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Research Article

Glucagon-like peptide-2 May Assist to Protect against Valproic Acid Induced Hepatic Injury in Rats

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Abstract

VPA is widely used in epilepsy and other psychological disorders, increasing the probability of developing non-alcoholic liver disease in long-term treatments. GLP-2 is a proglucagon belonging to the peptide family expressed in the intestine, pancreas and brain to date. Although there are many studies on the use of GLP-2 for therapeutic purposes on the gastrointestinal system, its effect on liver toxicity is unknown. We aimed to investigate the effect of GLP-2 administration on hepatic function in a rat model with VPA-induced hepatotoxicity. Rats were injected intraperitoneally at 500 mg/kg and GLP-2 5µg/kg a day. The rats (200-250g) were separated into four groups (n=7). Group C was administrated 1 mL of 0.9% SF, Group GLP treated with GLP-2 (5µg/kg/day), Group GLP+VPA were received GLP-2 (5µg/kg) 1 h prior to VPA (500 mg/kg), Group VPA received VPA (500 mg/kg), 1 h prior to 1 mL of 0.9% SF ip (n=7). Liver tissues were used to investigate effects of VPA and GLP-2 in the liver 15 days after application. While VPA caused moderate but significant liver damage according to biochemical results, mRNA expression of cytokines were found to significantly increase after the day 15. VPA administration significantly induced expression of Interleukin 1 beta (IL-1β), Tumor necrosis factor alpha (TNF-α), Interleukin 10 (IL-10). In contrast, GLP-2 treatment reduced expression of IL-1β, TNF-α and IL-10. Also malondialdehyde (MDA), glutathione s-transferase (α-GST), superoxide dismutase activities (SOD), total antioxidant status (TAS) and total oxidant status (TOS) levels were estimated. GLP-2 had positive effects on both liver enzymes and oxidative stress markers in VPA-induced hepatotoxicity. These results suggest that endogenous GLP-2 administration is associated with a mechanism that moderately protects liver tissue.

Keywords: Glucagon-like peptide-2 (GLP-2), Valproic acid (VPA), Hepatoprotective effect.

Glukagon benzeri peptit-2 Sıçanlarda Valproik Asite Bağlı Hepatik Yaralanmaya Karşı Korunmaya Yardımcı Olabilir

Öz

Valproik asit (VPA), epilepsi ve diğer psikolojik bozuklukların uzun süreli tedavilerinde yaygın olarak kullanılmakta, alkolik olmayan yağlı karaciğer hastalığı gelişme riskini artırmaktadır. Bugüne kadar, GLP-2, bağırsak, pankreas ve beyinde eksprese edilen peptit familyasına ait bir proglukagondur. GLP-2'nin gastrointestinal sistem üzerinde tedavi amaçlı kullanımına dair birçok çalışma olmasına rağmen, karaciğer toksisitesine olan etkisi bilinmemektedir. Bu çalışmada VPA ile hepatotoksisite oluşturulmuş sıçan modelinde GLP-2 uygulamasının hepatik fonksiyon üzerindeki etkisini araştırmayı amaçladık. Sıçanlara intraperitonal olarak 500 mg/kg/gün ve GLP-2 (5 µg /kg/gün) enjekte edildi. Sıçanlar (200-250g) dört gruba ayrıldı (n=7). C grubuna 1 mL % 0.9 tuzlu su verildi, GLP-2 grubuna 5 µg /kg/gün GLP-2 verildi, Grup GLP+VPA'ya, VPA'dan (500 mg/kg) 1 saat önce GLP-2 (5 µg/kg) verildi. Grup VPA, 1 mL %0.9 tuzlu sudan 1 saat önce VPA (500 mg/kg) aldı. Uygulamadan 15 gün sonra karaciğerdeki VPA ve GLP-2 etkilerini araştırmak için karaciğer dokuları kullanıldı. VPA, biyokimyasal sonuçlara göre orta fakat önemli karaciğer hasarına neden olurken, sitokinlerin mRNA

