Exp Biomed Res 2022; 5(1): 1-9
Doi: 10.30714/j-ebr.2022173846

Experimental Biomedical Research **Original article**

Effects of the ATP-dependent K (+)-channel effectors pinacidil and glibenclamide on liver tissue in an experimental model of epilepsy: A histopathological study

Selma Erdogan Duzcu[1], Ayhan Cetinkaya[2], Hayriye Soyturk[3], Serif Demir[4]

[1]*Department of Pathology, Bolu Abant Izzet Baysal University, Faculty of Medicine, Bolu, Turkey*
[2]*Department of Physiology, Bolu Abant Izzet Baysal University, Faculty of Medicine, Bolu, Turkey*
[3]*Interdisciplinary Neuroscience, Bolu Abant Izzet Baysal University, Institute of Graduate Studies, Bolu, Turkey*
[4]*Department of Physiology, Duzce University, Faculty of Medicine, Duzce, Turkey*

ABSTRACT

Aim: It is known that most of the antiepileptic drugs have negative effects on the liver. Pinacidil is a nonselective opener of KATP channels, including the plasma membrane and mitochondria. Glibenclamide is an ATP -dependent K channel blocker ensuring the intake of calcium. Our aim in this experimental study was to examine the effects of pinacidil and glibenclamide on the liver tissue of rats with focal epilepsy.
Method: Sixty male Sprague Dawley rats (2-4 months old, 200-250 gr) were used in the study. The rats were divided into 4 groups, 15 in each group. The groups were divided into control group, penicillin group, penicillin + pinacidil group and penicillin + glibenclamide group. The craniums of the rats in the control group were opened and normal saline was given; Penicillin (2 µl 500 IU) was intracortically administered to other groups and an experimental epilepsy model was created. At the end of the study, liver tissue of rats was taken and evaluated in terms of vacuolar degeneration, lymphocyte infiltration, vascular congestion, sinusoidal dilatation, necrosis, and Kupffer cell proliferation, radial alignment of hepatic cords, central vein and portal vein dilatation in hepatocytes.
Results: Venous congestion, cytoplasmic vacuolization, Kupffer cell proliferation, portal vein dilatation and necrosis were distinct in the group to which pinacidil was administered, and distortion was present in the radial sequence *(p<0.001)*. In addition, inflammation, venous congestion and hepatocyte necrosis were found to be lower in the glibenclamide given group compared to the control group *(p<0.001)*.
Conclusion: It can be suggested that pinacidil treatment caused negative results in liver histopathological parameters, whereas glibenclamide was more protective by reducing inflammation, venous congestion and hepatocyte necrosis.

Key words: Epilepsy, liver, pinacidil, glibenclamide, ATP dependent K (+)-channel, histopathology, rat.

✉ *Dr. Selma Erdogan Duzcu*
Department of Pathology, Bolu Abant Izzet Baysal University, Faculty of Medicine, Bolu, Turkey
E- mail: serdoganduzcu@hotmail.com
Received: 2021-05-25 / Revisions: 2021-07-12
Accepted: 2021-07-17 / Published online: 2022-01-01

Introduction

Epilepsy is one of the frequent central nervous system disorders, affecting many organs and system [1]. Unbalanced potassium homeostasis is one of the important factors playing a role in the pathogenesis of epileptic seizures [1]. The concentration of extracellular potassium increases during the seizures, and the potential of cellular membrane decreases [1]. Therefore, the studies examining the pathophysiological mechanisms are critical [1]. Drug toxicity may occur owing to the role of liver in the

elimination of drugs and potential toxins [2]. Inflammation occurs through the activation of inflammasome that is a significant factor for hepatocyte damage and hepatic stellate cell activation, and acute and chronic liver diseases occur [3]. Most of anti-epileptic drugs may cause hepatotoxicity [2].

ATP-sensitive K channels (KATP) are present in many cells and tissues such as pancreas β cells, skeletal muscles, neurons, glial cells, kidney and liver [4]. KATP channels play key roles in hyperglycemia, hypoglycemia, ischemia and hypoxia [4]. ATP-dependent potassium channels consists of two sub-units as Kir6.x and sulphonylurea receptor [5]. They are found in the mitochondrial inner membrane of rat liver [6]. They directly take part in the glucose metabolism of in the intra-cellular ATP concentrations with liver KATP channels [4]. Moreover, they have different physiological and pharmacological functions based on KATP channels' subunits [4]. However, the information on the roles of K channels in hepatocytes is limited [5].

