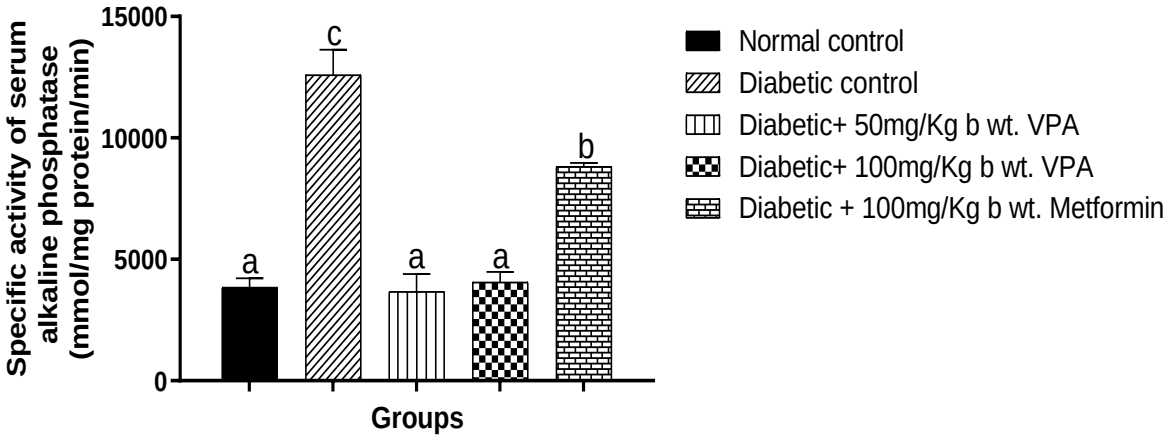


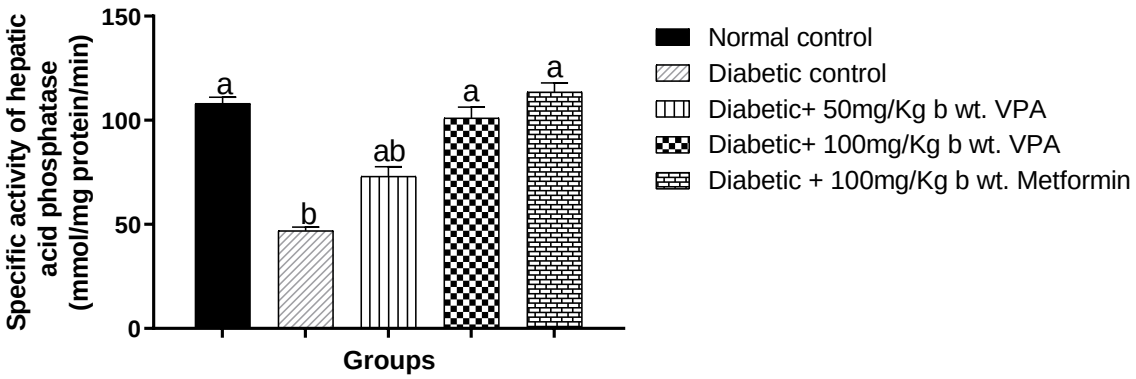
Figure 3: Specific activity of alkaline phosphatase in the liver (a), kidney (b) and serum (c) high-fat diet and streptozotocin-induced diabetic rats treated with valproic acid. The average of four measurements represents each value. Bars marked with dissimilar superscripts exhibit a significant difference ($p < 0.05$).

The presence of alkaline phosphatase (ALP) on the membrane of the hepatic drainage system makes it a promising biomarker for evaluating the integrity of this system in diabetic conditions. ALP is widely used for assessing liver injury (Shamban *et al*., 2018) and is abundantly found in various tissues, particularly the liver, bone, and kidney (Abubakar *et al*., 2020). As a biomarker of the plasma membrane, a decrease in liver ALP activity and the corresponding increase in activity of serum ALP suggest altered membrane integrity (Pérez-Burillo *et al.,* 2019). In this study, VPA treatment reversed the alteration in liver and serum ALP activities of diabetic rats (Figures 3a and 3c). Treatment with valproic acid ameliorated the damage done to the kidney (Figure 3b). The increase in ALP activity in the kidney may be a result of conditions that are associated with hyperfiltration, common in diabetic conditions as reported by Toshifumi *et al*. (2020). This further confirms that VPA confers protection to the liver of diabetic rats.