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ekspresyonunun kontrol grubuna göre 15. günden sonra önemli ölçüde arttığı bulunmuştur. VPA uygulaması Interleukin 1 beta (IL-1β), Tümör nekroz faktörü alfa (TNF-α), İnterlökin 10 (IL-10) ekspresyonunu artırırken, aksine, GLP-2, IL-l β, TNF-α ve IL-10 ekspresyonunu azaltmıştır. Ayrıca malondialdehit (MDA), glutatyon s-transferaz (α-GST), süperoksit dismutaz aktiviteleri (SOD), toplam antioksidan status (TAS) ve toplam oksidan status (TOS) seviyeleri ölçüldü. GLP-2'nin VPA kaynaklı hepatotoksisitede hem karaciğer enzimleri hem de oksidatif stres belirteçleri üzerinde olumlu etkileri olmuştur. Bu sonuçlar endojen GLP-2 uygulamasının, karaciğer dokusunu orta derecede koruyan bir mekanizma ile ilişkili olduğunu düşündürmektedir.

Anahtar Kelimeler: Glukagon benzeri peptit-2 (GLP-2), Valproik asit (VPA), Hepatoprotektif etki

1. Introduction

Valproic acid (VPA) is used to treat epilepsy in all age groups, including childhood. It is also a suggested drug in migraine, bipolar, mood, anxiety and psychiatric disorders. However, adverse drug reactions have limited clinical practice, particularly hepatotoxicity, teratogenicity, and pancreatitis (Ghodke-Puranik et al, 2013; Chen et al, 2019). In particular, the use in children younger than 2 years old, appears to be an increased risk of hepatotoxicity with susceptibility to a variety of metabolic disorders (Diederich et al, 2010). Various factors are involved in the formation of hepatotoxicity, including lipid peroxidation, reactive oxygen species (ROS) production, proinflammatory mediators such as cytokines and chemokines (Chen et al, 2019).

VPA, which is among anticonvulsants, primarily acts through the combination of many mechanisms such as inhibition of sodium channels and calcium conductivity. Important features that play a role in the toxicity of anticonvulsants are metabolism and elimination. VPA metabolizes 50-80% in mitochondria and endoplasmic reticulum with ß-oxidation in liver. As a result of beta oxidation, 4-en-VPA and propionate products are formed. It causes the inhibition of carbamoylphosphate synthase 1, which is necessary for the inclusion of 4-en-VPA ammonia, which is hepatotoxic, in urea production. With this enzymatic inhibition, serum ammonia concentration increases (Springer & Nappe, 2019; Guo et al, 2019).

GLP-2 has been shown to reduce gastric acid release and movement, increase enteric food carriage, peptic activity of enzyme, intestinal barrier concern, food use, and suppress food intake. Except for its effects on food intake, all reports are concentrated in the gastrointestinal effects of GLP-2. Conversely, in the generality of other investigate, pharmacological doses of GLP-2 or analogous have been used, and the effects of GLP-2 on physiological doses have not been fully elucidated (Lim et al, 2016).

Among all intestinal hormones, GLP-2 appears to be an interesting treatment to protect against VPA-induced hepatotoxicity. GLP-2 is a proglucagon belonging to the peptide family expressed in the intestine, pancreas and brain. It has a trophic (cell division and maturation) effect on epithelial cells, especially in the gastrointestinal tract and small intestine. It also has an effect of increasing intestinal mucosal blood flow. A study has been report to possess a beneficial effect on liver ischemic injury. It also has an inhibitory effect on apoptosis (Lim et al, 2014).

Numerous studies have been conducted showing the beneficial potency of GLP-2 on intestine extension and improvement (Lim et al, 2016; Drucker,2002; Boushey et al, 1999). Treatment of GLP-2 in dextran-sulfate-induced colitis and indomethacin-induced enteritis has prolonged life span and decreased bacteremia, tissue damage, inflammation and expression of inflammatory cytokines (Boushey et al, 1999; Drucker et al,1999). Arda-Pirincci and Bolkent investigated possible of GLP-2 on programmed cell death, cell proliferation, and oxidant–antioxidant balance on a mouse model of intestinal injury induced by TNF-α/ActD. They found pretreatment of GLP-2 prevented TNF-a /Act D-induced oxidative damage by reducing GSH levels, lipid peroxidation, GPx and SOD activities and caspase3 expression.To the best of our information, a study on the protective effects of GLP-2 against VPA hepatotoxicity has not been reported in the literature yet. It was thought that GLP-2 was found protective on VPA-induced hepatotoxicity. With these results, GLP2 will play a protective role in drug-induced liver toxicity. The current study was organized to evaluate the possible therapeutic profit of GLP-2, in the hepatotoxic-induced rat example of VPA.

