Sedative-hypnotic and muscle relaxant activities of propoxazepam in animal models and investigation on possible mechanisms

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ABSTRACT

Introduction: Analgesic (for both neuropathic and nociceptive pain) and anticonvulsant action of 7-bromo-5-(*o*-chlorophenyl)-3-propoxy-1,2-dihydro-3H-1,4-benzodiazepin-2-one (propoxazepam) make it perspective for further studying. Objectives: Propoxazepam was evaluated for sedative-hypnotic-like activity in mice and involved mechanisms in a battery of tests: acute oral toxicity, motor coordination and muscle relaxation studies. Materials and Methods: Acute toxicity (rats) and thiopental-induced sleeping (mice) were determined as motor coordination and muscle relaxation. Results: As relatively nontoxic substance (LD_{50}>5000 mg/kg) propoxazepam belong to fifth category according to GHS toxicity classification. At high doses (700, 1100 and 1300 mg/kg, i.p.) a delay in the righting reflex was observed but not a total loss. The results indicated that substance induces hypnotic effects. Because propoxazepam produces any effect on rotarod test, seems that its effects on sleeping time and sleep latency are mediated by affecting motor movement. Conclusion: Based on previous results and present data, it should be presumed that GABAergic mechanisms may be involved in the potentiating effect of propoxazepam on thiopental-induced sleeping time and sleep latency in mice. Propoxazepam potentiates sleep induced by hypnotics like thiopental, but can not induce hypnosis by itself.

Keywords: propoxazepam; acute oral toxicity; hypnotic; motor coordination; muscle relaxation

1. INTRODUCTION

Benzodiazepines (BDZ) are the drugs, which physiological effects (anticonvulsant, anxiolytic, sedative-hypnotic, muscle-relaxant) mediated through $GABA_A$ receptors ($GABA_ARs$), containing the defined benzodiazepine-binding site [1]. They demonstrate the wide spectrum of pharmacological effects and one of their application was some kinds of pain management. Propoxazepam demonstrated the analgesic activity in the models of nociceptive and neuropathic pain [2]. Similar to gabapentin and pregabalin, which are well-known anti-epileptic drugs used in medical practice for neuropathic pain treatment, propoxazepam also has an anticonvulsant effect [3], which explains the analgesic component of the pharmacological spectrum.

Our data suggest that the mechanism of propoxazepam anticonvulsant properties includes GABAergic and glycinergic systems [4, 5]. Antibradykinin and antileukotriene action, dopaminergic system, NMDA, and alpha-1 adrenergic receptors, except the antiprostaglandin component, are involved in the mechanisms of propoxazepam analgesic effect [6]. Now the preclinical trials are enrolled for this promising substance [7].

Before the proboxazepam clinical studies initialising and continius human administration conducting, the side effects of this substance according to the guidelines have to be made. This study aimed to evaluate the possible sedative-hypnotic and muscle relaxant profiles of propoxazepam single and repeated dose activity in rats and mice. In addition, the safety of this substance was examined by determination of LD_{50}.

2. MATERIALS AND METHODS

Drugs and chemicals

Propoxazepam was synthesized according to the method described in [8]. The structure of the substance was determined and approved by a complex of physico-chemical methods (IR and mass spectroscopy, X-ray diffraction analysis). Chemical purity was confirmed by elemental analysis (99%). Thiopental sodium (Sigma) was used in the study as a sleeping inducing agent.

Animals

Rats and mice were obtained from the animal house of the Institute Pharmacology and Toxicology NAMS of Ukraine. Male and non-pregnant female rats (200–220 g) and mice (25-30 g)), were housed in a temperature and light-controlled room (24.0 ± 2 ºC; 12 h light/dark cycle). Before starting the experiments, animals were randomly selected and kept in their cages for 5 days to allow for acclimatization to the laboratory conditions. The fulfilling of experiments was made in accordance with recommendations of the Committee for Research and Ethical Issues of the IASP (1983) and were approved by the regional ethical committee for animal research. The procedures were undertaken for minimizing of animal suffering and their number reducing.

