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EFFECTS OF VALPROATE SODIUM ON LIVER AND KIDNEYS OF ALBINO RATS AND THE ROLE OF METFORMIN AS ADJUVANT THERAPY

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ABSTRACT

Background: Valproate (VPA) which usually prescribed as its sodium salt, was approved for treatment of epilepsy either as monotherapy or in combination with other anticonvulsant drugs. It is also used in the treatment of a variety of neuropsychiatric illnesses. It was reported that it has sever toxic effects on different organs of body. Metformin is one of the most widely used oral antidiabetic drugs for the treatment of type 2 diabetes. **Aim** of the study was to assess the effect of the administration of valproate sodium on liver and kidneys of albino rats as and compare effect of its administration alone and with administration of metformin to study metformin possible protective effect. **Methods:** Four groups of 80 rats formed of a control group, Valproate sodium -treated group that received Valproate sodium dissolved in water for 12 weeks, Metformin -treated group that received Metformin dissolved in water for 12 weeks and Valproate sodium plus metformin -treated group that received Valproate sodium plus Metformin for 12 weeks. For all, liver and kidneys function tests and hepatic, kidneys histopathologic examinations were done. **Results:** There was a statistically significant difference (P <0.05) among rats' liver function tests in different study groups, with high mean of liver enzymes (ALT, AST and ALP) levels in Valproate sodium group. Also, among the valproate sodium about 40% of liver tissues showed Grade 2 hepatic necrosis. There was liver fibrosis in Valproate sodium group, about 50% of liver tissues of valproate sodium shows Grade 2 hepatic fibrosis, 50% showed Grade 1. There was a statistically significant difference (P <0.05) among rats' kidneys function tests(urea and creatinine) in different study groups, with high mean of urea and creatinine levels in Valproate sodium group. Also, among the valproate sodium kidneys tissues revealed moderate vascular congestion dense inflammation, fibrosis and vacuolar degeneration (ballooning) of some of the tubular cells with marked tubular dilatation and intratubular casts.Adding metformin to Valproate sodium was found to produce significant improvement in liver, kidneys function, liver and kidneys histopathological findings. **Conclusion:** Long term use of Valproate sodium in albino rats produces hepatotoxicity and nephrotoxicity and metformin restored the altered liver and kidneys function and possessed hepatoprotection and nephroprotection against Valproate sodium induced hepatotoxicity and nephrotoxicity.

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INTRODUCTION

Valproate (VPA) which typically prescribed as its sodium salt (McLaughlin *et al*., 2000), was approved for treatment of epilepsy either as monotherapy or in blend with other anticonvulsant drugs. It is additionally utilized in the treatment of a variety of neuropsychiatric illnesses as mania, bipolar affective disorder, migraine, headache and several anxiety disorders as It raises the entire brain gamma amino butyric acid (GABA) a major inhibitory neurotransmitter in the brain, and inhibits GABA degradation (Ibrahim, 2012). It was reported that it has sever toxic effects on liver tissue (Pourahmad *et al*., 2012). It was demonstrated that use of VPA in patients with epilepsy is associated with an increase in body weight by increasing serum insulin and insulin/glucose levels may or by stimulating appetite (Kanemura *et al*., 2012). Metformin is one of the most widely used oral antidiabetic drugs for the treatment of type 2 diabetes (Jiang *et al.*, 2014) as

it controls blood glucose levels by increasing insulin sensitivity and improving glucose uptake in the liver (Mori *et al*., 2017). It additionally finds place in the treatment of many clinical conditions other than type 2DM as heart disease, kidney disease, decreased sexual ability and circulation problems (Gao *et al*., 2010). It was reported that metformin has hepatoprotective potential against hepatotoxicity resulted from methotrexate therapy and the antioxidant properties, antiapoptotic and anti-inflammatory effects of metformin were probably the contributing factors for this hepatoprotection (Hadi *et al.,* 2012). The aim of the study was to assess the effect of the administration of valproate sodium on liver and kidneys of albino rats as and compare effect of its administration alone and with administration of metformin to study metformin possible protective effect.

MATERIAL AND METHODS

Materials

- Valproate sodium obtained from Sanovi Company in the form of 200 mg tablets.
- Metformin obtained from Merk Company in the form of 500 mg tablets.

Experimental animals: The study was conducted on 80 albino rats weighing between (134-290 g). The rats were acclimatized for 7days before the onset of the experiment. The chosen animals were individually housed in plastic cages with good aerated covers at normal atmospheric temperature (25 ± 5ºC) as well as under good ventilation and received water and standard balanced diet.

Experimental Design: Rats were divided into 4 groups, each group formed of 20 rats. Group A: Control group (20 rats): Animals untreated and served as negative control. Group B: Valproate sodium -treated group (20 rats): Animals were treated by valproate sodium at a dose of 200 mg/kg/day via oral route for 12 weeks. Group C: Metformin -treated group (20 rats): Animals were treated by metformin at a dose of 100 mg/kg/day via oral route for 12 weeks. Group D: Valproate sodium plus metformin -treated group (20 rats): Animals were treated by valproate sodium at a dose of 200mg/kg/day plus metformin at a dose of 100 mg/kg/day via oral route for 12 weeks. At the end of the experimental period animals were anesthetized using diethyl ether, blood samples were collected from the orbital sinus. The blood samples were centrifuged at 3000 round per minute (r.p.m.) for 20 minutes to obtain serum. The supernatant sera were separated and frozen at -80ºC for biochemical analysis:

A) Liver function tests: ALT (Alanine aminotransferase, AST (Aspartate aminotransferase, Alkaline phosphatase and Albumin

B) Kidney function tests: Urea serum level and Creatinine serum level

Tissue Harvesting Procedures: At the end of experiment, the animals sacrificed by cervical decapitation and laparotomy was carried out to remove the tissues (liver & kidneys) that were stored in a formalin solution.Sample of 0.5cm3 of the organs were removed and fixed in 10% neutral formalin for 24 hours followed by washing, dehydration in ascending grades of alcohol, clearing in xylene and embedding in hard paraffin. Samples were then serially sectioned at thickness of 5-6µ, mounted on albuminized slides and left for 24 hours at 37°C to dry and to avoid detachment of sections during subsequent steps of staining. The tissue sections were stained by Hematoxylin and Eosin stain and then examined under the light microscope. The histopathological examination of liver& kidneys was carried out to determine any associated changes and compare between groups. A numerical scoring system for histologically assessing the extent of fibrosis was adapted from the formula of (Schcuer,1991), with minor modifications (Hsu *et al*., 2005). Briefly, fibrosis was graded as:0: No fibrosis. Grade 1: Enlarged, fibrous portal tracts.Grade 2: Periportal or portal- portal septa, but intact architecture.Grade 3: Fibrosis with architectural distortion.Grade 4: Probable or definite cirrhosis. Additionally, hepatocyte necrosis or degeneration severity was also graded as:

0: No hepatocyte necrosis or degeneration. Grade 1: Focal necrosis or degeneration of hepatocytes (mild lesion no. ≤ 3). Grade 2: Multifocal necrosis or degeneration of hepatocytes (moderate lesion no.> 3). Grade 3: Locally extensive or diffuse necrosis or degeneration of hepatocytes (severe). Hepatocyte degeneration is mainly associated with cytoplasmic vacuolation and swelling, with the nuclear contour generally intact, whereas hepatocyte necrosis is associated with karyopyknosis (nuclear shrinkage) and karyorrhexis (nuclear rupture), in addition to degenerative changes (Weng *et al*., 2009).

