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Anti-inflammatory and protective effects of royal jelly against hepatic and renal damage induced by valproic acid in rats

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Genet. Mol. Res. 22 (1): gmr19063
Received May 10, 2022
Accepted December 21, 2022
Published January 18, 2023
DOI http://dx.doi.org/10.4238/gmr19063

ABSTRACT. Valproic acid (VPA) is a drug that is often used to treat epilepsy, seizures, and similar diseases. However, it is known to have serious toxic effects on the liver and the kidney. Oxidative stress and other metabolites of VPA have been suggested to be responsible for VPA induced hepatotoxicity and nephrotoxicity. We evaluated the possible protective role of royal jelly (RJ) against the effects of VPA through toxicity tests on livers and kidneys of rats. Twenty-four male albino rats were separated into three groups; group (1): healthy control received no drug, group (2): administrated VPA (500 mg/kg/day by oral gavage), group (3): received VPA (500 mg/kg/day by oral gavage) one hour prior to RJ (500 mg/kg/day by oral gavage). After two weeks, the rats' livers and kidneys were removed for histopathologic investigation with hematoxylin and eosin staining while biochemical assessment was performed on blood samples. The VPA group had a significant increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine and pro-inflammatory cytokines (IL-1a, IL-1b and IL-6). Histopathological observations in liver and kidney tissues also were related with the biochemical parameters. VPA causes hepato-renal

damage by promoting inflammation, oxidative stress, and fibrosis. RJ enhanced the functions of the liver and kidneys by reducing ALT, AST, urea and creatinine compared with the VPA treatment group and reduced serum pro-inflammatory cytokines. In addition, the histopathological impairment of liver and kidney tissues were reversed by RJ treatment. In conclusion, RJ can protect hepato-renal functions against VPA acid-induced organ damage.

Key words: Valproic acid; Royal jelly; Hepatotoxicity; Nephrotoxicity; Cytokines

INTRODUCTION

With the popular names Depakine and Valpakine, the valproic acid (VPA) salt is sodium valproate. It is one of the oldest and most commonly administered epilepsy treatments, and it is also used for migraine and bipolar disorder prevention (Peterson and Naunton, 2005). Additionally, it used in the management of a range of neuropsychiatric diseases, including mania, prophylaxis, and migraine, among other seizure illnesses (Perucca, 2002; Rosenberg, 2007). Furthermore, VPA treatment is linked to significant side influences such as hepatotoxicity, platelet aggregation, thrombocytopenia and pancreatitis (Ibrahim, 2012). The liver is a target organ for antiepileptic medication metabolism and it is susceptible to drug-induced harm (Ardianto et al., 2020). VPA administration is correlated to liver failure. Hepatotoxicity is believed inhibit hepatic mitochondrial β-oxidation in addition to the damaging effects of unsaturated VPA-derivates (Wafa et al., 2015). VPA-induced hepatotoxicity can also be caused by reactive oxygen species (ROS) causing the release of free radicals, a well-documents oxidative stress mechanism (Tong et al., 2005; Kiang et al., 2010). The major mediator of VPA toxicity, according to various studies, is reactive oxygen species (ROS). For example, neurotoxicity (Auinger et al., 2009), liver injury (Tong et al., 2005), teratogenicity (Tung and Winn, 2011), and kidney injury have all been linked to VPA-induced ROS production (Chaudhary et al., 2015). Redox signaling regulates inflammation (Lei et al., 2014), and inflammation causes fibrosis (Eddy, 2000). To date, however, there has been no clear-cut evidence on the pathogenic mechanism of VPA-induced kidney damage. Additionally, Pourahmad et al. (2012) and Sokmen et al. (2012) stated a decrease of hepatocyte glutathione (GSH) stores in experimental animals treated with VPA. For the reason that oxidative stress seems to have a significant role in VPA-induced hepatotoxicity, various natural products have been employed to reduce hepatic injury produced by VPA-induced oxidative damage, owing to their antioxidant properties and ability to scavenge free radicals (Jurima-Romet et al., 1996; Sokmen et al., 2012; Abdella et al., 2014). The concentration of liver enzymes (ALT, AST, ALP, LDH and GGT) are known to rise after induction of damage (Englund et al., 2011). Hematological toxicity, idiosyncratic hepatotoxicity, teratogenicity, obesity, and significant endocrine dysfunctions are all possible side effects of VPA medication (Verrotti et al., 2005; Dutheil et al., 2008; Zhang and Wang, 2009). In the kidneys, due to electrolyte imbalances, VPA poisoning damages renal tubules, resulting in acute renal failure and proximal tubular damage and necrosis (Hawkins and Brewer, 1993; Raza et al., 1997; Pourahmad et al., 2012; Jafarian et al., 2013). Galaly et al. (2014) also found significant histological