Acute Toxicity Studies

The acute oral toxicity test was performed using methods described in OECD Test Guidelines 425 [9]. Male and female rats and mice (6 animals per group) were orally treated with propoxazepam (2500; 3500; 4000; 4500; 5000 mg/kg). The oral administration (using an gavage cannula) expenses less and is painless to the animals. The test compounds were administered in suspension stabilized with Tween-80 (Tw), and the control animals received corresponding amount of vehicle (Tw). The LD_{50} was evaluated and propoxazepam classified according to the Globally Harmonized System (GHS) for the classification of chemicals [10].

Pharmacological Evaluation (Sedative-hypnotic and Muscle relaxant activity).

Sedative-hypnotic and muscle relaxant activity was evaluated using following models:

- Thiopental sodium -induced sleeping time and sleep latency.
- Righting-reflex test
- Rotarod test

Thiopental Sodium -induced Sleeping Time and Sleep latency

The effect of propoxazepam on thiopental-induced sleeping time and sleep latency in mice was measured as described by [11]. Animals were divided into groups and the following solutions were injected (*i.p.*) to each group (n=8 for each group): Group 1 - 0.05 %Tween 80 as the negative control forpropoxazepam. Substance administration was made half an hour later after intraperitoneal thiopental sodium (32 mg/kg) injection. At these and followed studies the positive control was not used (for example, diazepam) because propoxazepam belongs to typical 1.4-benzodiazepine derivatives and it mechanism of action directly involves the $GABA_A$-Rs. The type of latency sleeping (time interval between thiopental sodium administration and the righting reflex loss) and sleeping time (between loss and

appearing of righting reflex) were recorded. The time interval between administration of thiopental l and onset of sleep was considered as sleeping latency. Propoxazepam was administered in doses of 1,0; 1,4; 2,0 and 2,8 mg/kg. Thiopental (32 mg/kg, *i.p.,* after 30 mins of drug administration) ws used as sleeping agent. The hypnotic effect was estimated as time interval between righting reflex loss and recovery [12].

Test for righting reflex.

The righting reflex test is a widely used assay of motor coordination and sedation [13, 14]. Rats were placed inside a tube with wood shavings and were placed on their back by the experimenter. The time taken to right (time taken to place all four paws on the ground) was recorded. If time was longer than 60 s, this was recorded as maximal time. The average of three trials at each time point was obtained.

Rotarod test

The test aimed to estimate the drug possible motor coordination influence. The apparatus consists of a horizontal metal rod(3 cm in diameter) with a textured surface, attached to a motor with the speed adjusted to 10 rpm [15]. The rod is placed 50 cm above the table. Previously animals were "trained" at rotarod for 60 sec. Propoxazepam in doses of1,0; 2,0; 5,0 or 10,0 mg/kg was injected i.p. to mice with test repeating 30 min later. The time taken by the animals to fall down from the revolving rod was noted.

Statistical analysis

The data were presented as a relative number (partial contribution, M ± m) of total pharmacological effect. The significance of the differences in indices of pharmacological action between the control and experimental groups (after a preliminary analysis for compliance with the normal distribution law), as well as the final experimental data, were evaluated by the help of Student's t test (unpaired), or by Wilcoxon–Mann–Whitney test. Statistical significance was set at $P<0.05$.

The calculation of dose-effect curve slope was performed according $S = (ED_{82}/ED_{50}+ED_{50}/ED_{18})*0.5$, where ED_{18}, ED_{50} and ED_{82} – calculated doses corresponding to the effect of 18 %, 50 % or 82 % of animals (doses in the range ±1s).