Statistical analysis

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 18 (SPSS Inc, USA). For quantitative data, the mean and standard deviation were calculated. ANOVA (Analysis of variance) was used to test the difference about mean values of measured parameters among groups, multiple comparison between pairs of groups were performed using LSD (Post hoc range test). Paired t test was used in comparison between the difference of body weight in before and after intervention. For qualitative data the number and percent distribution was calculated, chi square ($\chi 2$) was used as a test of significance. For interpretation of results of tests of significance, significance was adopted at $P \leq 0.05$.

RESULTS

The body weight of rats was determined in the beginning of the study and after finishing it (after 12 week) (Table 1). There was significant increase in bodyweight gain ($p<0.05$) in the valproate sodium treated group with high mean of body weight gain when compared to rats of all other groups. There is significant decrease in bodyweight gain ($p<0.05$) in the metformin treated group when compared to rats of all other groups. The results of biochemical studies done to estimate the liver function were compared between different study groups (Table 2).There is statistically significant difference with p-value <0.05 among rats' liver function tests in different study groups, with high mean of liver enzymes (ALT, AST and ALP) level in valproate sodium group and there is significant increase in ALT level in valproate sodium plus metformin group when compared to control and metformin group but within normal range. Also, insignificant difference in albumin level in different study groups. Additionally, the kidney functions were compared between different study groups (Table 3). There is statistically significant difference with p-

value <0.05 in rats' kidney function tests (urea &creatinine) in different study groups, with high mean level in valproate sodium group when compared to other groups. There is statistically significant difference betweenvalproate sodium plus metformin group and control group.

Histopathological results

Liver Microscopically, Livers of rats of the control group (Figure 1): revealed normal histological structure of hepatic lobule with normally looking hepatocytes, normal portal areas

Table 1. Comparisons of body weights between study groups before and after interventions

	Mean	SD	P-value	Significance
Initial weight				
Control group (Group A)	177.1	25.3		
Valproate sodium group (Group B)	180	26.7	B vs. A: 0.975	NS
Metformin group (Group C)	156.6	16.1	C vs. A: 0.022	S
			C vs. B: 0.006	S
Valproate sodium plus metformin (Group D)	156.7	18	D vs. A: 0.022	S
			D vs. B: 0.007	S
			D vs. C: 1.000	NS
Last weight				
Control group (Group A)	237	24.9		
Valproate sodium group (Group B)	280.3	26	B vs. A:<0.0001	S
Metformin group (Group C)	137.6	14.7	C vs. A:<0.0001	S
			C vs. B:<0.0001	S
Valproate sodium plus metformin (Group D)	166.8	18.9	D vs. A:<0.0001	S
			D vs. B:<0.0001	S
			D vs. C:<0.0001	S

NS = Non-significant S = Significant SD= Standard deviation

Table 2. Comparison of liver function tests in different study groups

	Mean	SD	P-value	Significance
ALT				
Control group (Group A)	26.2	26.2		S
Valproate sodium group (Group B)	71.7	71.7	B vs. A:<0.0001	S
Metformin group (Group C)	25	25	C vs. A: 0.979	S
			C vs. B:<0.0001	S
				S
Valproate sodium plus metformin (Group D)	35.8	35.8	D vs. A: 0.012	S
			D vs. B:<0.0001	S
			D vs. C: 0.004	S
AST				
Control group (Group A)	90.5	30.8		
Valproate sodium group (Group B)	191.9	70.1	B vs. A:<0.0001	S
Metformin group (Group C)	90.1	30.7	C vs. A: 1.000	NS
			C vs. B:<0.0001	S
Valproate sodium plus metformin (Group D)	117.3	43.4	D vs. A: 0.273	NS
			D vs. B:<0.0001	S
			D vs. C: 0.261	NS
ALP				
Control group (Group A)	91.9	28.2		
Valproate sodium group (Group B)	214.8	50.6	B vs. A:<0.0001	S
Metformin group (Group C)	90.6	28.4	C vs. A: 0.999	NS
			C vs. B:<0.0001	S
Valproate sodium plus metformin (Group D)	113.5	25.9	D vs. A: 0.208	NS
			D vs. B:<0.0001	S
			D vs. C: 0.168	NS
Albumin				
Control group (Group A)	3.9	0.5		
Valproate sodium group (Group B)	3.8	0.5	B vs. A: 0.884	NS
Metformin group (Group C)	4	0.6	C vs. A: 0.940	NS
			C vs. B: 0.562	NS
Valproate sodium plus metformin (Group D)	3.7	0.3	D vs. A: 0.407	NS
			D vs. B: 0.841	NS
			D vs. C: 0.152	NS

NS = Non-significant, S = Significant, SD= Standard deviation

Table 3. Comparison of kidney function tests in different study groups

	Mean	SD	P-value	Significance
Urea				
Control group (Group A)	19.7	1.8		
Valproate sodium group (Group B)	33.3	7.7	B vs. A:<0.0001	S
Metformin group (Group C)	19.8	1.9	C vs. A: 1.000	NS
			C vs. B:<0.0001	S
Valproate sodium plus metformin (Group D)	20.1	2	D vs. A: 0.993	NS
			D vs. B:<0.0001	S
			D vs. C: 0.996	NS
Creatinine				
Control group (Group A)	0.8	0.2		
Valproate sodium group (Group B)	2.2	0.7	B vs. A:<0.0001	S
Metformin group (Group C)	0.8	0.2	C vs. A: 1.000	NS
			C vs. B:<0.0001	S
Valproate sodium plus metformin (Group D)	1	0.3	D vs. A: 0.251	NS
			D vs. B:<0.0001	S
			D vs. C: 0.251	NS