alterations in the kidney of mice treated with VPA. Renal corpuscles and tubules both showed these alterations. Glomerular atrophy, mesangial proliferation, and corpuscular adhesion with glomerular tufts were the most obvious symptoms of renal corpuscle destruction. The influence of VPA on modulating the immune system is also evident (Loscher, 1993), includes the level of serum immunoglobulin has been shown to be decreased (Joubert et al., 1977; Fujiwara et al., 1983; Garzon et al., 1985; Lenti et al., 1991). Furthermore, VPA was shown to enhance the plaque-creating cells count in the spleen and increases the risk of bacterial contamination (Queiroz and Mullen, 1992). The role of VPA in the expression of inflammatory cytokines and mediators has been controversy. According to Ichiyama et al. (2000), VPA inhibited the creation of pro-inflammatory mediators including the tumor necrosis factor alpha (TNF-a) and (IL-6) encouraged by lipopolysaccharide (LPS) in patients monocytic leukaemia cells and this suppression was related to the inhibition of nuclear transcription factor kappa B (NF-kB) stimulation. In human glioma cells, same inhibitory impact was also observed (Ichiyama et al., 2000). By contrast, in kids and youths with epilepsy, Verrotti et al. (2001) demonstrated that chronic VPA usage caused an important rise in the release of IL-1a, IL-1b, IL-6 and monocyte chemoattractant protein-1 (MCP-1). These findings were supported by the observations of Pacifici et al. (1995) in mature epileptic patients, but were not demonstrated by De Sarro et al. (1998) in similar patients.

Royal jelly (RJ) is a natural material that has been utilized as a therapeutic product for the treatment of many diseases and health promotion since ancient times due to its beneficial effects and minimal side effects (Khan, 2018; Vida et al., 2019). It is a creamy substance produced by the nurse bees' mandibular and hypopharyngeal glands for the sustenance of young larvae and queen honey bees (*Apis mellifera*) (Viuda-Martos et al., 2008). RJ is rich in a range of proteins, carbohydrates, and lipids, in addition to free amino acids (FAA), vitamins, minerals and trace salts (Ahmed et al., 2014). In previous studies, RJ has demonstrated anti-inflammatory properties (Karaca et al., 2012; Karaca et al., 2015), anti-oxidant (Viuda-Martos et al., 2008; Abdel-Hafez et al., 2017), anti-tumor (Vucevic et al., 2007; Zhang et al., 2017), anti-microbial (Chan et al., 2009), anti-allergic (Oka et al., 2001), anti-hypercholesterolemic (Ibrahim, 2014), and immunomodulatory (El-Nekeety et al., 2007; Gasic et al., 2007) properties. Inoue et al. (2003) showed that dietary RJ increased the average life span of C3H/HeJ mice, apparently through the mechanism of decreased oxidative injury. The aim of this study was to investigate the potential protective role of RJ against the toxic effects of VPA for the liver and kidney.

MATERIAL AND METHODS

Experimental animals

Twenty-four male albino rats weighing between 140 and 160 g were used in this research. They were supplied by King Abdul-Aziz University's main animal facility in Jeddah, Saudi Arabia. Animals were acclimatized for two weeks in the animal facility of Taibah University prior to experimentation. All rats were kept in standard circumstances in wire-covered plastic cages, including a temperature of (25±2°C), a 12/12 hour light/dark cycle, and enough ventilation. They were given water and standard balanced rat diet ad libitum. Every effort was made to avoid inflicting pain.

Drugs and chemicals

The medication sodium valproate (VPA) was acquired in syrup form from El-Nahdi pharmacy (Almadinah Almonawarah, Saudi Arabia) under the brand name Depakine (convulex®, made by Sanofi Aventis (France). Convulex syrup contains 200 mg sodium valproate per 1 mL. The RJ was purchased from the Natural Remedy Medical Pharmacy (Almadinah Almonawarah, Saudi Arabia). Made from fresh RJ, each capsule contains 1000 mg of natural Royal Jelly® in a base of soya oil and soya lecithin (Manufactured by: Martinez Nieto, S.A., Cartagena, Spain R.S. No. 26.861/MU). The RJ was diluted with distilled water to adjust the dose given for each rat according to weight.

Dose and treatment procedure

The VPA dose was 500 mg/kg body weight (Niaraki et al., 2013; Gezginci-Oktayoglu et al., 2016). This dose was given orally every day for 14 days by gastric intubation. The dose of RJ used was 500 mg/kg body weight (Zhang et al., 2017) For 14 consecutive days, these doses were delivered orally through gastric intubation.

Experimental grouping

The rats were separated into three groups, with eight rats in each group, as follows:

The 1st group: served as a healthy control group and received no treatment.

The 2nd group: given VPA orally at a dose of (500 mg/kg b. wt.) daily for 14 days (Niaraki et al., 2013; Gezginci-Oktayoglu et al., 2016).