2. Material and Method

2.1. Animal model

Male Wistar rats were applied in this experiment (n=28). The experiment process were permitted by Çanakkale Onsekiz Mart University Institutional Animal Care and Use Committee (Protocol number:2018/02-09). Along the study, all rats were kept at 20±2°C temperature in 12 hours light/12 hours dark cycle (light 08:00-20:00, dark 20:00-08:00).

2.2. Experimental Design

A total of 28 rats (200-250g) were stated to four groups.

Group C: Rats were given 1 mL of 0.9% SF ip (n=7).

Group GLP: Rats were given only GLP (5µg/kg) ip (n=7).

Group GLP+VPA: Rats were given GLP (5µg/kg) 1h prior to VPA (500 mg/kg) ip (n=7).

Group VPA: Rats were given VPA (500 mg/kg), 1h prior to 1 mL of 0.9% SF ip (n=7)

GLP (Absolute GR for analysis, SIGMA) and VPA (Valproic acid sodium salt, Depakine, Sanofi) were prepared in sterile distilled water and rats were given daily by ip based on the body weight of the rats during the 15 days. On the 16th day of study, rats were anesthetized with 5 mg/kg xylazine (Rompun®, Bayer, Istanbul, Turkey) and 50 mg/kg ketamine hydrochloride (Ketalar®, Eczacibasi) with room temperature, and blood and liver tissue were removed.

2.3. Biochemical analysis

After sacrifice, all biochemical investigation of rat liver tissue of malondialdehyde (MDA) levels, glutathione s-transferase (αGST) and superoxide dismutase activities (SOD) from each sample were measured with sensitive ELISA spectrophotometry, respectively. Total antioxidant status (TAS) and total oxidant status (TOS; Rel Assay Diagnostics) levels were determined spectrophotometrically by accounted commercial kits. Oxidative stress index (OSI) value was accounted by using TAS and TOS level. The protein concentrations were evaluated by the Lowry method (Sigma Aldrich, Total protein kit-TP0300-1KT-(USA)). All the result was showed per mg of protein.

2.4. Genetic Analysis

Total RNA was separated from 10-30 mg liver tissue (Ambion Pure Link RNA). The purity and rate of the RNA was analyzed by calculating 260/280 absorbance ratio using a NanoDrop ND-1000 Spektrofotometre. Reverse transcription was applied using a kit (High Capacity cDNA Revere Transcription Kit). Synthesized cDNA samples were amplified for quantitative Real-Time PCR using Tagmanprob PCR master mix (ABI Stepone). Gene expression values were evaluated by Tagmanprob. Beta-actin was applied for genes normalization. Primer ID number of TNF-□, IL-1β, IL-10 and β-Actin are Rn01525859_g1, Rn00566700_m1, Rn01483988_g1, Rn00667869_m1respectively (Thermofischer).

2.5. Statistical Analysis

All results were assessed by SPSS Statistics for Windows, Software Version 20.0 (Armonk, New York, USA: IBM Corp.) All groups were compared to One Way Anova test, followed Tukey's test and $p < 0.05$ was statistically significant. Genes expression level were evaluated $2^{-\Delta\Delta Ct}$ method [$\Delta\Delta Ct$=(Ct Target gene- Ct reference gene)].

3. Results

All animals survived throughout the experimental process. When the study was completed, liver tissue was removed from the rats. α-GST, MDA, SOD, TAS, TOS and OSI levels, gene expression changes from liver tissue were examined. Compared with the control group, VPA administration significantly increased liver α-GST, MDA, TOS levels and IL-1β, TNF-α and Il-10 gene expression levels whereas SOD, TAS levels decreased.