3. RESULTS

Acute Toxicity Study Analysis

After a single oral dose of propoxazepam at doses of 2500; 3500; 4000; 4500 and 5000 mg/kg of body weight, mice and rats of both sexes had no death recorded during the observation period. Only after dosage of 5000 mg/kg the animals gradually became sedated in crouched or prone position in association with frequent eye-closure and decrease in right behavior to tactile stimulation, these sedative symptoms persisting for about 4 hours. There were no abnormalities found in rat`s body weight or pathological changes after necropsy. Under the experimental conditions, the results indicated that the median lethal dose (LD_{50}) of propoxazepam is greater than 5000 mg/kg of body weight. Thus, propoxazepam may be assigned to category V as the lowest class of toxicity according to the GHS.

Thiopental sodium -induced Sleeping Time and Sleep latency

The acute oral treatment with 1,0; 1,4; 2,0 and 2,8 mg/kg of propoxazepam 30 min before the thiopental injection significantly modified the latency to induce sleep as well as increasing duration of sleepiness induced by thiopental, as depicted in Table 1.

Table 1. Effect of propoxazepam on thiopental-induced sleeping time

Dose, mg/kg	logD,	Effect appearance frequency	Probit	Linear regression characteristics of probit-method	
1,0	0,0	0,2	4,092	Slope, k	7,01
1,4	0,1	0,5	5,000	Intercept, b	4,00
2,0	0,3	0,8	5,908		
2,8	0,4	1,0	7,287		
			$logD_{50}$	0,14	
			$logD_{18}$	-0,001	

			$logD_{82}$	0,28	
Mean effective dose, D_{50}				1,39 ± 0,27 mg/kg 3,4 ± 0,7 μmol/kg	
"Dose-effect" curve slope, s				0,25	

After propoxazepam administration to experimental animals the increase of hypnotic effect manifestation had been noticed (the increase of sleeping animals number) what characterizes the studying compound as potentiating the barbiturate action. The mean effective dose of propoxazepam (table 1) in this test 1,39 ± 0,27 mg/kg (3,4 ± 0,7 μmol/kg) was higher in compare to the doses for other classical benzodiazepines and is close to that of clonazepam (~ 1 mg/kg). So, for nitrazepam, which pharmacological spectrum is mostly presented as hypnotic, this indicator is ~0.12 mg/kg and for phenazepam ~ 0.1 mg/kg. According to this indicator propoxazepam is close to substances which pharmacological spectrum has no significant hypnotic action.

The "dose-effect" curve slope (s) in the ranges of the mean effective dose is 0.25 what corresponds to rapid increase of effect and reversible (receptor-dependent) interaction mechanism.

In the thiopental-induced sleeping test, propoxazepam at 1,0 1,4 2,0 and 2,8 mg/kg doses showed significant reducing in the sleeping time onset as well as duration of sleep in a dose-dependent manner. The results were found to be statistically significant ($p < 0.05$) (Table 1).

The thiopental-induced sleeping latency time of animals shows no statistically significant difference depending on the propoxazepam dose administered (Fig. 1). Only at propoxazepam doses 1.0 and 2.0 mg/kg the calculated significance level ($p \leq 0,06$ and $p \leq 0,06$ correspondingly) approaches the significant values (at least 0.05). The latency time changes, possibly, are due to the individual features of animals and one have to mention that despite the different sleeping effect appearance frequency in experimental groups the effect itself is due to the thiopental concentrations in the brain.

At the same time the common duration of sleeping time of animals (Fig. 2) demonstrated individual differences (at doses 1.4 and 2.0 mg/kg) with certain trend of non-dose-dependent increase. So, in the range of doses 1.0-2.0 mg/kg there is nongradual increase of this indicator from 7,0 ± 0,7 min (control) to 18,5 ±10,6 min (2,0 mg/kg with highly deviating data). At the dose 2.8 mg/kg the mean sleeping time was 173 ±35 min, what corresponds to effect development at 100 % of animals and exceeded the supervision time.

According to the results of the study it was proved that propoxazepam can be regarded as substance which can potentiate the thiopental-induced sleeping in mice.