The 3rd group: given VPA orally at a dose of (500 mg/kg body weight) daily for 14 days (Niaraki et al., 2013; Gezginci-Oktayoglu et al., 2016), and after one hour from VPA administration then treated with RJ orally at a dose of (500 mg/kg body weight) daily for 14 days (Zhang et al., 2017).

Ethical considerations

This study was carried out in accordance with the recommendations for the care and the use of laboratory animals. Every effort was made to reduce the quantity of used animals as well as their suffering. The research project received the approval of the Ethical Committee for Animal Research of Taibah University, KSA (IRB Reg. No. COPTU-RIC 20210113), which approved the protocol for this study.

Biochemical study

Diethyl ether was used to anesthetize the animals, and blood was obtained from the retro-orbital venous plexus 24 hours following the most recent treatment. Then blood was collected and left to coagulate for 40 minutes at room temperature. For biochemical analysis, sera were separated by centrifugation at 5000 rpm for 20 minutes at 20°C and then frozen at -20°C. The levels of urea, creatinine, (AST), and (ALT) were then measured with commercially available kits (Muslco SJ, Saudi Arabia).

Evaluation of pro-inflammatory interleukins

Evaluation of pro-inflammatory cytokine (interleukins) such as some pro-inflammatory markers (IL-1a, IL-1b and IL-6) was tested in serum using ELISA (enzyme-linked immunosorbent assay kits), as directed by the manufacturer.

Histopathological investigation

Additional liver and kidney tissue sections were merged in 10% buffered formalin, wash away with tap water, and dehydrated in various dilutions of 100% ethanol. Microtome sections of paraffin wax tissue with a thickness of 4 microns were created. Hematoxylin and eosin was used to stain the tissue sectors after they were placed on glass slides before being studied under a microscope for histological purposes (Bancroft and Gamble, 2002).

Statistical study

The data were analyzed by one-way ANOVA, after that post hoc multiple comparisons LSD's test using the SPSS statistical package v22.0 for Windows (IBM, Armonk, NY, USA). P values less than 0.05 were regarded as statistically significant differences. The analyzed data are presented as the mean ± standard error of mean (SEM).

RESULTS

The influence of RJ on serum liver (AST, ALT) and kidney (urea and creatinine) concentration in VPA-induced hepato-renal injury in rats.

In rats, VPA (500 mg/kg/day, P.O.) caused liver damage, as demonstrated by significant increases in AST and ALT to 22.93% and 15.81 %, respectively, versus the typical control group. When compared to the VPA - control group, RJ (500 mg/kg/day, P.O.) significantly decreased high serum AST to 19.34 % and reduced elevated serum ALT to 16.69 % (Table 1 and Figures 1 and 2).

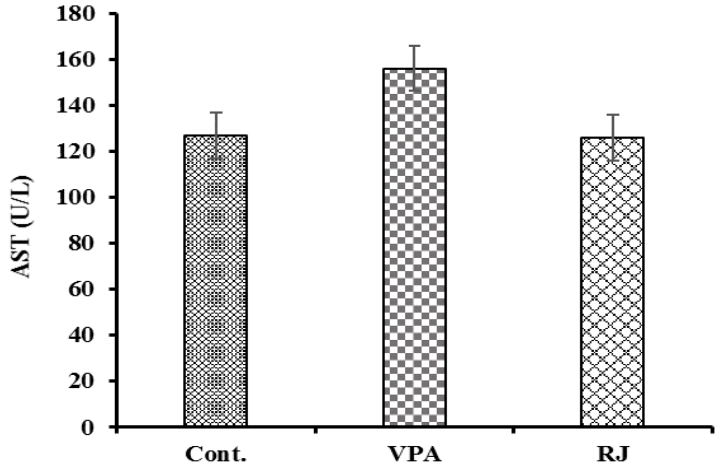

Figure 1. The influence of royal jelly (RJ) on valproic acid (VPA)-treated albino rat serum AST activity.

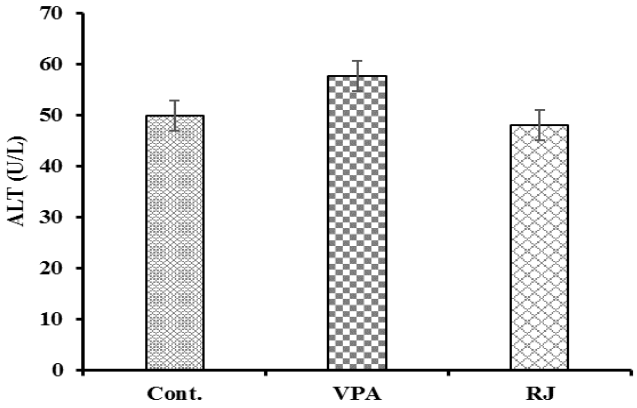

Figure 2. The influence of royal jelly (RJ) on valproic acid (VPA)- treated albino rat serum ALT activity.

Table 1. Effects of royal jelly on serum AST, ALT, creatinine and urea levels in valproic acid (VPA)-induced hepato-renal injury in rats.