The specific activity of liver acid phosphatase (ACP) was significantly ($p < 0.05$) reduced by a high-fat diet and STZ in rats, but administering VPA at doses of 50 and 100 mg/kg bw significantly ($p < 0.05$) increased ACP activity in a dose-dependent manner in diabetic rats. Metformin at 100 mg/kg bw had a similar effect to VPA at 100 mg/kg bw (Figure 4a). Kidney acid phosphatase specific activity was increased by a high-fat diet and STZ compared to normal control, but treatment with VPA at doses of 50 and 100 mg/kg bw significantly reduced ($p < 0.05$) and normalized the specific activity of acid phosphatase in rats' kidneys. Treatment with 100 mg/kg body weight metformin also significantly reduced ($p < 0.05$) specific activity of acid phosphatase in rats' kidneys (Figure 4b). In addition, HFD and STZ increased serum ACP activity, but administering VPA at both doses, as well as 100 mg/kg bw metformin, significantly ($p < 0.05$) reduced and normalized serum ACP activity in diabetic rats (Figure 4c). Increased serum acid phosphatase (ACP) in liver disease is connected with intra- or extra-hepatic obstruction, diabetes, obstructive jaundice (Shamban *et al.,* 2018), infectious mononucleosis, and biliary cirrhosis (Khanna and Kumar, 2016). In this study, VPA significantly increased the reduced activities of serum ACP (Figure 4c) in diabetic rats and significantly increased the liver ACP activity (Figure 4a), while in the kidney VPA was able to normalize the activity of ACP. This suggests that VPA might have ameliorated extra or intra-hepatic obstruction in diabetic rats.

The specific activity of liver aspartate aminotransferase (AST) in rats was reduced by high-fat diet and STZ-induced diabetes, but treatment with 50 and 100 mg/kg bw VPA or 100 mg/kg bw metformin significantly increased and normalized AST activity (Figure 5a). Similarly, high-fat diet and STZ increased the specific activity of AST in the rats, but treatment with metformin and VPA at both doses significantly decreased and normalized AST activity in the kidney of the treated rats (Figure 5b). In addition, serum AST activity was significantly increased by HFD and STZ in rats, but treatment with VPA and metformin at both doses significantly reduced and normalized serum AST activity in the diabetic rats (Figure 5c).

(a)



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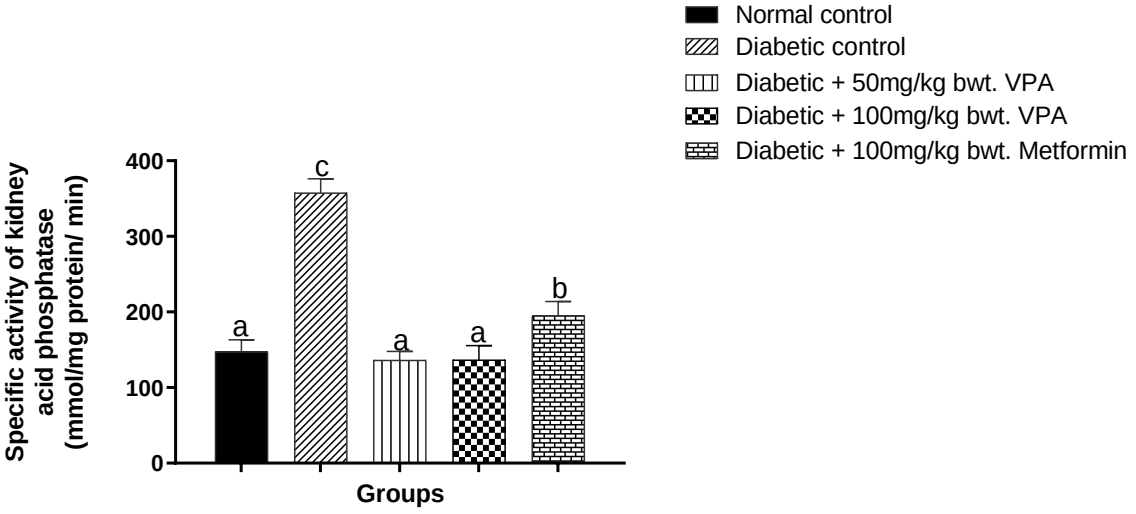