Table 1 represent the toxic effect of VPA in rat hepatocytes with an increased α-GST levels. For protective assessment, GLP-2 administration has rendered a significant modification as compared to VPA group (Table 1). α-GST was markedly increased in VPA treatment group ($p<0.05$) when compared to other groups (C, GLP, GLP+VPA). Conversely, VPA treatment evoked a significant increase on MDA ($p=0.000$) and TOS ($p=0.001$) levels when compared to C group. Additionally, GLP-2 pretreatment showed a significant decrease on MDA, and TOS levels as compared to VPA treatment group ($p<0.05$). GLP-2 administration caused an elevation on antioxidant enzymes concentrations. The levels of TAS were increased in GLP group compared to VPA group. GLP-2 only exposure led no significant change on OSI level as compared to control group ($p=0.755$). MDA level was significantly different between groups ($p<.001$), although the MDA level was lower GLP+VPA group (0.68±0.03 nmole.ml^{-1}.mg protein^{-1}) than VPA group (0.72±0.04 nmole.ml^{-1}.mg protein^{-1}), there was no statistically significant difference between the GLP+VPA and the VPA group after posthoc test ($p=0.870$). SOD level was significantly different between experimental groups ($p = 0.002$). After pairwise comparison, SOD value was found statistically significant between VPA treated group and other groups ($p<0.05$) (Table 1).

Table 1. α-GST and SOD activities, MDA, TAS and TOS levels, and OSI value of rats in the groups.

	Groups			
Biochemical parameters	**C** (n=7)	**GLP** (n=7)	**GLP+VPA** (n=7)	**VPA** (n=7)
α-GST (ng/ml.mg protein)	1543±3.3	1595±29.7	1497±74.9	1809±68.9*****
MDA (nmole.ml^{-1}.mg protein^{-1})	0.43±0.02$^{¥}$	0.37±0.01^{α}	0.68±0.03	0.72±0.04
SOD (U.ml^{-1}.mg protein^{-1})	15.77±0.8	17.34±0.9^{α}	13.35±0.5	6.43±0.3*****
TAS (mmol/L)	0.16±0.1	0.14±0.1	0.12±0.0	0.11±0.0$^{∞}$
TOS (μmol/L)	0.58±0.0	0.47±0.0	0.55±0.0	0.82±0.0*****^{δ}
OSI (mmol/L)/(μmol/L)	0.28±0.0	0.32±0.0	0.24±0.0	0.14±0.0^{π}

All outcomes are showed as mean ± standard error (m± SE). *****:compared to other groups, $^{¥, α}$:compared to GLP+VPA and VPA, $^{∞}$**:** compared to C, ^{δ}**:** compared to GLP and GLP+VPA, ^{π}: compared to C and GLP, $p<0.05$.