Groups	Hepatic function test		Renal function test	
	AST (U/L)	ALT (U/L)	Creatinine (mg/dL)	Urea (mg/dL)
Normal control	127 ± 1.13	49.85 ± 0.604	85.65 ± 1.04	46.24 ± 0.810
Valproic acid-control (500 mg/kg, P.O)	156.12 [a] ± 2.24	57.73 [a] ± 0.740	107.65 [a] ± 0.850	54.8 [a] ± 0.998
Valproic acid + RJ (500 mg/kg, P.O.)	125.92 [b] ± 1.01	48.1 [b] ± 1.18	83.06 [b] ± 0.633	42.53 [b] ± 0.889

Data is shown as mean ± SEM. [a]Significantly different from the normal control group at $p < 0.05$ (LSD test). [b]Significantly different from the valproic acid -control group at $P < 0.05$ (LSD test).

In rats, VPA caused kidney impairment, as demonstrated by significant increases in serum urea and creatinine to 20.7 % and 25.68 %, respectively, as compared to the normal control group. Urea and creatinine levels in the blood are recognized indicators of severe renal damage (Mehta et al., 2007; Bonventre et al., 2010). When compared to the VPA - control group, RJ reduced raised serum urea by 22.39 % and reduced elevated serum creatinine by 22.84 %, respectively (Table 1) and (Figures 3 and 4).

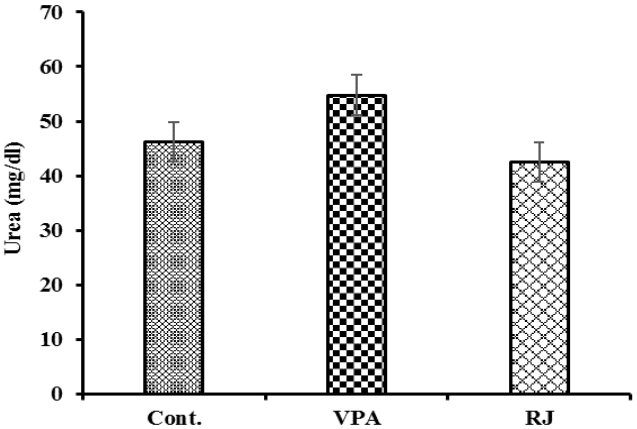

Figure 3. The influence of royal jelly (RJ) on valproic acid (VPA)- treated albino rat serum urea concentration.

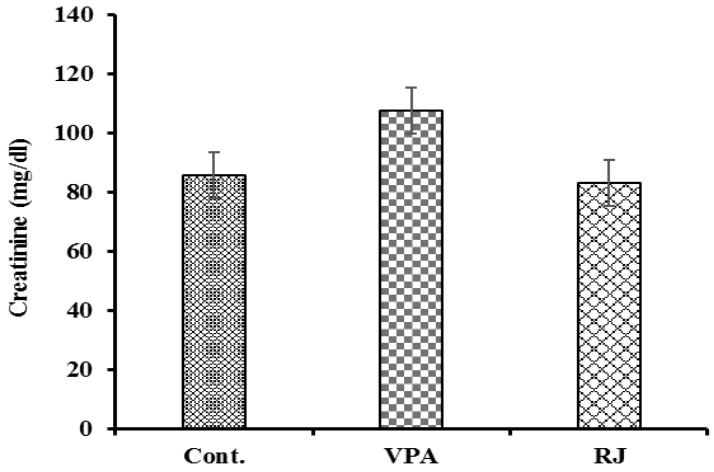

Figure 4. The influence of royal jelly (RJ) on valproic acid (VPA)- treated albino rat serum creatinine concentration.

The influence of RJ on serum pro-inflammatory cytokines level in VPA-induced hepato-renal injury in rats.

The amount of pro-inflammatory cytokine (IL-1a, IL-1b and IL-6) in serum of VPA -treated rats revealed a significant increment ($P < 0.05$) as compared with controls. However, VPA (500 mg/kg/day, P.O.) resulted in the significant elevation of the amount of pro-inflammatory cytokines (IL-1a, IL-1b and IL-6) in serum to 95.78%, 106.8% and 8.4% respectively in comparison to the typical control group. Combined treatment with RJ (500 mg/kg/day, P.O.) significantly reduced ($P < 0.05$) the elevated level of pro-inflammatory cytokines (IL-1a, IL-1b and IL-6) in serum to 15.66%, 23.41% and 22.64% respectively in comparison to VPA - control group (Table 2) and (Figures 5, 6 and 7).