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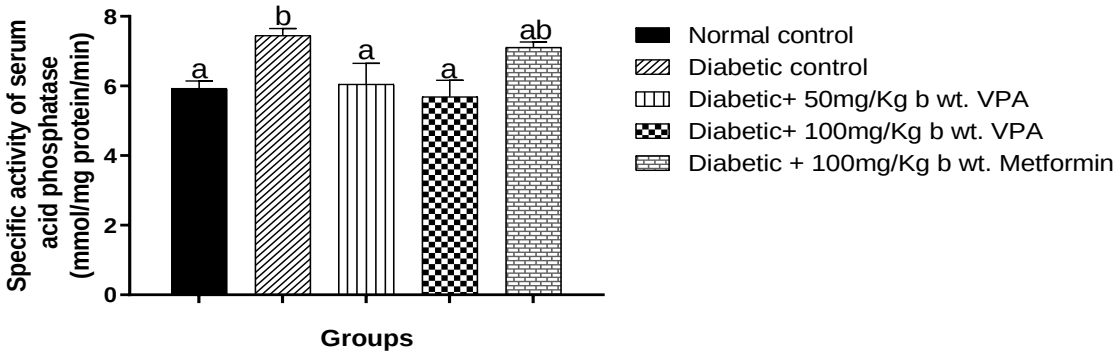
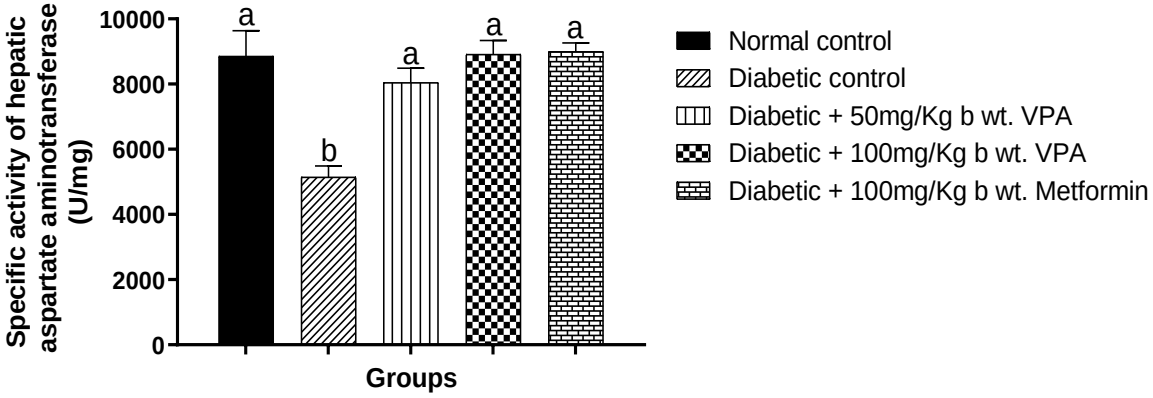
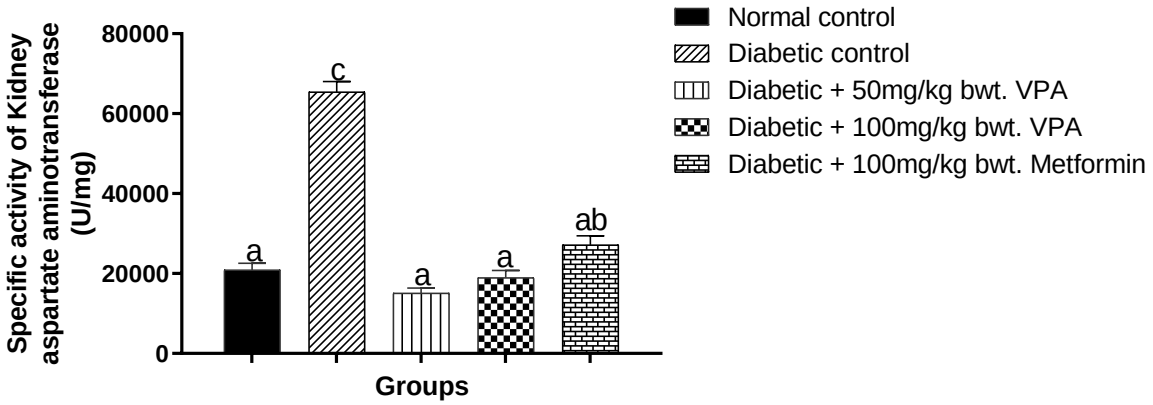


Figure 4: Specific activity of acid phosphatase in the liver (a), kidney (b) and serum (c) of the high-fat diet and streptozotocin-induced diabetic rats treated with valproic acid. The average of four measurements represents each value. Bars marked with dissimilar superscripts exhibit a significant difference ($p < 0.05$).