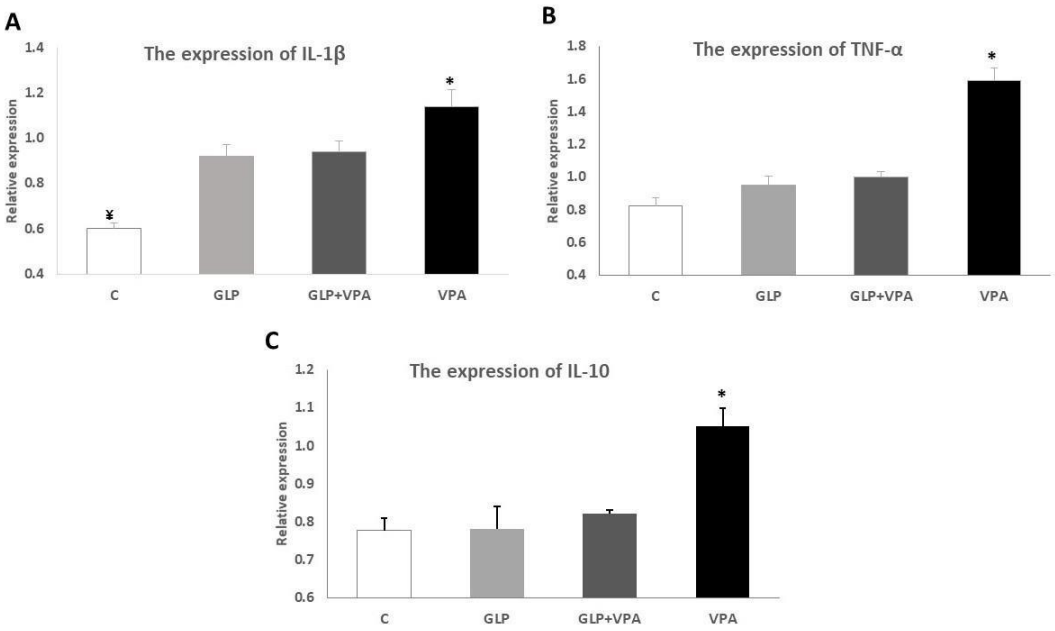

Figure1: Relative total RNA expression levels of *IL-1β* (**A**), *TNF-α* (**B**) and *IL-10* (**C**) were assessed in after treatment with VPA and GLP. Expression levels were normalized to β-Actin. The expression levels of IL-1β, *TNF-α* and *IL-10* were significantly lower in VPA treatment groups than in the non-treatment group (*:compared to other groups, ¥:compared to C and GLP. $p < 0.05$).

Gene expression levels of, IL-1β, TNF-α and IL-10 were analyzed from liver tissues. The significant differences were observed in the VPA group, IL-1β, TNF-α and IL-10 levels compared to the C group. When the GLP+VPA group and the VPA group were compared, it was observed that in the GLP+VPA group, IL-1β, TNF-α and IL-10 levels decreased by approximately a third (p=0.037, p=0.000, p=0.001) respectively. When the GLP group was compared with the C group, results close to the C group were obtained; in other words, TNF-α and IL-10 expression levels were similar (p=0.015, p=0.378, p=1.000) respectively (Figure 1).

4. Discussion

The consequence of VPA on hepatotoxicity was studied to assess biochemical data and liver gene expression levels. The liver is an organ in which various drugs are metabolised, constantly encountering various toxins and detoxifying them. Due to the functional structure of the liver, many factors can be injured. When this process is not respond by an effective regeneration and repair, the liver structure is impaired. Some therapeutic drugs can cause degeneration in liver cells or vascular parts of the liver (Neuman, 2019).

Alteration the level of biomolecules in the liver provide information about hepatocyte injury. The increase of biomarkers such as aminotransferase does not give information about the prognosis because the circulating enzymes cannot always give the actual dimension of hepatic damage (Neuman, 2019), Aminotransferases may increase in the initial period of severe liver injury, decrease after a short time, so the rate of increase in aminotransferase activities is not directly related to the significance of liver damage (Akşit et al, 2015). Studies have shown that α-GST is more specific and sensitive than other liver enzymes such as transaminases to reveal liver damage (Clarke,1997). As a result of this present experiment, alanine aminotransferase and aspartate aminotransferase were analyzed colorimetrically in the blood; however, the results were variable among animals (data not shown).

The present study, liver toxicity was determined by measuring α-GST levels because of the controversial status of aminotransferases. VPA treatment altered the activities of glutathione using enzymes such as glutathione-S-transferase. In this study, α-GST was statistically significant between groups as it served as a marker of liver damage ($p<0.05$); After GLP-2 treatment, α-GST concentrations of GLP + VPA group were found to be 1497±74.92 ng/ml.mg protein and VPA group was 1809 ± 68.93 ng/ml.mg protein ($p =0.001$) (Table 1). Similar to our study, Tong et al. reported that rats administered 500 mg of VPA daily for 14 days, resulting in increased serum α-GST levels on day 4, and increased incidence of inflammation in liver cells, which corresponded to hepatotoxicity (Tong et al, 2005).

There are different types of cells in the liver, normal hepatocytes have villae and are interconnected. If there is noxa, the villae disappear and the stellate cells accumulate lipids. When drug toxicity develops, macrophages produce proinflammatory cytokines and cell inflammation develops and the cell goes either to apoptosis or necrosis. VPA often induces microvesicular steatosis with necrosis (Neuman, 2019). VPA toxicity also is related to enhanced ROS level, which is an important risk factor for liver injury (Fourcade et al, 2010). Some study reports have shown that mechanism of induces toxicity of VPA is increased free radicals and oxidative stress (Kiang et al, 2010; Ahangar et al, 2017). Increased oxidative stress and ROS for various reasons damages lipids, proteins and DNA. Various functions have been submitted to clarify the inhibition of mitochondrial mechanism due to VPA (Tong et al. 2005). ROS takes part in many cellular events, such as mitogenesis, gene expression, various signaling pathways and apoptosis (Birben et al. 2012). Oxidative stress in hepatocytes frequently occurs throughout the an inbalance between pro-oxidants and antioxidants (Mercan, 2004). In this study, in the VPA group, oxidant MDA and TOS values were higher and antioxidant SOD and TAS values were lower than other groups