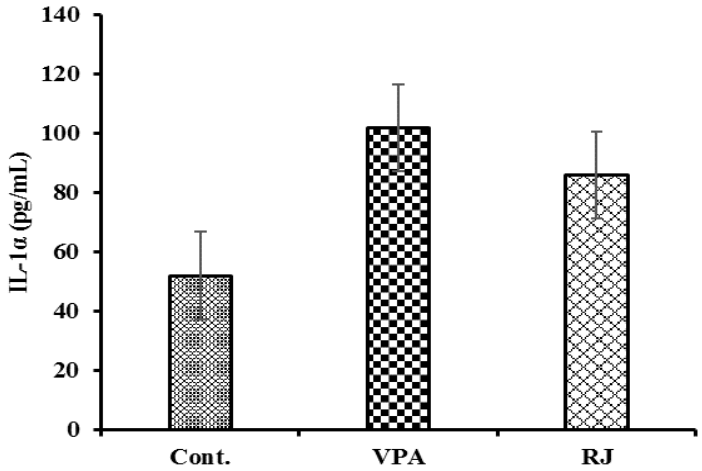

Figure 5. The influence of royal jelly (RJ) on valproic acid (VPA)-treated albino rat serum IL-1α levels.

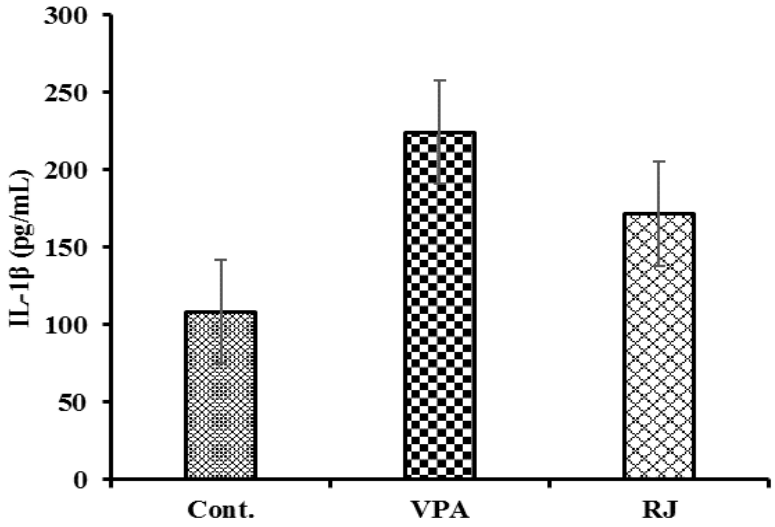

Figure 6. The influence of royal jelly (RJ) on valproic acid (VPA)- treated albino rat serum IL-1β level.

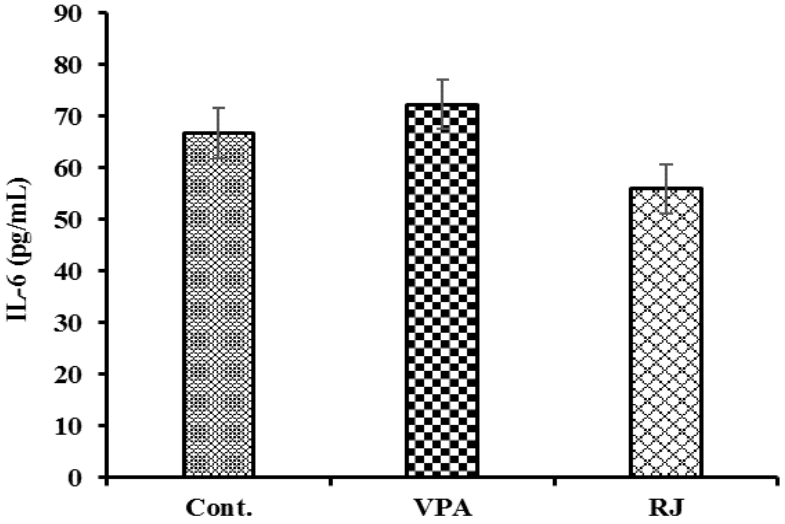

Figure 7. The influence of royal jelly (RJ) on valproic acid (VPA)- treated albino rat serum IL-6 level.

Table 2. Effects of royal jelly (RJ) on serum pro-inflammatory cytokines levels in valproic acid-induced hepato-renal damage in rats.

Groups	IL-1a (pg/mL)	IL-1b (pg/mL)	IL-6 (pg/mL)
Normal control	51.98 ± 0.355	108.52 ± 0.451	66.72 ± 0.194
Valproic acid-control (500 mg/kg, P.O)	101.77 [a] ± 0.349	224.42 [a] ± 0.282	72.32 [a] ± 0.225
Valproic acid + RJ (500 mg/kg, P.O.)	85.84 [b] ± 0.210	171.87 [b] ± 0.258	55.95 [b] ± 0.284

Data is shown as mean ± SEM. [a]Significantly different from the normal control group at $p < 0.05$ (LSD test). [b]Significantly different from the valproic acid -control group at $p < 0.05$ (LSD test).