(a)



(b)



(c)

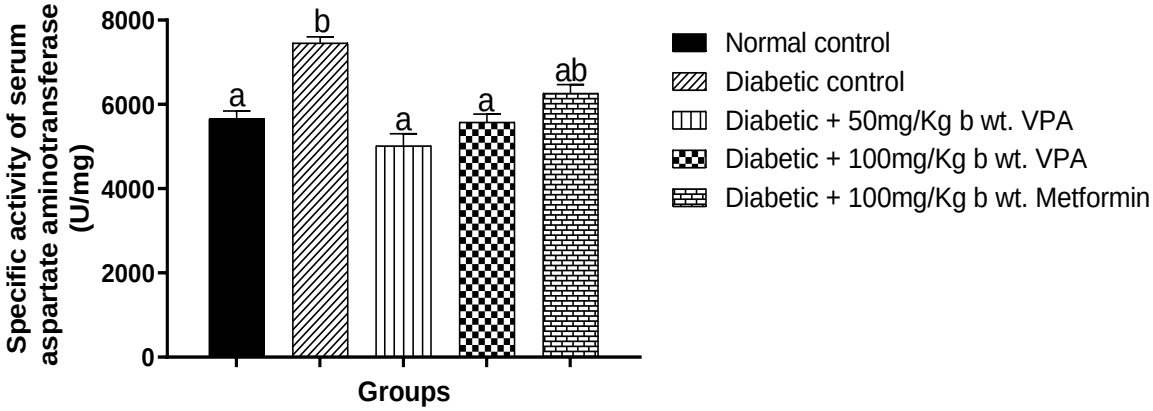
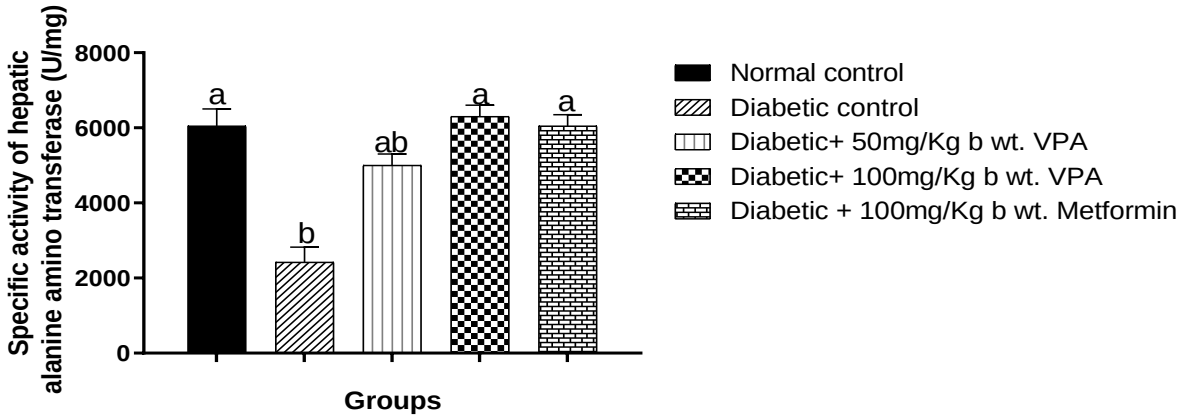
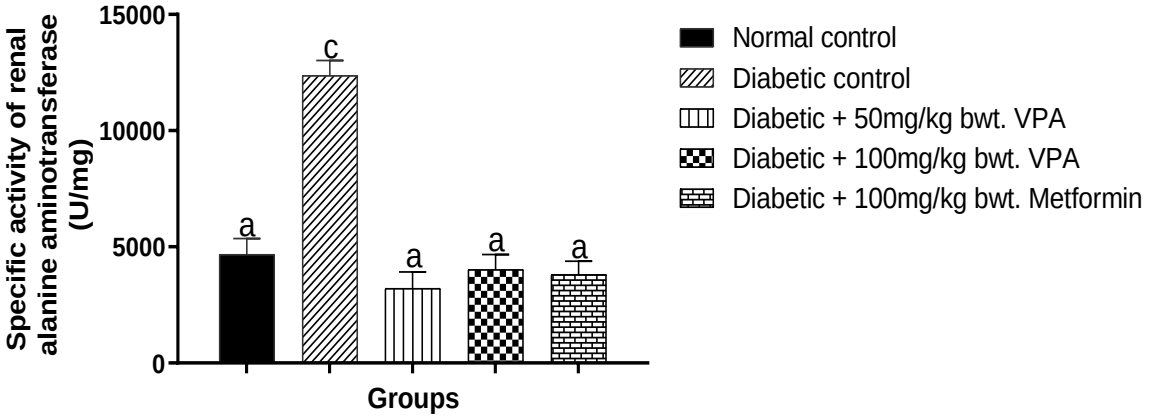


Figure 5: Specific activity of aspartate aminotransferase in the liver (a), kidney (b) and serum (c) of high-fat diet and streptozotocin-induced diabetic rats treated with valproic acid. The average of four measurements represents each value. Bars marked with dissimilar superscripts exhibit a significant difference ($p < 0.05$).