(Table 1). These results showed that the statement of oxidant/antioxidant was exchanged for the good of the oxidative stress by VPA treatment. MDA is one of the most important products resulting from lipid peroxidation. Likewise, SOD is one of the antioxidants that prevents oxidation produced by free radicals and has the capability to control and stabilize free radicals. VPA-induced hepatotoxicity promotes oxidative stress and elevate the production of ROS (Karaca et al, 2014).

Ahangar et al. (2017) showed that as a result of VPA application of 200 mg/kg/day for 4 weeks, the level of MDA increased compared to the control group, and lipid peroxidation decreased with Zn and Se supplementation. In this study, MDA level increased in VPA group compared to control, although GLP-2 supplementation partially decreased MDA level, but it was not statistically significant. The current experiment also analyzed the result of VPA on the SOD levels of rat liver cells as a cursor of mitochondrial dysfunction. SOD values decreased significantly in other groups compared to the control group. Hence, a significant difference was observed between the group that received VPA and the C, GLP and GLP + VPA group (Table 1). In a study, they examined liver functions in the autism animal model using single dose VPA (600 mg/kg VPA), SOD values did not change compared to the control group (Bambini-Junior et al, 2011).

The second purpose of this research was to investigate whether there is a potential effect in reducing the inflammatory response in hepatotoxic rats treated with GLP-2. GLP-2 is a growth factor, specific for intestinal epithelium, which has been proven to reduce gut mucosal damage and inflammation (Zhang et al, 2008). Therefore, it is thought to be responsible for endocrine regulation of intestine growth and adaptation in a variety of experimental and pathophysiological conditions associated with the release of proglucagon-derived peptides. However, GLP-2 may have functions that have not yet appeared. Recently, GLP-2 has been proven from the expression of the receptor in the hypothalamus that activates adenylate cyclase in brain sections (Hartman et al,2000). The biologically active GLP-2 acts on the GLP-2 receptor, which performs class II glucagon secretion and works together with the G protein (Munrore et al, 1999).

A study by Lim et al. studied the effects of exogenous GLP-2 treatment to improve cholestasis in the neonatal parenteral nutrition–associated model of liver disease in piglet, and found that GLP-2 treatment significantly reduced aminotransferase levels (Lim et al,2016). Chronic hepatocellular injury often causes defects in erythrocyte production. When major inflammation is a component of chronic liver disease, hemoglobin and hematocrit level reduce. Recently, Guan et al. stated that GLP-2 stimulates portal blood flow and the intestinal hemoglobin concentration increases with infusion (Guan et al, 2003). In this study, external GLP-2 application has been shown to have ameliorative effects on the antioxidant system, causing marked reductions on inflammation markers. Although this research indicates that GLP-2 has an important role in the circulatory system, its effects on circulation are not fully known. Even though the nourishing impression of GLP-2 administration on the intestinal mucosa are well investigated, GLP - 2 is not known to have a beneficial effect on the liver. When liver damage develops, levels of inflammatory mediators such as IL-1β, IL-6, NF-κB, TNF-α increase (Jin et al, 2014).

In the present study, we examined the result of external GLP-2 administration on gene expression of IL-1, TNF-α and IL-10 inflammation markers. While the mRNA levels of IL-1 and TNF-α inflammation cytokines increased in the VPA-administered group, GLP-2 treatment reduced the gene expression of liver inflammation markers negligibly (Figure 1). In one study, there was no difference in caspase-3 staining after GLP administration, suggesting that there was no measurable difference in proliferation or apoptosis (Lim et al, 2016). In another study, GLP-2 treatment significantly diminished the rate of mucosal injury, inflammation and submucosal edema, TNF and IFN-γ expression in HLA-B27 rats (Alavi et al, 2000).

5. Conclusions and Recommendations

In this study, it was determined that the application of GLP-2 has protective effects against hepatotoxicity caused by VPA at the cellular, inflammatory and oxidative level. Pre-administration with GLP-2, could inverse the VPA-induced toxic outcome in liver tissue of rat. Based on the hepatoprotective, antioxidant and immune suppressive effect of GLP-2, we suggest that this molecule can be assessed as a possible safe and beneficial aspect to reducing the side effects of VPA-induced hepatotoxicity.