Histopathological examination of hepatic and renal tissues

The normal control rats' hepatic sections show a normal histological image, with normal architecture, no disruption in the central vein, and no alterations in the architecture of the sinusoids and liver cells (Figure 8a). Hepatic section from VPA-treated rat shows different hepatic lesions; blood congestion, aggregation in the portal region of inflammatory cells (Figure 8b), highly congestion of central vein as well as hepatic sinusoids that appear dilated (Figure 8c), ballooning and vacuolar (cloudy) degeneration of hepatocytes and dilated sinusoid (Figure 8d). For the toxic VPA group treated with RJ reveals good recovery of the general histopathological image and liver looks normal (Figure 8e).

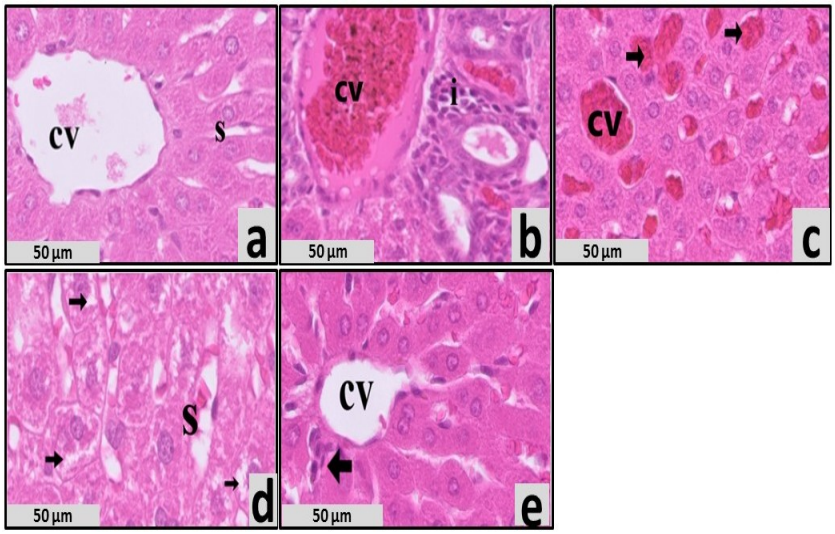

Figure 8. H & E-stained photomicrographs of the livers of control, valproic acid (VPA), and royal jelly (RJ)-treated rats showing: a. Normal liver viewing normal architecture of central vein (cv) with hepatic chords radiating and sinusoid (s). b. Liver from valproic acid (VPA) treated group showing different hepatic lesions; blood congestion, aggregation of inflammatory cells (i) in the portal area. c. Liver from valproic acid (VPA) treated group showing a highly congested central vein as well as hepatic sinusoids that appear dilated (arrows). d. Liver from valproic acid (VPA) treated group showing ballooning and vacuolar (cloudy) degeneration of hepatocytes (arrows) and dilated sinusoid (s). e. Liver looks normal, improvement of hepatocytes, central vein (cv), blood sinusoids and few inflammatory cells (arrow) are observed of valproic acid (VPA) plus royal jelly (RJ) treated rats.

The renal sections of the normal control rats show normal histopathological picture, the glomerulus and renal tubules (Figure 9a). Renal section from VPA- treated rat shows dilated Bowman's space, almost all renal tubules show a degree of deterioration (Figure 9b), some of them shows amorphous eosinophilic casts as well as inter-tubular capillary congestion (Figure 9c) For the toxic VPA group treated with RJ reveals good recovery of the general histopathological image and kidney looks normal appearance (Figure 9d).

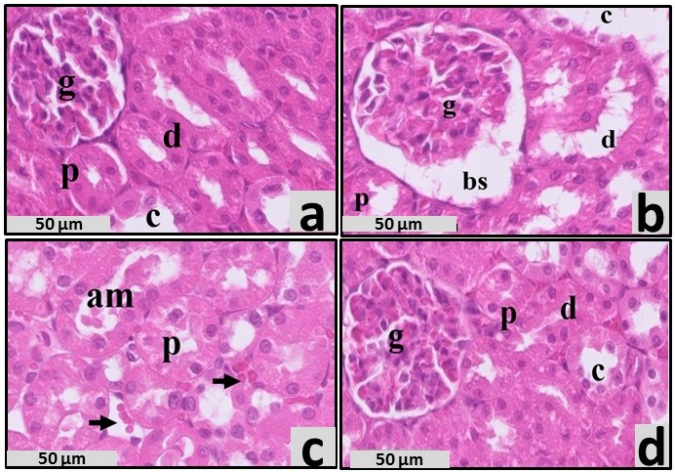

Figure 9. H & E-stained photomicrographs of the kidneys of control, valproic acid (VPA), and royal jelly (RJ)-treated rats showing: a. Normal kidney showing normal glomerulus (g), proximal (p), distal (d) and collecting (c) renal tubules. b. Kidney from valproic acid (VPA) treated group showing dilated Bowman's space (bs), almost all renal tubules show a degree of deterioration. c. Kidney from valproic acid (VPA) treated group showing some of renal tubules revealed amorphous eosinophilic casts as well as inter-tubular capillary congestion (arrow). d. Kidney with normal appearance, improvement of glomerulus (g), proximal (p), distal (d) and collecting (c) renal tubules are observed of valproic acid (VPA) plus royal jelly (RJ) treated rats.