Pinacidil is a nonselective opener of KATP channels, including the plasma membrane and mitochondria [7, 8]. Pinacidil has been shown to have significant cardioprotective and vasorelaxant effects cardiac ischemia/reperfusion injury, arrhythmia and hypertension [7, 8].

Pinacidil is a vasodilator agent used in the treatment of hypertension [9]. Pinacidil have also been shown to prevent hypoxia in rat cardiac myocytes [8].

Glibenclamide is an ATP -dependent K channel blocker ensuring the intake of calcium [10, 11]. It is one of the most commonly-administered anti-diabetic drugs from the sulfonylurea group [3, 12]. Having been used for type 2 diabetes since 1960s, it has been reported to have anti-inflammatory and anti-oxidant effects [3, 10].

In the literature, there is no study revealing the effects of pinacidil and glibenclamide on the liver tissue of rats in which a focal epilepsy model was created. Therefore, in this study, we aimed to histopathologically investigate the effects of pinacidil and glibenclamide on liver morphology by creating experimental epilepsy in rats.

Materials and methods

Sixty male Sprague Dawley rats (2-4 months old, 200-250 gr) were used in the study. The Institutional Animal Care and Use Committee of Bolu Abant Izzet Baysal University (Number: 2018/36/A1) approved for the study. All procedures complied with the Guide for the Care and Use of Laboratory Animals (1996). These rats were kept in the environment that was illuminated/dark for 12, had a room temperature of 22 ± 2°C and relative humidity of 60-70% and they were fed ad libitum (with water and food).

Epilepsy animal model and groups

Experimental focal epilepsy model was performed by intracortical administration of penicillin to the animals in the experimental groups under ketamine-xylazine anesthesia (10/90 mg/kg, intraperitoneally).

Group 1: used as negative control group. The craniums of the rats in this group were opened and 2 µl of normal saline was intracortically administered.

The craniums of the rats in group 2, group 3 and group 4 were opened as in the group 1 and 2 µl of 500 IU penicillin was intracortically administered.

Group 2: dimethyl sulfoxide (DMSO) was intravenously administered 15 minutes after the focal penicillin model was formed in the brain.

Group 3: KATP channel opener pinacidil monohydrate (0.1 mg/kg, Sigma-Aldrich, CAS:

85371-64-8) was intravenously administered 15 minutes after the focal penicillin model was formed in the brain. The solvent of this drug was DMSO.

Group 4: KATP channel blocker glibenclamide (0.1mg/kg, Sigma-Aldrich, CAS: 10238-21-8) was intravenously administered 15 minutes after the focal penicillin model was formed in the brain. The solvent of this drug was DMSO.

On the eighth day of work, the animals were euthanized by cervical dislocation under deep anesthesia using 5% halothane. Then, the liver tissues obtained from rats were fixed in 10% formaldehyde for histopathological examination.

Histopathological examination

Sections from liver tissues were followed in a manner to view the broadest surfaces, and then embedded into paraffin. 3 μm sections were cut from the paraffin blocks and stained with hematoxylin eosin. Sections were assessed by a pathologist under LEICA DM 2000 LED light microscope.

Vacuolar degeneration, lymphocyte infiltration, vascular congestion, sinusoidal dilatation, necrosis, Kupffer cell proliferation, radial alignment of hepatic cords, central vein and portal vein dilatation in hepatocytes were assessed in the pathological examination of liver tissue and semi-quantitatively scored with points from 0 to 3 [13, 14]. Based on this score, absence of pathology was scored as 0, while presence of mild (focal) pathology was scored as 1, presence of moderate (multifocal) pathology was scored as 2, and presence of severe (diffuse) pathology scored as 3. Sections were stained with hematoxylin eosin were photographed at different scales through the Infinity 3 Analyze Release 6.5 system.

Statistical analyses

Number and percentage values were used for the descriptive statistics. To determine whether there was a significant difference between the groups, Kruskal-Wallis test was used. In cases where there were differences between the groups, paired comparisons (post hoc) test was used to determine the groups as the sources of this difference. Significance level was used as $p<0.05$. Analyses were performed on Statistical Package for the Social Sciences (SPSS) v.21 by IBM.