NS = Non-significant S = Significant SD= Standard deviation

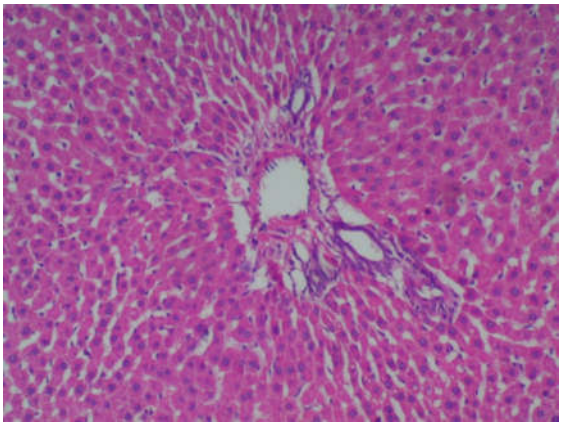

Figure 1. A micrograph of liver from control group showing no histopathological changes (H&E X200)

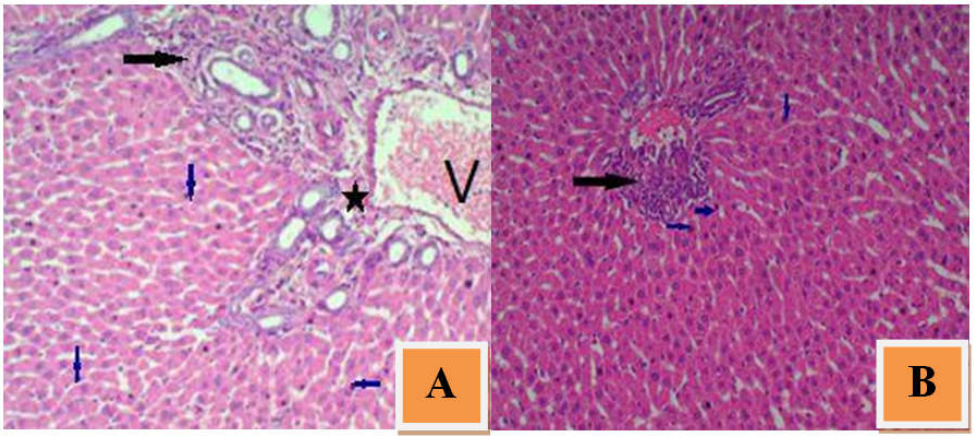

Figure 2. A micrograph of liver from the valproate sodium -treated group (A) Showing moderate inflammatory cells (the black arrow) many apoptotic cells (the blue arrows) fibrosis and proliferated portal ducts, the star, and dilated congested blood vessels (the letter V) (B) Showing moderate inflammatory cells (the black arrow) many apoptotic cells, the blue arrows, (H&E X200)

and central veins. Livers of rats of valproate sodium -treated group: All examined livers of rats (n=20) revealed central vein dilatation, half of examined livers of rats (n=10) revealed mild inflammation and mild fibrosis (Grade 1 hepatic fibrosis) but the other half (n=10) showed moderate inflammation, moderate fibrosis (Grade 2 hepatic fibrosis), many apoptotic cells, and proliferated portal ducts and dilated congested blood vessels (Figure 2).

Some (n=6) livers of rats of the same group showed cloudy degeneration of the hepatocytes (Figure 3A), while some (n=8) showed vacuolar degeneration of hepatocytes and fatty accumulation (steatosis) with signet ring appearance of the hepatocytes (Figure 3B) and revealed spotty hepatic necrosis (Grade 2 hepatic necrosis) (Figure 4). Livers of rats of metformin -treated group: Most (n=17) of examined livers of rats revealed normal histological structure of hepatic lobule

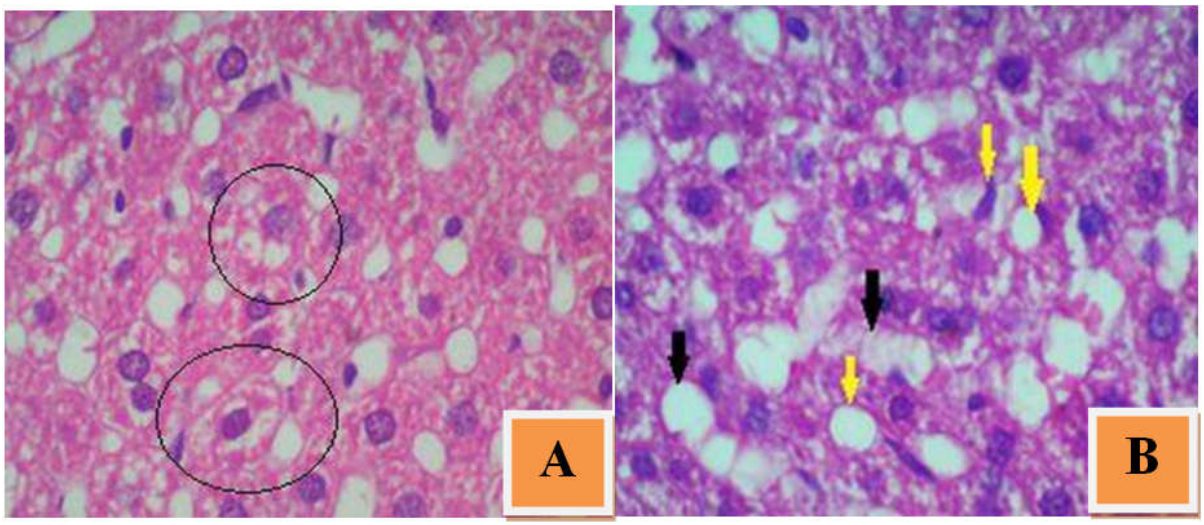

Figure 3. A micrograph of liver from the valproate sodium -treated group, (A) Showing cloudy degeneration of the hepatocytes (the circles), (B) Showing vacuolar degeneration of the hepatocytes (the black arrows) and fatty accumulation (steatosis) with signet ring appearance of the hepatocytes (the yellow arrows) (H&E, X400)

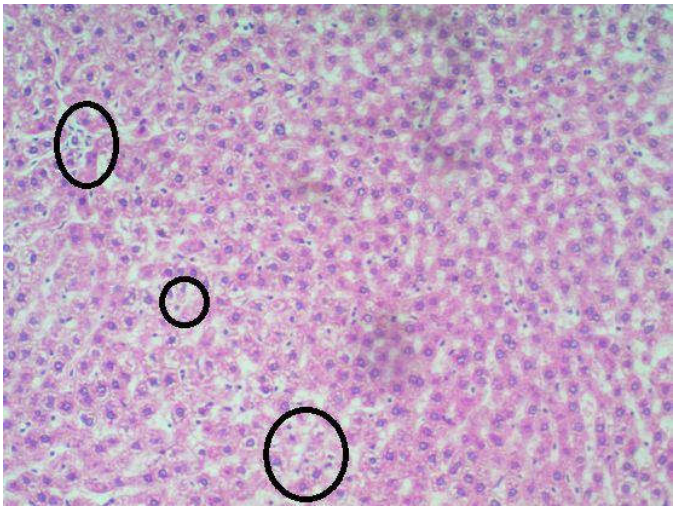

Figure 4. A micrograph of section from the liver in valproate sodium -treated group Showing some foci of spotty necrosis. (H&E x200)