DISCUSSION

VPA is a popular choice as an antiepileptic agent. Despite being effective in this regard, VPA is also known to have minor to severe adverse effects sometimes leading to death (Baran and Akkus, 2004). Natural products such as RJ was hypothesized to have protective effects against the biochemical and histological changes caused by VPA. Several biochemical/molecular mechanisms have been proposed to explain VPA toxicity, includes interactions with the metabolic pathways for folate methionine (Dawson et al., 2006), inhibition of histone deacetylase activity (Eikel et al., 2006), modulation of gene expression (Okada et al., 2005), lipoxygenases or the prostaglandin synthetase oxidation pathway to produce reactive intermediates (Miranda et al., 1994), changes in antioxidant enzymes (Graf et al., 1998) or a rise in the synthesis of VPA toxic metabolites (Baran and Akkus, 2004) such as 2, 4-diene-VPA, which can disrupt glutathione homeostasis by forming glutathione conjugates (Tabatabaei and Abbott, 1999). Tabatabaei et al. (1997), also showed that an induction of oxygen free radical flux during VPA metabolism causes DNA damage in nucleus, which results in cell death. Additionally, free radicals perform an essential regulatory mediator in cellular signaling cascades. Overproduction of free radicals was also correlated to oxidative stress in epileptic people administrating VPA (Martinez-Ballesteros et al., 2004; Tong et al., 2005). Chronic use of VPA also affects the kidneys causing nephrotoxicity in rats (Raza et al., 2000; Chaudhary et al., 2015) as well as in patients (Watanabe et al., 2005; Dhillon and Högler, 2011). VPA can also affect the levels of hepatic enzymes, elevating lipid peroxidation, impairing the antioxidant function, and causing pathohistological changes (Tanvir et al., 2015). Regarding oxidative stress, hydrogen peroxide and hydroxyl radicals were shown to be produced during VPA metabolism (Tabatabaei and Abbott, 1999). In accordance with former studies, VPA

treatment resulted in hepatic and renal damage, as evidenced by increases in serum AST, ALT, urea and creatinine. The damaged hepatocytes and bile duct development shown in this study may explain the increase in AST and ALT (Okdah and Ibrahim, 2014). This is in contrast with the study by Omidipour et al. (2021) who reported that the group injected with VPA (500 mg/kg/day) for 14 days show lower amounts of liver enzymes ALT and AST and higher amount of GGT levels. According to Ibrahim (2012), VPA was shown to induce damage to mice liver tissues, as evident by vascular congestion and degeneration, hypertrophied nuclei with irregular chromatin, and inflammation. Another study showed similar histopathological changes in the liver and kidney of animals administrated with VPA (500 mg/kg/day) for 15 days including microvesicular steatosis, changes in cell architecture, hepatic necrosis, tubular disturbance in kidneys, especially in the glomerular and proximal tubules, in addition to the mononuclear cells infiltration and the gathering of fibers in necrotic zones (Gezginci-Oktayoglu et al., 2016). These findings were also confirmed by several previous investigations (Raza et al., 1997; Raza et al., 2000; Natarajan et al., 2006; Raza et al., 2006; Thaakur and Chandravadana, 2008; Amrani et al., 2013; Gezginci-Oktayoglu et al., 2016). In the current study, the injection of VPA (500 mg/kg/day, P.O.) for 14 days produced a considerable rise in liver enzymes, including AST, ALT and renal dysfunction including urea and creatinine. These findings demonstrated that VPA treatment caused substantial hepatic membrane damage, consistent with previous research (Giridharan et al., 2017; Adeyemi and Olayaki, 2018).