Results

Two rat each in the groups 1, 2 and 3 died on the fourth day and were excluded from the study. While no epileptic activity was observed in the control group by electrophysiological evaluation of the experimental groups, it was shown by spike waves that the epilepsy model was formed in the other groups.

The histopathological parameters of the groups are presented in table 1. In addition, examples of microscopic images of the groups are presented in figures 1-4. The inflammatory cells in the portal area had a significant difference ($p<0.001$). The penicillin group (PE) had a significant increase compared to the control group. Moreover, compared to the PE group, penicillin+glibenclamide (PE+GLI) group had a significantly less inflammation. A significant difference regarding the sinusoidal dilatation values was present ($p<0.001$). Penicillin group had a significance increase compared to the control group.

A significance difference regarding the venous congestion values was present between the groups ($p<0.001$). PE and penicillin+pinacidil (PE+PI) groups had a significant increase in the venous congestion compared to the control group. PE+PI and PE+GLI group had a statistically significant decrease in venous congestion compared to the penicillin group. PE+PI group had a more distinctive congestion compared to the PE+GLI group.

Table 1. Distribution of histopathological parameters among the groups.

Parameters		Groups				
		Control (N=13) N (%)	**PE (N=13) N (%)**	**PE + PI (N=13) N (%)**	**PE + GLI (N=15) N (%)**	***p***
Inflammation	**0**	4 (30.8)	0 (0.0)	0 (0.0)	0 (0.0)	***0.001***
	1	9 (69.2)	6 (46.2)	10 (76.9)	15 (100.0)	
	2	0 (0.0)	4 (30.8)	2 (15.4)	0 (0.0)	
	3	0 (0.0)	3 (23.1)	1 (7.7)	0 (0.0)	
Average rank		17.73[a]	38.04[b]	30.19[a,b]	24.50[a]	
Sinusoidal dilatation	**1**	12 (92.3)	0 (0.0)	6 (46.2)	6 (40.0)	***0.001***
	2	1 (7.7)	10 (76.9)	4 (30.8)	9 (60.0)	
	3	0 (0.0)	3 (23.1)	3 (23.1)	0 (0.0)	
Average rank		14.35[a]	39.96[b]	28.88[a,b]	26.90[a,b]	
Congestion	**1**	5 (38.5)	0 (0.0)	0 (0.0)	9 (60.0)	***0.001***
	2	8 (61.5)	2 (15.4)	6 (46.2)	5 (33.3)	
	3	0 (0.0)	11 (84.6)	7 (53.8)	1 (6.7)	
Average rank		18.27[a]	41.92[b]	35.77[b]	15.83[a]	
Vacuolization	**0**	11 (84.6)	0 (0.0)	0 (0.0)	4 (26.7)	***0.001***
	1	2 (15.4)	4 (30.8)	8 (61.5)	7 (46.7)	
	2	0 (0.0)	9 (69.2)	3 (23.1)	4 (26.7)	
	3	0 (0.0)	0 (0.0)	2 (15.4)	0 (0.0)	
Average rank		10.77[a]	38.81[b]	34.50[b]	26.13[b]	
Necrosis	**0**	10 (76.9)	4 (30.8)	2 (15.4)	10 (66.7)	***0.001***
	1	3 (23.1)	4 (30.8)	8 (61.5)	5 (33.3)	
	2	0 (0.0)	5 (38.5)	2 (15.4)	0 (0.0)	
	3	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	
Average rank		18.81[a]	34.62[b,c]	36.38[b]	21.17[c]	
Kupffer cell proliferation	**0**	12 (92.3)	3 (23.1)	0 (0.0)	3 (20.0)	***0.001***
	1	1 (7.7)	4 (30.8)	12 (92.3)	11 (73.3)	
	2	0 (0.0)	6 (46.2)	0 (0.0)	1 (6.7)	
	3	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	
Average rank		11.27[a]	35.27[b]	34.15[b]	29.07[b]	
Distortion in radial alignment	**0**	11 (84.6)	10 (76.9)	5 (38.5)	14 (93.3)	***0.010***
	1	2 (15.4)	2 (15.4)	6 (46.2)	0 (0.0)	
	2	0 (0.0)	0 (0.0)	1 (7.7)	1 (6.7)	
	3	0 (0.0)	1 (7.7)	1 (7.7)	0 (0.0)	
Average rank		24.35[a]	26.88[a,b]	36.96[b]	22.57[a]	
Central vein dilatation	**0**	7 (53.8)	4 (30.8)	3 (23.1)	4 (26.7)	*0.192*
	1	5 (38.5)	4 (30.8)	6 (46.2)	10 (66.7)	
	2	1 (7.7)	5 (38.5)	4 (30.8)	1 (6.7)	
Average rank		20.81	31.31	31.58	26.47	
Portal vein dilatation	**0**	9 (69.2)	7 (53.8)	1 (7.7)	10 (66.7)	***0.003***
	1	4 (30.8)	3 (23.1)	6 (46.2)	2 (13.3)	
	2	0 (0.0)	3 (23.1)	5 (38.5)	3 (20.0)	
	3	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	
Average rank		20.46[a]	26.69[a,b]	39.85[b]	23.60[a]	