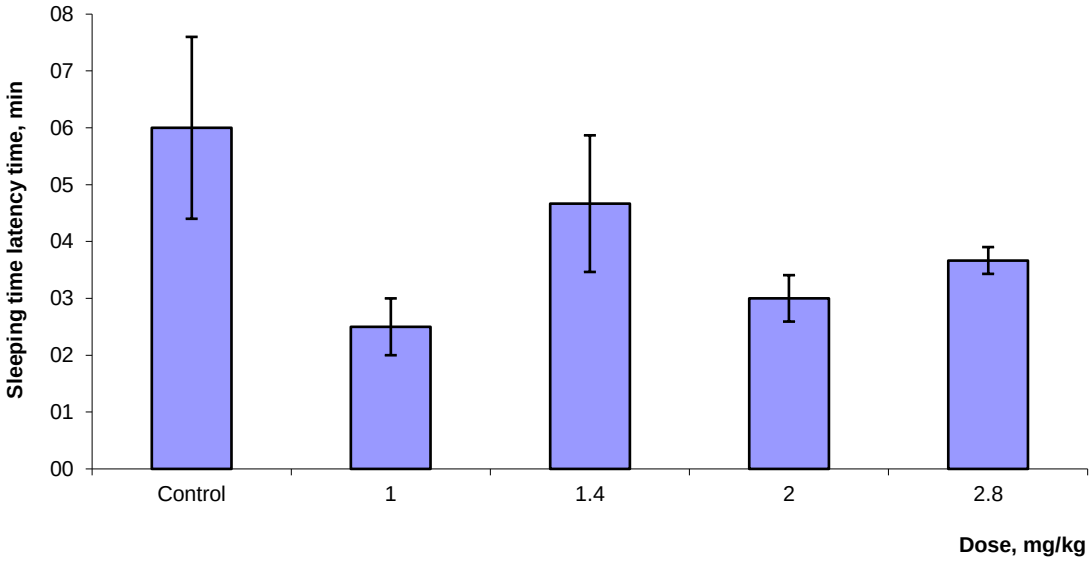

Figure 1: Effect of propoxazepam on thiopental– induced sleep latency in mice
Bars are mean values + S.E.M. (n=8).
***Indicates significant difference from control. p<0.05.**