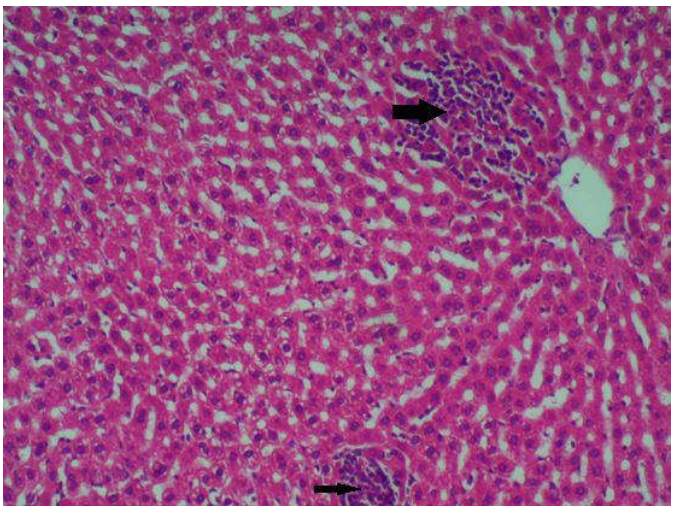

Figure 5. A micrograph of liver from metformin -treated group Showing within normal liver apart from mild inflammatory cells (the arrows) (H&E X200)

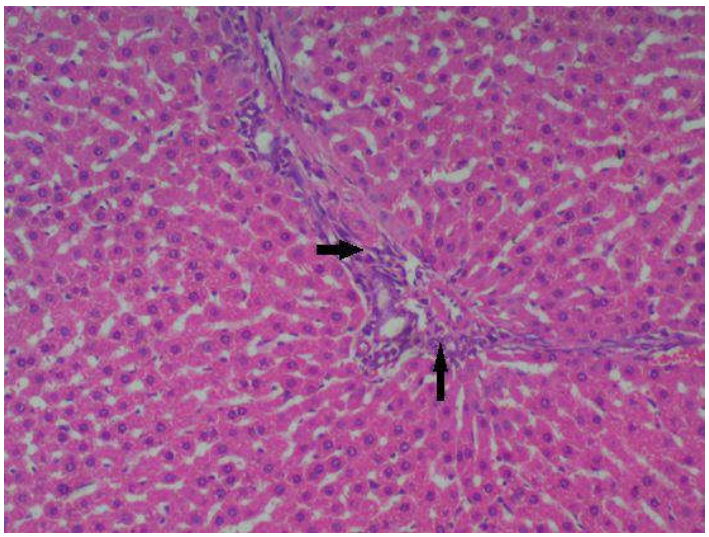

Figure 6. A micrograph of liver from valproate sodium plus metformin –treated group Showing within normal liver apart from minimal inflammatory cells in the portal tracts and early fibrous expansion (the arrows) (H&E X200)

except some (n=3) showed mild inflammation (Figure 5) and mild fibrosis (Grade 1 hepatic fibrosis). Livers of rats of valproate sodium plus metformin -treated group (Figure 6): Most (n=16) of examined livers of rats revealed normal histological structure of hepatic lobule within normal liver except some (n=4) revealed early fibrous expansion (Grade 1 hepatic fibrosis) and minimal inflammatory cells in the portal tracts. There was a statistically significant difference (P <0.0001) in rats' liver necrosis in valproate sodium -treated group ,about 40%liver tissues shows Grade 2hepatic necrosis and 60%shows no hepatic necrosis(Figure 7) but other groups showed no necrosis.Also, there was a statistically significance difference (P <0.0001) in rats' liver fibrosis in different study groups with higher effect on liver tissues amongvalproate sodium -treated group. Regarding liver tissues of the valproate sodium group, about 50% show Grade 2 hepatic fibrosis and 50% show Grade 1, in liver tissues of the metformin group about 85% show no fibrosis but 15% show Grade 1 hepatic fibrosis. In liver tissues of the valproate sodium plus metformin group 80% no fibrosis and 20% show Grade 1 hepatic fibrosis. There is no hepatic fibrosis effect among control group (Figure 8).

aggregates. The glomeruli are of average cellularity. The tubules are not dilated with no degeneration or cast formation.

The kidneys of rats of valproate sodium -treated group (Figure 10): All examined kidneys of rats (n=20) revealed vacuolar degeneration (ballooning) of some of the tubular cells with marked tubular dilatation and intratubular casts (Figure 10A). Some (n=10) revealed moderate vascular congestion dense inflammation and fibrosis (Figure 10B).

The kidneys of rats of metformin -treated group (Figure 11): Most kidneys of rats (n=15) revealed mild vascular congestion of the glomerular tufts (Figure 11A) and some (n=8) showed vacuolar degeneration (ballooning) of some of the tubular cells. The stroma showing no inflammatory cells aggregates. The glomeruli are of average cellularity. The tubules are not dilated with no degeneration or cast formation (Figure 11 B).

The kidneys of rats of valproate sodium plus metformin -treated group (Figure 12): All examined kidneys of rats (n=20) revealed normal pattern, the stroma showing no inflammatory cells aggregates.

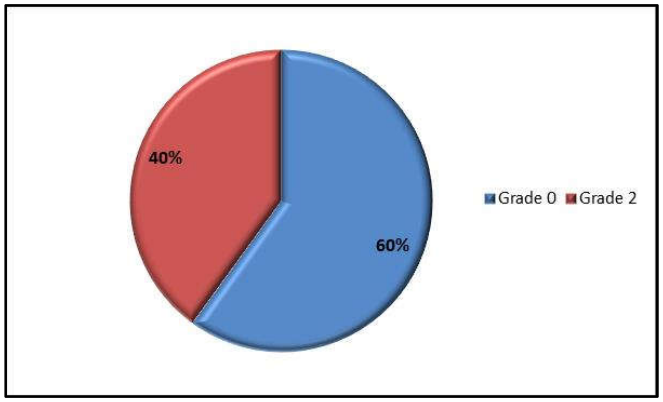

Figure 7. The variation in hepatic necrosis in valproate sodium treated group

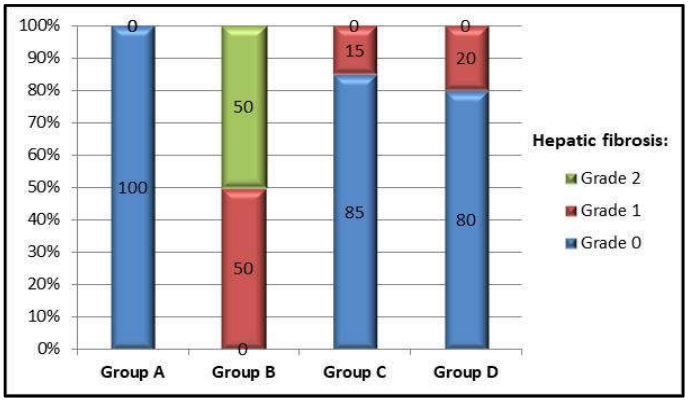

Figure 8. The variation in hepatic fibrosis according to study groups of the rats