** Kruskal Wallis test ** letters a, b indicate the paired comparison (post-hoc) test results.*

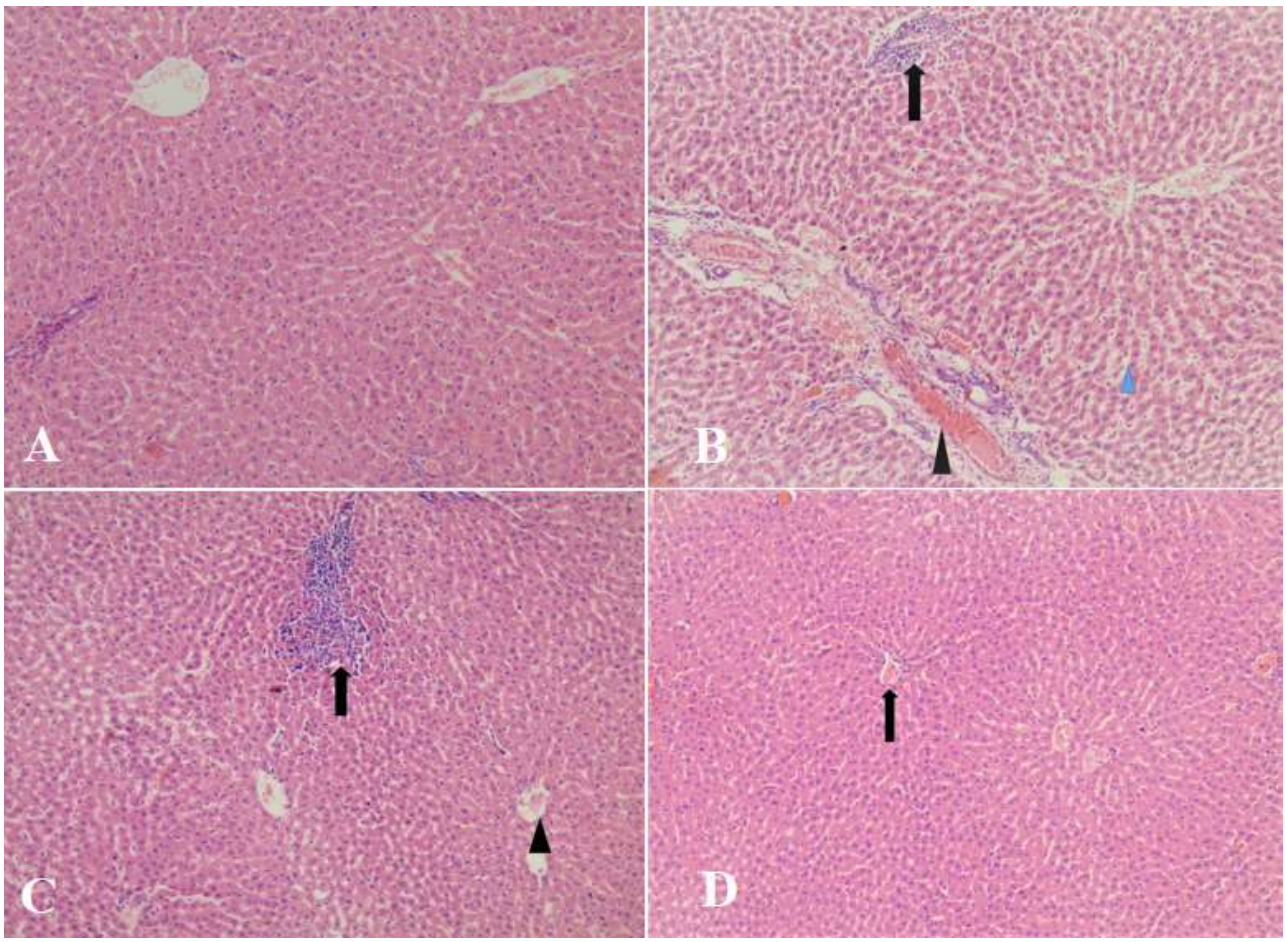

Figure 1. A. Control group, regular radial alignment present, inflammation and necrosis absent, HEX100. **B.** PE group; distinctive inflammation in the portal area (black arrow), portal venous congestion (tip of black arrow), sinusoidal dilatation (tip of blue arrow), HEX100. **C**. PE+PI group; distinctive portal inflammation (black arrow), central vein dilatation (tip of black arrow), HEX100. **D.** PE+GLI group; mild congestion in the central vein (black arrow), no distinctive inflammation present, HEX100.

The groups had a significant difference in terms of cytoplasmic vacuolization values (p<0.001). Cytoplasmic vacuolization statistically and significantly increased in other groups compared to the control group.

The groups had a significant difference in terms of necrosis values (p=0.001). Necrosis statistically and significantly increased in PE and PE+PI groups compared to the control group. PE+PI group had more necrosis compared to the PE+GLI group.

The groups had a significant difference in terms of Kupffer cell proliferation values (p<0.001). Kupffer cell proliferation statistically and significantly increased in other groups compared to the control group.

The groups had a significant difference in terms of the values of radial sequence distortion (p=0.010). Compared to the control group and PE+GLI group, radial sequence of PE+PI group had a statistically significant distortion. The groups had no significant difference in terms of central vein dilatation (p=0.192).

The groups had a significant difference in terms of portal venous dilatation values (p=0.003). Portal vein dilatation statistically and significantly increased in PE+PI group compared to the control group. PE+PI group

had more portal vein dilatation compared to the PE+GLI group.

Discussion

Penicillin is administered intracortically or parenterally to form the experimental epilepsy model, and chemical convulsions are formed [15]. Based on the hypoxic processes in the cells of all systems, seizures may cause reversible and irreversible damages [15].