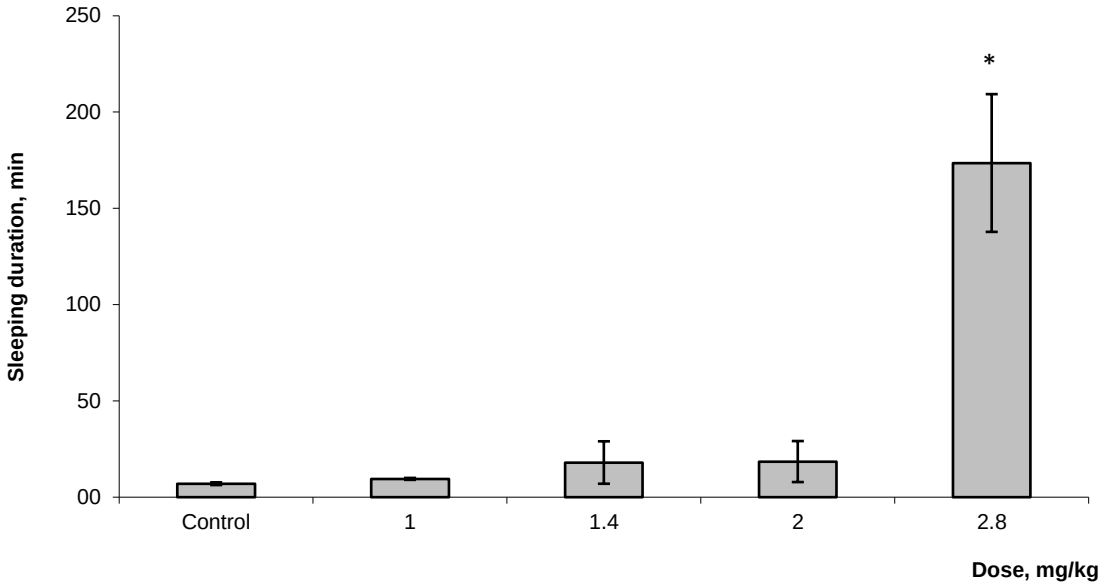

Figure 2: Effect of propoxazepam on thiopental – induced sleeping time in mice
Bars are mean values + S.E.M. (n=8).
***Indicates significant difference from control. p<0.05.**

The test of righting reflex

The loss of righting reflex is a widely used laboratory standard which allows to test anesthetic sensitivity in different animal spices [13]. In experiments on mice it was shown that from the dose 700 mg/kg propoxazepam induced torpidity of animals, their sluggishness and inactivity. At the dose 1100 and 1300 mg/kg the mice state could be characterized as "numbed", but the animals were at physiological state. After the attempting to give them the lateral position the animals immediately returned to usual state (at four extremities). The animal reacted on the external stimuli but in the case of their absence again turned "numbed". Thus propoxazepam at high doses demonstrates moderate sedative action which is not raises to hypnotic.

Rotarod test

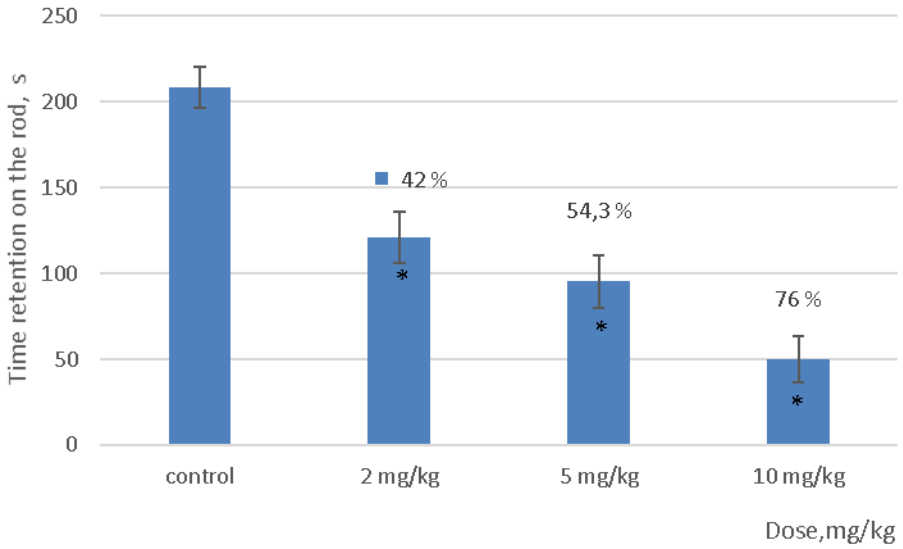

Figure 3: The influence of different doses of the studied substance of time retention (in min and % of decrease in compare to control) on rotarod.
Bars are mean values + S.E.M. (n=8).
***- Indicates significant difference from control. p<0.05.**

There is a dose-depending trend for reducing the time of retention on the rod: for experimental group of 2 mg/kg these indicator reduced on 42 %, for group of 5 mg/kg - on 54.3 % and for group 10 mg/kg - on 76 % in compare to control group (fig. 3).

In some cases myorelaxant properties of the compound can be positive, especially being combined with nociceptive action. In case of high impact of muscle spasm in pain formation the myorelaxants are prescribed [16]. It also have to be noted that muscle myorelaxants act is on the level of spinal cord but not on the muscles thus having different action points.

4. DISCUSSION

In the present study, we evaluated the hypnotic effects of propoxazepam for the first time. Also, the safety of this substance was examined by determination of LD_{50} in acute toxicity test.