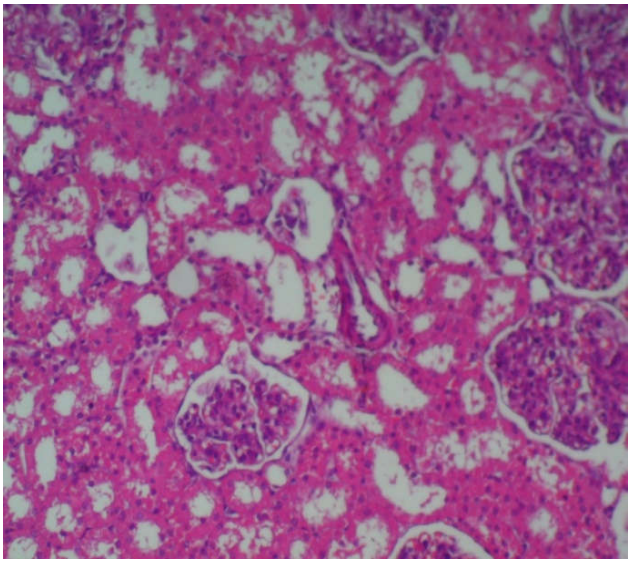

Figure 9. A micrograph for kidney from control group

Kidneys: Microscopically, kidneys of rats of the control group (Figure 9) revealed normal histological structure of renal parenchyma; the stroma showing no inflammatory cells

The glomeruli are of average cellularity. The tubules are not dilated with no degeneration or cast formation. Mild vascular congestion is seen.

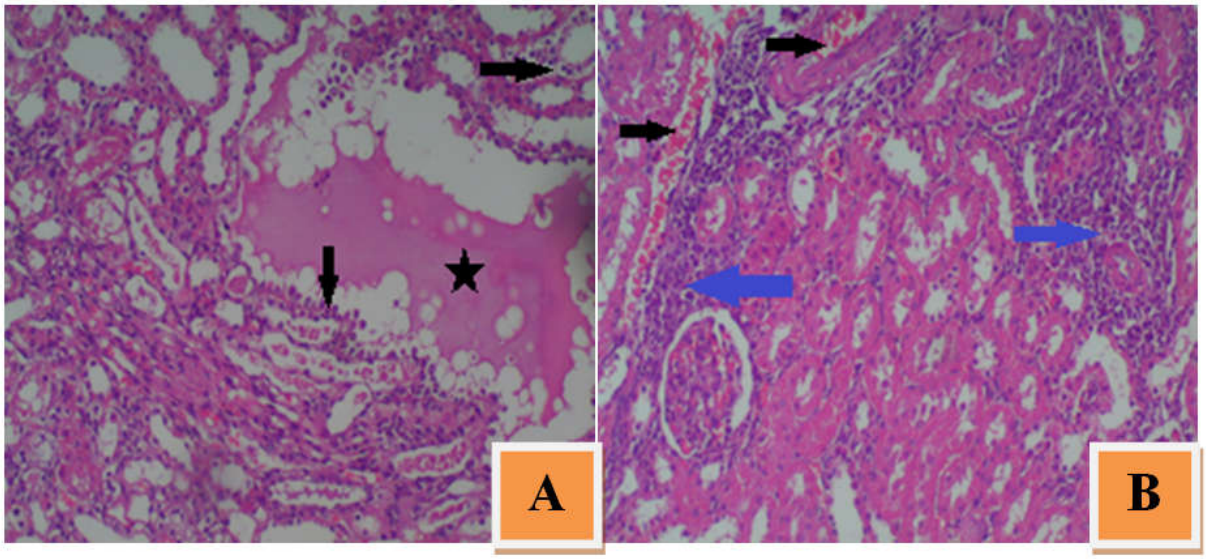

Figure 10. A micrograph for kidney from valproate sodium -treated group, (A) Showing focal vacuolar degeneration (ballooning) of some of the tubular cells (the black arrows) with marked tubular dilatation and intratubular casts (the star) (H&E X200), (B) Showing moderate vascular congestion (the black arrows), dense inflammation and fibrosis (the blue arrows) (H&E X200)

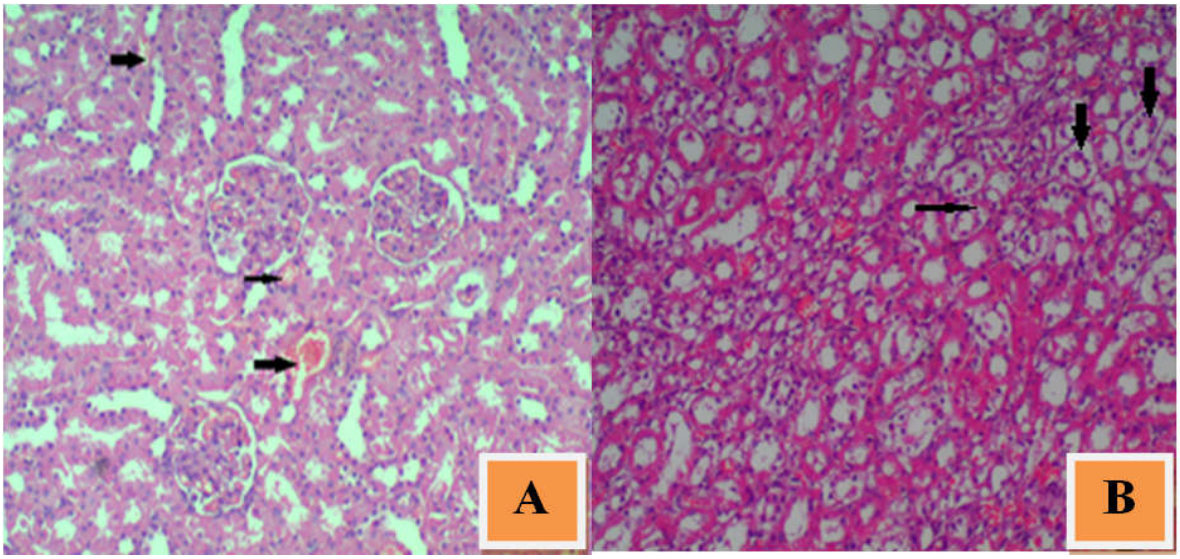

Figure 11. A micrograph for kidney from metformin -treated group showed mild changes, (A) Showing mild vascular congestion is seen (the arrows) (H&E X200), (B) Showing focal vascular degeneration (ballooning) of some of the tubular cells (H&E X200). The stroma showing no inflammatory cells aggregates. The glomeruli are of average cellularity. The tubules are not dilated with no degeneration or cast formation