KATP channels are important for electrical activity [1]. KATP channels close when intracellular ATP concentration is high, and open in case of ischemia [4]. These channels consist of four pore-forming subunits Kir6.x (Kir6.1 or Kir6.2) and four regulatory subunit sulfonylurea receptors SUR (SUR1, SUR2A, or SUR2B) [4]. SUR1 and Kir6.2 are sensitive to diazoxide, the activator of KATP channels, and Kir6.2 with SUR2A is sensitive to pinacidil [4]. Genetic, physiological and pharmacological findings have demonstrated that K channels also have a role in epilepsy management and neuronal excitability [16, 17]. Thus, it has been reported that KATP channels may be a potential treatment for novel drugs [16, 17]. Acar et al. showed that pinacidil reduced epileptic activity, but glibenclamide did not have an effect [16]. Another study reported that glibenclamide showed an anticonvulsant effect by inhibiting generalized tonic clonic and absence seizures [18].

Ateş et al. examined the impact of epileptic seizure experienced by rats during their pregnancy on the liver of newborn rat [15]. Under the electron microscope, expansion and decrease in the number of mitochondria was seen and assessed in favor of latency in the hepatogenesis [15].

Inflammation, venous congestion, cytoplasmic vacuolization in hepatocytes, necrosis and Kupffer cell proliferation increased in the livers of rats that were exposed to epilepsy seizure through the intracortical administration of penicillin, and inflammatory impact was seen in the livers of rats that suffered epileptic seizures, indicating hepatotoxic effect.

Pinacidil is an ATP-dependent mitochondrial K channel opener [19]. Pinacidil has been reported to have cardioprotective impacts by protecting the cardiac tissues from mitochondrial damage in ischemia reperfusion that occurs in vivo and in vitro [19]. In addition, pinacidil suppressed the inflammation around the incision line, as seen in a study conducted with rats [20]. However, the literature did not have data on the morphological effects of pinacidil on liver. This study indicated distinctive venous congestion, cytoplasmic vacuolization, Kupffer cell proliferation, portal vein dilatation and necrosis in the PE+PI group, and distortion was seen in the radial alignment. Compared to the control group, hepatotoxic impact was seen, but no significant difference with PE group was seen.