4. Acknowledge

The authors declare that they have no conflict of interest.

References

1. Ahangar, N., Naderi, M., Noroozi, A., Ghasemi, M., Zamani, E., & Shaki, F. (2017). Zinc deficiency and oxidative stress involved in valproic acid induced hepatotoxicity: protection by zinc and selenium supplementation. *Biological trace element research*, *179*(1), 102-109.
2. Akşit, H., Akşit, D., Bildik, A., Hatibe, K. A. R. A., Yavuz, Ö., & Seyrek, K. (2015). Deneysel karaciğer intoksikasyonunda N-asetil sistein'in glutatyon metabolizması ve lipid peroksidasyonuna etkileri. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *62*(1), 1-5.
3. Alavi, K., Schwartz, M. Z., Palazzo, J. P., & Prasad, R. (2000). Treatment of inflammatory bowel disease in a rodent model with the intestinal growth factor glucagon-like peptide-2. *Journal of pediatric surgery*, *35*(6), 847-851.

4. Arda-Pirincci, P., & Bolkent, S. (2011). The role of glucagon-like peptide-2 on apoptosis, cell proliferation, and oxidant– antioxidant system at a mouse model of intestinal injury induced by tumor necrosis factor-alpha/actinomycin D. *Molecular and cellular biochemistry*, *350*(1-2), 13-27.
5. Bambini-Junior, V., Rodrigues, L., Behr, G. A., Moreira, J. C. F., Riesgo, R., & Gottfried, C. (2011). Animal model of autism induced by prenatal exposure to valproate: behavioral changes and liver parameters. *Brain research*, *1408*, 8-16.
6. Boushey, R. P., Yusta, B., & Drucker, D. J. (1999). Glucagon-like peptide 2 decreases mortality and reduces the severity of indomethacin-induced murine enteritis. *American Journal of Physiology-Endocrinology And Metabolism*, *277*(5), E937-E947.
7. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012). Oxidative stress and antioxidant defense. World Allergy Organ J 5:9–19
8. Chen, G. W., Chen, T. Y., & Yang, P. M. (2019). Differential effect of herbal tea extracts on free fatty acids-ethanol-and acetaminophen-induced hepatotoxicity in FL83B hepatocytes. *Drug and chemical toxicology*, 1-6.
9. Chen, Y., Zhou, J., Xu, S., Liu, M., Wang, M., Ma, Y., Zhao, M., Wang, Z., Guo, Y. & Zhao, L. (2019). Association between the perturbation of bile acid homeostasis and valproic acid-induced hepatotoxicity. *Biochemical Pharmacology*, *170*, 113669.
10. Clarke, H., Egan, D. A., Heffernan, M., Doyle, S., Byrne, C., Kilty, C., and Ryan, M. P. (1997). Alpha-glutathione S-transferase (alpha-GST) release, an early indicator of carbon tetrachloride hepatotoxicity in the rat. *Hum. Exp. Toxicol.* 16 , 154–157.
11. Diederich, M., Chateauvieux, S., Morceau, F., & Dicato, M. (2010). Molecular and therapeutic potential and toxicity of valproic acid. *J Biomed Biotechnol*, *2010*, 201.
12. Drucker, D. J. (2002). Biological actions and therapeutic potential of the glucagon-like peptides. *Gastroenterology*, *122*(2), 531-544.
13. Drucker, D. J., Yusta, B., Boushey, R. P., DeForest, L., & Brubaker, P. L. (1999). Human [Gly2] GLP-2 reduces the severity of colonic injury in a murine model of experimental colitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *276*(1), G79-G91.
14. Fourcade,S., Ruiz, M., Guilera, C., Hahnen E, Brichta L, Naudi A, Portero-Otín M, Dacremont G, Cartier N, Wanders R, Kemp S, Mandel JL, Wirth B, Pamplona R, Aubourg P, Pujol A (2010). Valproic acid induces antioxidant effects in X-linked adrenoleukodystrophy. Hum Mol Genet 19:2005–2014 .