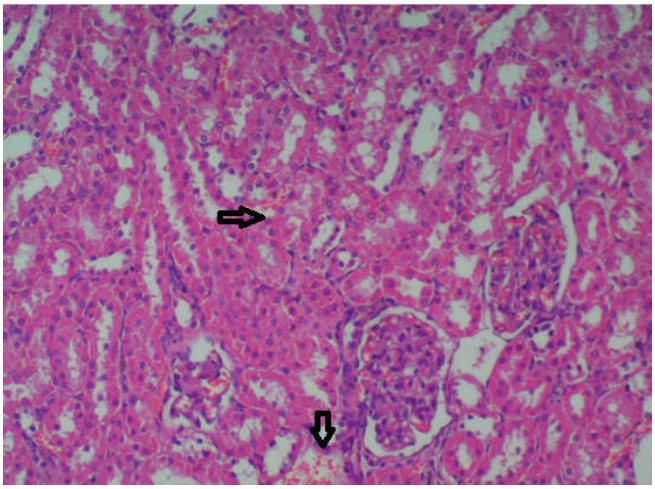

Figure 12. A micrograph for kidney from sodium plus metformin -treated group

Showing normal pattern, the stroma showing no inflammatory cells aggregates. The glomeruli are of average cellularity. The tubules are not dilated with no degeneration or cast formation. Mild vascular congestion is seen (the arrows) (H&E X200)

DISCUSSION

Valproic acid (VPA) is a medicine widely prescribed as an anticonvulsant and mood stabilizer. It has been commonly used in the treatment of epilepsy and bipolar disorder. VPA has been prescribed as anti-epileptic drugs (AEDs) over the past 40 years (Al-amoudi, 2017). Metformin is biguanide compound which has been used for the alleviation of the hyperglycemia associated with type 2 diabetes for decades. Some studies have reported that it increases peripheral glucose utilization and causes reduction of basal hepatic glucose output (Cleasby *et al*., 2004).

According to body weight, this study revealed that there was significant increase in bodyweight gain in the valproate sodium treated group with high mean of body weight gain when compared to rats of all other groups. These results agree with Luef *et al*., 2009 whoreported that weight gain is one of the most common side-effects of VPA therapy. This coincide with Stephen *et al*., 2001 who observed that VPA patients were more likely to be overweight. Also, it was demonstrated that long-term antiepileptic therapy with VPA in adults and children is associated with high body weight and body fat (El-khatib *et al*., 2007;Rauchenzauner *et al*., 2008; Sidhu *et al*. 2018). Similarly , it was noted a greater increase in mean body weight during the first year of valproate therapy (Morrell *et al*., 2008). These results disagree with Roste *et al.,* 2002 who demonstrated that there was no significant weight gain observed in any of the valproate treated rats compared to controls. According to this studythere was significant decrease in body weight gain in the metformin treated group when compared to rats of all other groups and the body weight gain of rat of valproate plus metformin treated group was within normal ranges. These results agree with Tao *et al*., 2019 who observed significant decrease in the body weights in rats after daily administration of metformin for 4 weeks. Similarly, (Meng *et al*., 2017) noted that the body weight in rats treated with metformin per day for 4 weeks were significantly lower than other groups. The mechanism of reducing body weight in metformin was reported in many studies as Lin *et al*., 2000 who suggested that metformin suppress feeding behavior and excessive caloric intake which plays a role in the genesis of obesity. Another study reported that metformin has anorexigenic effects which mediated via an increase in the central sensitivity to leptin (Aubert *et al*., 2011) which is an adipocyte-derived hormone, contributes to body weight homeostasis by regulating food intake (Kim *et al*., 2006). These resultsdisagree with (Ko *et al*., 2017) who demonstrated that the bodyweight was not significantlydifferent between the metformin-treated and control groups.

Regarding to the liver enzymes, this study showed variations to show effect of valproate sodium and compare effect of its administration alone and with administration of metformin. Firstly the variation in ALT, AST, ALP and albumin values showed the normal range in control group and metformin -treated group. The variation in ALT, AST and ALP showed higher values in group treated by valproate sodium when compared to those of the other groups (control, metformin -treated group &valproate sodium plus metformin -treated group). ALT level slightly increased in valproate sodium plus metformin group but other enzymes levels were within the normal range. Albumin levels are within normal ranges in all groups. Both aminotransferases are highly concentrated in the liver however an increase in ALT serum levels is, consequently, more specific for liver damage. ALP is a membrane associated enzyme and its increased activity is an indication of liver damage. In addition determining serum albumin levels is considered "test of liver function". This is mainly because hepatic synthesis of albumin tends to diminish in end-stage liver disease (Giannini *et al*., 2005). These results agree with Zeng *et al*., 2010 who reported mild aminotransferase elevations among patients who take VPA as epilepsy treatment and Al-amoudi, 2017 concluded that SVP treatment resulted in a significant increase in hepatic enzymes level, total-bilirubin after six weeks of administration when compared to control rats. Also, elevated aminotransferase levels are observed in bipolar disorder patients treated with VPA (Bowden *et al*., 2008).

Another supporting study noted elevated aminotransferase levels in patients after using VPA for painful diabetic neuropathy(Kochar *et al*., 2004).Similarly, Fenichel and Greene, 1985 also observed rise in serum transaminases activities and ammonia concentrations during the first three months of VPS therapy. These results disagree with Akindele *et al*., 2015 whoreported reduction in the levels of AST, ALT, ALP and bilirubin diabetic rats after treatment by valproic acid. This coincides with Lee *et al*., 2007 who noted that serum alanine aminotransferase and aspartate aminotransferase levels were not significantly altered in the experimental animals after VPS treatment. Additionally, Khan *et al*., 2015 noted that VPA treatment reduced the levels of AST, ALT and ALP when compared with diabetic rats.