Glibenclamide inhibits ATP-sensitive potassium channels in pancreas beta cells [11]. Glibenclamide reduces proinflammatory cytokine generation, vasogenic edema and caspase-3 activation in bacterial infection cases among diabetic patients [3]. It has a protective role against the inflammation-related damage in the respiratory system, digestive system, kidneys, bladder and heart, in ischemia reperfusion damage and septic shock [3, 10]. Its anti-inflammatory impact is inhibited by the NLRP3 inflammasome, and it makes its contributions by reducing the generation of proinflammatory cytokines, preventing the spread of inflammatory cells into the inflammation area and boosting the nitric oxide generation [12].

Dwivedi et al. found that glibenclamide decreased thioacetamide-related

inflammasome activation, inflammatory lymphocyte infiltration, collagen deposition, necrotic hepatocytes and fibrosis [3]. Sokolovska et al. indicated that glibenclamide treatment reduced the liver tissue damage among the rats that had streptozotocin-related diabetes [11]. Liu et al. examined the effects of glibenclamide on the liver tissue damage related to acute radiation on rats [10]. Glibenclamide was found to decrease hepatocyte vacuolization, hepatocellular edema and hepatic sinusoids [10]. Administration of glibenclamide before the radiation stimulated the Akt-NF-κB pathway and decreased reactive oxygen derivatives, creating a protective impact on the hepatocytes [10].

Malhi et al. indicated that ATP-dependent K channels had an important role in the arrangement of hepatocyte proliferation [5]. A limited DNA synthesis was seen in cell culture and pinacidil increased DNA synthesis, while glibenclamide inhibited DNA synthesis [5]. Ymazaki et al. conducted a study on pigs and examined the impact of nicorandil and glibenclamide on ischemia reperfusion damage [6]. Glibenclamide corrected the hepatocyte damage like nicorandil, but no difference was found when compared to the control group [6]. Hai et al. indicated that nicorandil (KATP channel opener) was protected from ischemia reperfusion damage through apoptosis inhibition [21].

This study indicated that glibenclamide decreased inflammation, venous congestion and hepatocyte necrosis, and that glibenclamide might have a hepatoprotective effect, which is in line with the literature. Pinacidil, whose impacts on the liver morphology has not been detailed in the literature, created hepatotoxic impact compared to the control group, but no significant difference with the data of PE group was found.

The study was solely based on histopathologic examination, and biochemical and other clinical tests were not present. We see this situation as the limitation of our study.

Conclusion

As a results, it can be suggested that pinacidil treatment caused negative results in liver histopathological parameters, whereas glibenclamide was more protective by reducing inflammation, venous congestion and hepatocyte necrosis. In addition to, studies that will reveal the impact mechanisms of ATP-dependent K channel opener and blocker drugs for liver will be beneficial for the therapeutic use of these drugs.

Funding: *The author(s) received no financial support for the research, authorship, and/or publication of this article.*

Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement:

The study was approved by Local Clinical Research Ethics Committee Number: (2018/36/A1), and written informed consent was obtained from each subject.

Open Access Statement

References

[1]Venediktova NI, Gorbacheva OS, Belosludtseva N V, et al. Energetic, oxidative and ionic exchange in rat brain and liver mitochondria at experimental audiogenic epilepsy (Krushinsky–Molodkina model). J Bioenerg Biomembr. 2017;49(2):149–58.

[2]Poorrostami A, Farokhi F, Heidari R. Effect of hydroalcoholic extract of ginger on the liver of epileptic female rats treated with lamotrigine. Avicenna J Phytomedicine. 2014;4(4):276–86.

[3]Dwivedi DK, Jena GB. Glibenclamide protects against thioacetamide-induced hepatic damage in Wistar rat: investigation on NLRP3, MMP-2, and stellate cell activation. Naunyn Schmiedebergs Arch Pharmacol. 2018;391(11):1257–74.

[4]Zhou M, Yoshikawa K, Akashi H, et al. Localization of ATP-sensitive K + channel subunits in rat liver . World J Exp Med. 2019;9(2):14–31.