14. Ghodke-Puranik, Y., Thorn, C. F., Lamba, J. K., Leeder, J. S., Song, W., Birnbaum, A. K, Altman R.B & Klein, T. E. (2013). Valproic acid pathway: pharmacokinetics and pharmacodynamics. *Pharmacogenetics and genomics*, *23*(4), 236.
15. Guan, X., Stoll, B., Lu, X., Tappenden, K. A., Holst, J. J., Hartmann, B., & Burrin, D. G. (2003). GLP-2-mediated up-regulation of intestinal blood flow and glucose uptake is nitric oxide-dependent in TPN-fed piglets. *Gastroenterology*, *125*(1), 136-147.
16. Guo, H. L., Jing, X., Sun, J. Y., Hu, Y. H., Xu, Z. J., Ni, M. M., ..& Wang, T. (2019). Valproic Acid and the Liver Injury in Patients with Epilepsy: An Update. *Current pharmaceutical design*, *25*(3), 343-351.
17. Hartmann, B., Johnsen, A. H., Ørskov, C., Adelhorst, K., Thim, L., & Holst, J. J. (2000). Structure, measurement, and secretion of human glucagon-like peptide-2. *Peptides*, *21*(1), 73-80.
18. Jin, J., Xiong, T., Hou, X., Sun, X., Liao, J., Huang, Z., ..& Zhao, Z. (2014). Role of Nrf2 activation and NF-κB inhibition in valproic acid induced hepatotoxicity and in diammonium glycyrrhizinate induced protection in mice. *Food and chemical toxicology*, *73*, 95-104.
19. Karaca, Ö., Sunay, F. B., Kuş, M. A., Gülcen, B., Özcan, E., Ögetürk, M., & Kuş, İ. (2014). Kadmiyum ile Oluşturulan Deneysel Karaciğer Hasarına Karşı Melatoninin Etkilerinin Biyokimyasal ve Histopatolojik Düzeylerde İncelenmesi. *Fırat Tıp Derg*, *19*(3), 110- 115
21. Kiang,TK., Teng, XW, Karagiozov S, Surendradoss J, Chang TK, Abbott FS (2010). Role of oxidative metabolism in the effect of valproic acid on markers of cell viability, necrosis, and oxidative stress in sandwich-cultured rat hepatocytes. Toxicol Sci 118:501–509
22. Lim, D. W., Wales, P. W., Josephson, J. K., Nation, P. N., Wizzard, P. R., Sergi, C. M., .. & Turner, J. M. (2016). GlucagonLike Peptide 2 Improves Cholestasis in Parenteral Nutrition–Associated Liver Disease. *Journal of Parenteral and Enteral Nutrition*, *40*(1), 14-21.
23. Mercan, U. (2004). Toksikolojide serbest radikallerin önemi. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, *15*(1), 9196.
24. Munroe, D. G., Gupta, A. K., Kooshesh, F., Vyas, T. B., Rizkalla, G., Wang, H., ... & McCallum, K. (1999). Prototypic G protein-coupled receptor for the intestinotrophic factor glucagon-like peptide 2. *Proceedings of the National Academy of Sciences*, *96*(4), 1569-1573.
25. Neuman, M. G. (2019). Biomarkers of drug-induced liver toxicity. *Therapeutic drug monitoring*, *41*(2), 227-234.
26. Springer, C., & Nappe, T. M. (2019). Anticonvulsants Toxicity. In *StatPearls [Internet]*. StatPearls Publishing.
28. Tong, V., Teng, X. W., Chang, T. K., & Abbott, F. S. (2005). Valproic acid I: time course of lipid peroxidation biomarkers, liver toxicity, and valproic acid metabolite levels in rats. *Toxicological Sciences*, *86*(2), 427-435.
29. Tong, V., Teng XW, Chang TK, Abbott FS (2005b).Valproic acid I: time course of lipid peroxidation biomarkers, liver toxicity, and valproic acid metabolite levels in rats. Toxicol Sci 86:427–435
30. Zhang, W., Zhu, W., Zhang, J., Li, N., & Li, J. (2008). Protective effects of glucagon-like peptide 2 on intestinal ischemia reperfusion rats. *Microsurgery: Official Journal of the International Microsurgical Society and the European Federation of Societies for Microsurgery*, *28*(4), 285-290.