The effect of metforminto reduce the effect of valproate sodium was clearly observed in the reduction of ALT, AST and ALP values in group of metformin with valproate sodium. The reduction in ALT, AST and ALP values in these cases mainly related to hepatoprotective effect of metformin. This agree with study by Hadi *et al*. 2012 who found that there is a significant reduction and restoration of the activity of serum transaminases was achieved after administration of metformin during methotrexate induced hepatotoxicity which was explained by the antioxidant properties, anti-apoptotic and anti-inflammatory effects of metformin. Similarly, this coincide with Lavine *et al*., 2011who demonstrated a decrease in ALT, AST and ALP levels over 96 weeks of treatment of metformin.

Additionally, it was reported that metformin treatment resulted in normalization of serum liver enzyme activities in mice and improved viability of hepatocytes (Lin *et al*., 2000). Another supporting study noted that metformin pretreatment in cases of acetaminophen hepatotoxicity leaded to progressively decreased plasma levels of ALT and AST (Kim *et al*., 2015).Also, Poon *et al*., 2003 reported that metformin treatment protects against hepatotoxicity induced by chronic repeated administration of carbon tetrachloride in mice as it significantly decreased the plasma ALT activity. These results disagree with Ko *et al*., 2017 who noted that the plasma levels of AST and total bilirubin were also not significantly different in rats with induced biliary cirrhosis via common bile duct ligation (CBDL) which treated by metformin.Similarly, It was reported that the group treated with metformin only

showed a non-significant decrease in the level of ALT as compared to the untreated diabetic group (Elattar *et al*., 2017). Regarding to the liver histology , this study showed that no histopathological changes occur in the control groups but the group treated by valproate sodium showed histopathological changes in the liver tissue in the form of central vein dilatation,50% of liver tissues showed mild inflammation and mild fibrosis (Grade 1 hepatic fibrosis) but the other 50% showed moderate inflammation , moderate fibrosis (Grade 2 hepatic fibrosis), many apoptotic cells, proliferated portal ducts and dilated congested blood vessels . 40% of livers of rats of the same group revealed vacuolar degeneration of hepatocytes and fatty accumulation (steatosis) with signet ring appearance of the hepatocytes and revealed spotty hepatic necrosis (Grade 2 hepatic necrosis), while 30% of livers of rats of the same group showed cloudy degeneration of the hepatocytes. These changes in liver histopathology agree with Al-amoudi, 2017 who demonatrated that the liver sections obtained from the SVP treated rats revealed clear hepatocytes disorganization, hepatocytes nuclei were shrinked, pyknotic or apoptotic, congestion of the intrahepatic blood veins, infiltrations by masses of leukocyte inflammatory cells have cytoplasmic vacuolation with pyknotic nuclei, congestion, fibrosis and bile duct necrosis around the portal tract and also, fatty infiltrations.

Additionally, the study performed by Lee *et al*., 2007 described liver injury as the most serious side effect of SVP and reported microvesicular steatosis, hepatocellular necrosis, cholestatic liver injury. It was reported intra hepatic synthesis of triglyceride rich lipoproteins and fat accumulation which leaded to development of steatohepatitis which progress to cirrhosis in 25% of patients (Luef *et al*., 2004). Another study reported that mice showed a higher incidence of hepatocellular necrosis, microvesicular steatosis and polymorphic infiltration and centrilobular necrosis after SVP treatment (Qureshi *et al*, 1985). One more supporting source of hepatocellular affection by valproic acid revealed partial distortion of liver architecture, accompanied with vacuolar degenerative changes seen focally in hepatocytes, congested portal vein and scattered focal aggregates of inflammatory cells seen in portal areas and between hepatocytes after 15 days of valproic acid treatment and revealed marked distorted hepatic architecture, scattered multifocal necrotic areas and hypertrophied nuclei with fragmented chromatin after 30 days of valproic acid treatment (Ibrahim, 2012). Another supporting study demonstrated that sodium valproate causes cell death through apoptosis in a rat liver cell (Phillips *et al*., 2003).Naviaux, 2005 noted micronodular cirrhosis and bile ductular proliferation in patient after VPS treatment. Additionally, another study reported severe microvesicular fatty change, degeneration and necrosis, extensive bile duct proliferation and massive fibrosis (Pronicka *et al*., 2011). Also, Al-amoudi, 2017 concluded that SVP treatment resulted in hepatocytes disorganization and hepatocytes nuclei were shrinked, pyknotic or apoptotic, congestion of the intrahepatic blood veins, infiltrations by masses of leukocyte inflammatory cells, cytoplasmic vacuolation with pyknotic nuclei, congestion, fibrosis and bile duct necrosis around the portal tract and fatty infiltrations. Another supporting study by Heidari *et al*., 2018 whodetected liver histopathological changes in VPA-treated groups including steatosisnecrosis and inflammation. Additionally, Akindele *et al*., 2015 noted that valproate leaded to congested vascular channels and scattered focal haemorrhages and vacuolations in many hepatocytes.

The mechanism of VPA hepatotoxicity was explained by Fenichel and Greene, 1985 whodemonasterated that theretwo kinds of hepatotoxicity may happen among people treated with valproate. One is dose-related (due to direct injury by the drug), the other is idiosyncratic. Another explanation by Pourahmad *et al*., 2012 who reported that the cytotoxic action of VPA is mediated by reactive oxygen species (ROS) formation and noted that incubation of hepatocytes with VPA also caused rapid hepatocyte glutathione depletion which is another marker of cellular oxidative stress. There are studies disagree with these results as Khan *et al*., 2015 whoreported thatVPA prevents the hepatic fibroblast activation in vitro and in vivo experiments as it exerts anti-inflammatory and anti-oxidant activity which protects the multiple organ damage in several pathological conditions. The effect of metformin on liver histology was clearly observed in this study as 85% of examined livers of rats of metformin -treated group revealed no histopathological changes but 15% showed mild inflammation and mild fibrosis (Grade 1 hepatic fibrosis). The effect of metformin in the reducing the negative side effect of sodium valproate on liver histology was clearly observed in this study as most of examined livers of rats of valproate sodium plus metformin -treated group (80%) revealed normal histological structure of hepatic lobule except 20% of them showed early fibrous expansion (Grade 1 hepatic fibrosis) and minimal inflammatory cells in the portal tracts.