[5]Malhi H, Irani AN, Rajvanshi P, et al. K(ATP) channels regulate mitogenically induced proliferation in primary rat hepatocytes and human liver cell lines: Implications for liver growth control and potential therapeutic targeting. J Biol Chem. 2000;275(34):26050–57.

[6]Yamazaki H, Oshima K, Sato H, et al. The effect of nicorandil on ischemia-reperfusion injury in a porcine total hepatic vascular exclusion model. J Surg Res. 2011;167(1):49–55.

[7]Kopustinskiene DM, Liobikas J, Skemiene K, et al. Direct effects of K ATP channel openers pinacidil and diazoxide on oxidative phosphorylation of mitochondria in situ. Cell Physiol Biochem. 2010;25(2–3):181–86.

[8]Iguchi K, Saotome M, Yamashita K, et al. Pinacidil, a KATP channel opener, stimulates cardiac Na + / Ca 2 + exchanger function through the NO / cGMP / PKG signaling pathway in guinea pig cardiac ventricular myocytes. Naunyn Schmiedebergs Arch Pharmacol. 2019;392(8):949–59.

[9]Shaheen O, Patel J, Avant GR, et al. Effect of cirrhosis and debrisoquin phenotype on the disposition and effects of pinacidil. Clin Pharmacol Ther. 1986;40(6):650–55.

[10]Liu H, Wang S, Wu Z, et al. Glibenclamide, a diabetic drug, prevents acute radiationinduced liver injury of mice via up-regulating intracellular ROS and subsequently activating Akt-NF-κB pathway. Oncotarget. 2017;8(25):40568–82.

[11]Sokolovska J, Isajevs S, Sugoka O, et al. Comparison of the effects of glibenclamide on metabolic parameters, GLUT1 expression, and liver injury in rats with severe and mild streptozotocin-induced diabetes mellitus. Med. 2012;48(10):532–43.

[12]Zhang G, Lin X, Zhang S, et al. A Protective Role of Glibenclamide in Inflammation-Associated Injury. Mediators Inflamm. 2017;2017:3578702.

[13]Sabbah WS, Hanafy SM, Arafa MAA. Biochemical and histological study on the effect of levetiracetam on the liver and kidney of pregnant albino rats. Folia Morphol. 2019;78(4):809–17.

[14]Ahmed OM, Fahim HI, Ahmed HY, et al. The preventive effects and the mechanisms of action of navel orange peel hydroethanolic extract, naringin, and naringenin in N-Acetyl-p-aminophenol-induced liver injury in wistar rats. Oxid Med Cell Longev. 2019;2019:2745352.

[15]Ates U, Baka M, Uyanıkgil Y,et al. Histological study of liver tissue from

newborn rats of mothers. Ege Tıp Derg. 2004;43(2):73–77.

[16] Kobayashi K, Nishizawa Y, Sawada K, et al. K + -Channel Openers Suppress Epileptiform Activities Induced by 4-Aminopyridine in Cultured Rat Hippocampal Neurons. J Pharmacol Sci. 2008;108(4):517–28.

[17] Acar Y, Özmerdivenli R, Demir Ş, et al, Ankaralı S, Beyazçiçek Ö, et al. Agonist and Antagonist Effects of ATP-Dependent Potassium Channel on Penicillin Induced Epilepsy in Rats. Kafkas J Med Sci. 2016;6(1):38–45.

[18] Pithadia AB, Navale A, Mansuri J, et al. Reversal of experimentally induced seizure activity in mice by glibenclamide. Ann Neurosci. 2013;20(1):10–12.

[19] Yu T, Fu XY, Liu XK, et al. Protective effects of pinacidil hyperpolarizing cardioplegia on myocardial ischemia reperfusion injury by mitochondrial K ATP channels. Chin Med J (Engl). 2011;124(24):4205–10.

[20] Cao S, Qin Y, Chen J, et al. Effects of pinacidil on changes to the microenvironment around the incision site, of a skin/muscle incision and retraction, in a rat model of postoperative pain. Mol Med Rep. 2015;12(1):829–36.

[21] Hai S, Takemura S, Minamiyama Y, et al. Mitochondrial KATP channel opener prevents ischemia-reperfusion injury in rat liver. Transplant Proc. 2005;37(1):428–31.