RJ has been demonstrated to have antioxidant properties (Abdel-Hafez et al., 2017) in addition to anti-inflammatory properties (Karaca et al., 2012). In our hands, oral RJ (500 mg/kg/day, P.O.) administration for 14 days significantly lowered serum ALT, AST, urea, and creatinine concentration in VPA-administrated animals. This can be because of reduced liver enzyme leakage through the blood, preserved liver cells membrane integrity, and/or hepato-renal protective effects. RJ has indeed been shown to have a protective impact on hepatic tissue in previous investigations (Kanbur et al., 2009; Cemek et al., 2010). The RJ and honey's antioxidant effect as a free radical scavenger significantly reversed the alterations in liver enzymes brought on by cisplatin injection. (Silici et al., 2011; Bhalchandra and Alqadhi, 2019). Additionally, oral administration of RJ caused a considerable enhancement in the histological parameters of the hepatic and renal tissues in contrast to the VPA-control group. This was agree with the biochemical and immunohistochemical results discussed earlier. Furthermore, VPA administration has been shown to increase serum urea and creatinine levels (Fukuda et al., 1996; Watanabe et al., 2005). Most of these results are critical signs of kidney impairment in individuals given this medicine, indicating the urgent need for protective agents. According to other research, RJ was able to restore renal function in the case of cisplatin-caused kidney injury (Karadeniz et al., 2011; Ibrahim et al., 2016) and diclofenac-induced nephrotoxicity (Mostafa et al., 2020). VPA-induced kidney histopathological impairment was reversed by RJ treatment, which was consistent with the biochemical and immunohistochemical results and with previous reports (Ghanbari et al., 2015). On the other hand, El-Nekeety et al. (2007) demonstrated that the glomeruli and renal tubules of rats injected with fumonisin with RJ had nearly normal architecture. Furthermore, in rats given cisplatin as part of the RJ prophylaxis, necrosis and lymphocytic infiltration were reduced, and RJ treatment resulted in less degenerative changes and an improvement in kidney oxidative markers.

Inflammation is related to greater levels of reactive oxygen species (ROS). Pro-inflammatory cytokines, for example TNF-α and IL-1β, cause macrophages and neutrophils to produce reactive oxygen species (ROS) (Lei et al. 2014). VPA-activated tubules, according to Gezginci-Oktayoglu et al., (2016), yields MCP-1 to increase mononuclear cell penetration and differentiation into macrophages, which then release pro-inflammatory cytokines such as TNF-α and IL-1β. This situation is entirely consistent with previous findings (Eddy, 2000). Levels of pro-inflammatory cytokines are indicative of an ongoing inflammation (Jekarl et al., 2013). In acute and chronic liver pathology, serum levels of IL-6 were shown to be increased (Streetz et al., 2003). Additionally, elevated amounts of pro-inflammatory cytokines have been seen in serum and cerebral fluid samples from epileptic patients (Sinha et al., 2008). As per the present study, the level of pro-inflammatory cytokines (IL-1α, IL-1β and IL-6) rise significantly in the serum of VPA-treated animals. These results are in harmony with prior findings by Verrotti et al. (2001) who demonstrated that the VPA-treated individuals had an increased expression of serum IL-1α, IL-1β and IL-6. On the other hand, Shiaha et al. (2005) and Leu et al. (2016) showed that VPA treatment could increase the serum levels of IL-6. Furthermore, Sonmez et al. (2013) and Tsyvunin et al. (2020) investigations showed that VPA treatment can result in boosting IL-1α levels. Another body of evidence revealed a significant lowering in the serum level of IL-1β (Sonmez et al., 2013; Zheng et al., 2014; Himmerich et al., 2014; Amirzargar et al., 2017 and Mairuae and Cheepsunthorn, 2018) and IL-6 (Sonmez et al., 2013; Zheng et al., 2014 and Himmerich et al., 2014; Seet et al., 2019; Liu et al., 2020). Interestingly, the co-administration of RJ and VPA, significantly lowered the levels of these cytokines (IL-1α, IL-1β and IL-6) when compared to VPA-control rats. In this context, and in support to biochemical and histological findings, the supplementation of RJ prevented VPA-induced inflammation as demonstrated by the decreased levels of IL-1α, IL-1β and IL-6 in blood serum. These findings showed that RJ mediated suppression of inflammation plays a central role in their hepato-renal protective effects.

CONCLUSIONS

We conclude that RJ defends against VPA-induced hepatic and renal injury by improving biochemical parameters such as AST, ALT, urea, and creatinine, as well as preventing soft tissue destruction, oxidative stress, and inflammation in relation to VPA treatment. The results are in line with improvement of histological abnormalities in the liver and kidney. Therefore, RJ could be used as an adjuvant remedy to protect against VPA induced toxicity and inflammatory damage.

LIST OF ABBREVIATIONS

VPA: Valproic acid
RJ: Royal jelly
ALT: Alanine aminotransferase
AST: Aspartate transaminase
ALP: Alkaline Phosphatase
GGT: Gamma glutamyl transferase
LDH: Lactic dehydrogenase

LPS: Lipopolysaccharides
IL-1α: Interleukin 1 alpha
IL-1β: Interleukin 1 beta
IL-6: Interleukin 6
ROS: Reactive Oxygen Species
GSH: Glutathione
TNF-α: Tumour necrosis factor alpha
NF-kB: Nuclear transcription factor kappa B
MCP-1: Monocyte chemoattractant protein-1
FAA: Free amino acids
P.O: Per oral

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ACKNOWLEDGMENTS

The author would like to thank Dr. Tarek bin Ziad Abo Lebda the Dean of Faculty of Science, Islamic University, Almadinah Almonawara, Saudi Arabia for the support needed to carry out this research. Author also acknowledges all staff members of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Taibah University, Almadinah Almonawara, Saudi Arabia for their assistance during the research.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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