These results agree with Hadi *et al*. 2012 whoreported that histopathological changes of the group treated with metformin during methotrexate induced hepatotoxicity showed significant improvement in architecture. Another supporting study reported that in rats with induced biliary cirrhosis via common bile duct ligation (CBDL) histological changes were significantly ameliorated by metformin treatmentand improved hepatic fibrosis in cirrhotic rats (Ko *et al*., 2017). Similarly, Fan *et al*., 2017 noted that metformin could decrease carbon tetrachloride (CCl4)-induced liver fibrosis. Additionally, Kim *et al*., 2015 demonastrated that Acetaminopheninduced liver damage was also ameliorated by metformin pretreatment and it gradually reduced damaged area in liver parenchyma. Another supporting study reported that metformin treatment can reverse the liver injury and prevents nonalcoholic fatty liver disease (NAFLD) progression to more severe states(Karise *et al*., 2017). The hepatoprotective effect of metformin was explained by Zheng, 2015 who reported that metformin inhibit hepatocyte and macrophage inflammatory responses in rat so it ameliorates liver inflammation. Beside that it was reported that metformin has a protective effect against oxidative stress which induces apoptosis in hepatocytes and the majority of chronic liver diseases are accompanied by oxidative stress (de la Rosa *et al*., 2015). Also, Lin *et al*., 2000 demonasterated that the therapeutic mechanism of metformin involves inhibited hepatic expression of tumor necrosis factor (TNF) α and TNF-inducible factors that promote hepatic lipid accumulation and ATP depletion.

This study disagree with Lavine *et al*., 2011 whonoted lack of hepatoprotective effect of metformin as metformin did not result in any significant histological improvements compared with placebo over 96 weeks in cases of nonalcoholic fatty liver disease in children and adolescents. Regarding to the kidney function, this study showedkidney functionwas normal in control group, metformin -treated group and valproate sodium plus metformin -treated groupwhile highly increased in valproate sodium treated group. This agrees with Al-amoudi,

2017 who reported that SVP treatment resulted in a significant increase in creatinine and urea nitrogen after six weeks of administration as compared to control rats. Another supporting study noted a significant increase in the levels of urea and creatinine in the serum of VPA-treated rats (Gezginci-Oktayoglu *et al*., 2015). Also, it was demonasterated that valproic acid-treated animals developed elevated serum creatinine and urea(Fukuda *et al*., 1996; Heidari *et al*., 2018). These results disagree with Khan *et al*., 2015who reported that VPA treatment reduced the levels of creatinine and urea compared with diabetic rats. Similarly, Altunbaşak *et al*., 2001 noted that there is no statistically significant differences in serum creatinine and urea were found between patients treated by VPA and controls. Regarding the kidney histology, this study detected no histopathological changes occur in the control group. All examined kidneys of rats of valproate sodium -treated group revealed vacuolar degeneration (ballooning) of some of the tubular cells with marked tubular dilatation and intratubular casts, 50% of the kidneys revealed moderate vascular congestion, dense inflammation and fibrosis.

These results agree with Al-amoudi, 2017 who reported that the microscopical examination of kidney cortex of the animals treatedwith SVP showed congested and enlarged renal veins and vacuolar degeneration in some tubular epithelial cells, some celldebris scattered in tubular lumina. The renal tubules showedcytoplasmic vacuolation of epithelial lining and their lumenfilled with proteinaceous casts. Also, edematouslesion was observed between the tubules. The renal tubules appeared severely injured andthe glomeruli were fragmented and degenerated. There is another supporting study reported that VPA treatment leaded to architectural disruptions with focal cystic lesions in the proximal tubules, irregularities of epithelial cells and basement membranes. The interstitium had moderate fibrosis and marked infiltration of mononuclear cells (Fukuda *et al*., 1996).Another reported case of renal injury after valproate sodium treatment revealed infiltration of the interstitium by numerous lymphocytes and macrophages, and mild interstitial fibrosis, that was associated with diffuse tubular atrophy with loss of the proximal tubular brush border (Yoshikawa *et al*., 2002).Additionally, Akindele *et al*., 2015 noted that valproateleaded to congestion of glomerular capillaries with vacuolations in proximal convoluted tubular cells.

Many studies noted the mechanism of sodium valproate nephrotoxicity as Heidari *et al*., 2018 andGezginci-Oktayoglu *et al*., 2015who reported thatoxidative stress developed in renal tissue after VPA administration and it elevated kidney Reactive oxygen species(ROS) levels, reduced tissue antioxidant activity, increased lipid peroxidation and depleted renal glutathione stores. Moreover, they found that renal mitochondrial function was impaired in VPA-treated animals. Beside that Altunbaşak *et al*., 2001 found increased urinary Malondialdehyde (MDA) excretion in children on VPA therapy. MDA is a highly reactive aldehyde and it is formed from the peroxidation of polyunsaturated fatty acids due to the effect of oxygen free radicals and it forms covalent bounds with proteins, phospholipids and DNA, which leads to renal damage. In addition to another explanation by Fukuda *et al*., 1996 who reportd a case of hypersensitivity to VPA. These results disagree with Heidari *et al*., 2018 whofound no sign of kidney tissue fibrosis in VPA-treated animals. Similarly, Zhang *et al*., 2009 who observed that VPA treatment did not induce renal lesions in any segment of the cortex or the medulla. Also, Kawaoka *et al*., 2017 reported that VPA may be used for the treatment of renal fibrosis. The effect of metformin in the reducing the negative side effect of sodium valproate on kidneys function and histology was clearly observed in this study as kidneys function in sodium valproate plus metformin –treated were within normal ranges and all examined kidneys revealednormal pattern, the stroma showed no inflammatory cells aggregates, the glomeruli are of average cellularity, the tubules are not dilated with no degeneration or cast formation and mild vascular congestion is seen. These results agree with Kopaei *et al*., 2013 whoreported thatmetformin have curative and protective activity against gentamycin nephrotoxicity as the levels of urea and creatinine in group which received metformin were significantly lesser than group which receive gentamycin. Similarly, Amini *et al*., 2012 demonstrated that post-treatment with metformin or co-treatment with metformin could prevent the elevation of serum urea and creatinine induced by gentamycin and also attenuates the damage. Another supporting study noted that light-microscopic examination of kidneys from metformin-treated rats showed no structural alterations in renal tissues in rats treated with gentamycin (Morales *et al*., 2010).

Many studies explained the mechanism of nephroprotective effect of metformin as Kim *et al*., 2012 who reported that metformin has antioxidant effect through reducing ROS levels while the overproduction of ROS is an important mechanism for oxidative stress which causes cell death. These results disagree with Sahu *et al*., 2013 whonoted that metformin treatment did not show any significant protection against cisplatin-induced renal damage as blood urea nitrogen and serum creatinin levels were significantly elevated in cisplatin and were also elevated in metformin plus cisplatin group. Beside that Cisplatin rats showed prominent multiple tubular necrosis, degeneration, inflammatory cell infiltration, vacuolization and loss of architecture of tubules while in metformin plus cisplatin group metformin did not protect the kidney as evidenced by the presence of dilated tubules with degenerated epithelium vacuolated cells and lumen filled with esinophilic materials.

Conclusion

Long term use of valproate sodium in albino rats produces hepatotoxicity and nephrotoxicity.Metformin restored the altered liver and kidney function and possessed hepatoprotection and nephroprotective effect against valproate sodium toxicity.

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