Acute toxicity studies both with multiple dose studies are typically useful in evaluating the safety profile of innovative medicines. Many types of toxicity studies can be performed for evaluation of therapeutic agents` toxic effects that could pose threat to the lives of humans and animals. The traditional methods of determining toxic effects of chemicals and drugs include acute toxicity study [17] which is carried out to determine the short time toxicity effect of a toxicant (1 to 2 weeks), whereas subacute toxicity study is carried out to know the relative long term effect of a toxicant (4 weeks – 6 months).The present study demonstrated the preclinical safety of propoxazepam during acute administration in mice and rats. Mice and rats were used as two spices of experimental animals.

Under the experimental conditions, the results indicated that the median lethal dose (LD_{50}) of propoxazepam is greater than 5000 mg/kg of body weight. Thus, propoxazepam may be assigned to category V as the lowest class of toxicity according to the GHS. It should be noted that the toxicity of known 1,4-benzodiazepines drugs is significantly higher. Diazepam is 1240 mg/kg (rats), 720 mg/kg (mice); lorazepam is 3178 mg/kg (rats); nitrazepam is 980 mg/kg (rats); flunitrazepan is 415 mg/kg (rats); temazepam is 833 mg/kg (rats); alprazolam is 331-2171 mg/kg (rats); flumazenil is 100-300 mg. kg (mice), 100-1000 mg/kg (rats) [18].

We started our investigation for the sedative effects of propoxazepam by determining thiopental sodium-induced sleeping time and sleep latency, rotarod, and righting-reflex tests. Thiopental is a rapid-onset short-acting barbiturate general anesthetic that exerts its pharmacological effect on the central nervous system by enhancing inhibition of GABA-mediated neurotransmission The potentiation of thiopental-induced sleeping time was used to evaluate the possible sedative-hypnotic effects of propoxazepam. It is emphasized that the method employed for this assay is considered as a very sensitive way and denote agent with depressor activity on the CNS [19].

After propoxazepam administration to experimental animals the increase of hypnotic effect manifestations had been observed (increase of number sleeping animals), due to which the studied substance can be considered as potentiating the hypnotic effect of barbiturate. The “dose-effect” curve slope in the ranges of the mean effective dose is 0.25 what corresponds to rapid effect increase as well as concentration-dependent (receptor-mediated) mechanism of interaction.

The sleeping latency time after thiopental administration has no statistically significant changes in dependence of the propoxazepam dose administered (fig. 1). The total time of sleeping duration (fig. 2) has individual peculiarities (at doses 1.4 and 2.0 mg/kg) with certain trend to its increase in a non-dose dependent way.

The test of righting reflex estimation is widely used for coordination of motion and sedation. With the high doses of propoxazepam (700, 1100 and 1300 mg/kg, i.p.), a delay in the righting reflex was observed but not a total loss. Mice were defined as having a loss of the righting reflex when they were unable to right within 30 seconds after being placed on their back. The duration of the loss of the righting reflex was also assessed in the higher propoxazepam dose experiment by measuring how long after its administration it took mice to regain the ability to right within 30 s after being turned on their back. This study proved that propoxazepam potentiates sleep induced by hypnotics like thiopental, but cannot induce hypnosis by itself.

However, the myorelaxant effect was observed with doses 2,0; 5,0 and 10,0 mg/kg of propoxazepam which resulted in an increase in the number of falls and a decrease in the time on the bar as detected by the rotarod test.

The results of our study indicated that substance induce hypnotic effects. Because propoxazepam produce any effect on rotarod test, it seems that its effects on sleeping time and sleep latency are mediated by affecting motor movement.

The inhibition of motor coordination and induce hypnotic effects was possibly mediated through the $GABA_A$/benzodiazepine receptor and glycine inhibitory mechanisms [20].Similar observations we have been reported previously [5, 21].On the base of results of comparative quantile analysis for dose-effect data with different convulsions origin (picrotoxin, pentylenetetrazole, strychnine) there were distinguished stages of propoxazepam interaction with receptors of $GABA_A$ and glycine *in vivo*.