(a)



(b)



(c)

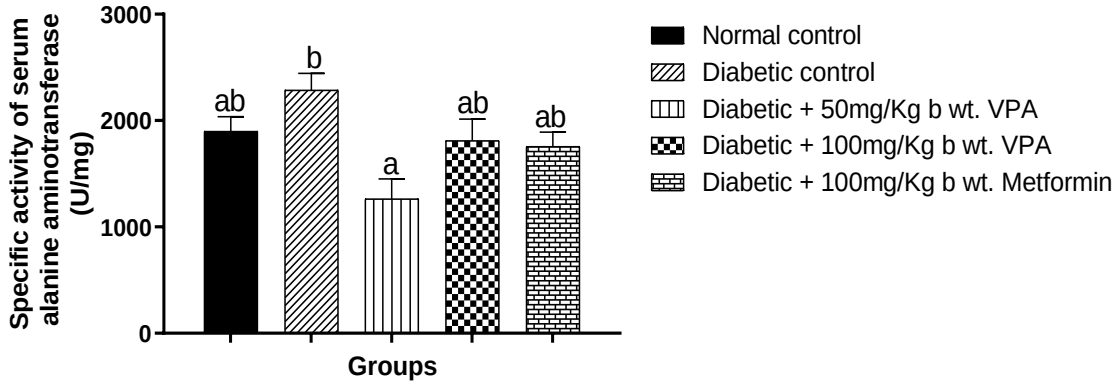


Figure 6: Specific activity of alanine aminotransferase in the liver (a), kidney (b) and serum (c) of high-fat diet and streptozotocin-induced diabetic rats treated with valproic acid. The average of four measurements represents each value. Bars marked with dissimilar superscripts exhibit a significant difference ($p < 0.05$).

The specific activity of rat liver alanine aminotransferase (ALT) was reduced by high-fat diet and STZ-induced diabetes, but treatment with VPA at doses of 50 and 100 mg/kg bw and metformin at 100 mg/kg bw significantly increased liver ALT activity in a dose-dependent manner in diabetic rats (Figure 6a). Furthermore, high-fat diet and STZ increased the specific activity of kidney ALT in rats, but treatment with VPA and metformin at all doses significantly decreased and normalized kidney ALT activity (Figure 6b). In addition, serum ALT activity was significantly increased by HFD and STZ in rats, but VPA treatment at all doses significantly decreased ALT activity in a dose-dependent manner, and metformin had a similar effect to VPA at a dose of 100 mg/kg bw (Figure 6c).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are crucial biomarkers for predicting potential organ toxicity (Lala *et al*., 2022), as they are primarily concentrated in the liver and kidney and are typically present in low quantities in the serum, indicating a healthy cell membrane. Elevated levels of AST and ALT in the serum may suggest cellular damage or dysfunction in these organs, as the enzymes are released into the bloodstream when the cell membrane is compromised (Shamban *et al*., 2018). These aminotransferases are of clinical importance, especially in the evaluation of liver and kidney functions (Lala *et al.,* 2022). The activities of both enzymes increase in many disorders associated with liver damage and kidney damage; hence suitable as reliable markers of liver damage (Pérez-Burillo *et al.,* 2019). The elevated levels of AST and ALT in the serum of diabetic rats and the reduced activities of these enzymes in the liver and kidney observed in this study suggest liver and kidney damage resulting from the diabetic condition and subsequent release of enzymes into the bloodstream (Galicia *et al*., 2020). This finding supports the idea that VPA can protect the liver and kidney by restoring membrane integrity and reversing damage caused by diabetes. The ability of VPA to reverse the changes in liver and serum AST and ALT activities in diabetic rats implies that VPA may be beneficial in restoring liver and kidney function.

4. Conclusion

The results of this study indicate that the administration of valproic acid have a positive impact on liver and kidney dysfunctions in rats with type 2 diabetes. While diabetic induction altered the functional parameters of liver and kidney in rats, administration of valproic acid ameliorated the liver and kidney dysfunctions in the diabetic rats.

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