Propoxazepam could interact with the GABAergic system to induce its hypnotic effect, since it has been reported that several neurotransmitters and endogenous molecules are involved in regulation of sleep and wakefulness. The sleep–promoting neurons located in the anterior hypothalamus release GABA to suppress activity of wake–inducing areas of the brain [22]. Thiopental is known to act at GABA receptors ionophore complex and favor the biding of GABA. Also benzodiazepine agonists such as propoxazepam [4] and diazepam [23] enhance the affinity of GABA for its receptor and hence prolong pentobarbital – induced sleep duration.

Benzodiazepines and barbiturates might amend the GABA system, at the level of the synthesis of it by potentiating the GABA-mediated postsynaptic inhibition through an allosteric modification of $GABA_A$ receptors [24]. The increase in chloride flux or potentiated GABA-induced chloride conductance with concomitant inhibition of voltage activated Ca^{2+} channel [25]. Consequently, it is anticipated that propoxazepam may potentiate inhibition, caused by GABAergic mechanisms. It may enhance affinity for GABA or an increase in the duration of the GABA-gated channel opening [24].

5. CONCLUSION

In conclusion, our results revealed that propoxazepam possess potent sedative–hypnotic properties. Furthermore, the results of the present study showed that substance exhibit very low toxicity, which is proved by the high LD_{50} values for i.p. administration. Additionally, this illustrates that enhancement may result from chloride channel activation and $GABA_A$-ergic transmission. Thus, propoxazepam requires further investigation to understand the underlying pharmacological characteristics. The present findings provide compelling evidence to support a critical role of this compound as an attractive candidate in an improved treatment for CNS disorder, a concept which is currently being tested in multiple pre-clinical studies.

Author`s contribution:

Mykola Golovenko - literature analysis, experiment aim and design, results analysis, article writing
Vitalii Larionov - experiment design and conducting, results analysis, article writing
Anatoliy Reder - experiment aim and design, results analysis
Iryna Valivodz' - experiment conducting, data analysis
Zhanna Tsapenko - experiment conducting, data analysis

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Conflict of Interest:

The authors declare that there are no conflicts of interests.

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Data and materials availability:

All data associated with this study are present in the paper.

REFERENCE

1. Sieghart W. Pharmacology of benzodiazepine receptors: an update. *J Psychiatry Neurosci*1994;19(1):24–9.
2. GolovenkoN Ya., Voloshchuk N I., Andronati S A., et al. Antinociception induced by a novel benzodiazepine receptor agonist and bradykinin receptor antagonist in rodent acute and chronic pain models. EJBPS 2018;5:79-88.
3. Desai A, Kherallah Y, Szabo C, Marawar R. Gabapentin or pregabalin induced myoclonus: A case series and literature review. J Clin Neurosci2019; 61: 225–34.
4. Golovenko NYa., Larionov VB., Andronati S.A., et al. Pharmacodynamics of interaction between Propoxazepam and a GABA-benzodiazepine receptor-ionophor complex. Neurophysiology 2018. 50: 2-10.
5. Golovenko N Ya. Larionov V B., Reder A S., Valivodz I P. An effector analysis of the interaction of propoxazepam with antagonists of GABA and glycine receptors. Neurochemical Journal 2017; 11: 302–8.
6. VoloshchukN I., Reder A S., Golovenko MYa., et al. Pharmacological analysis of neurochemical antinociceptive mechanisms of propoxazepam action. Pharmacology and drug toxicology2017;1:3-11.
7. Larionov V B., Reder A S. Propoxazepam, a novel analgesic with multifunctional mechanism of action: review of preclinical data. International scientific and practical conference «Prospects for the development of medicine in EU countries and Ukraine», Wloclavek, Republic of Poland, December 21–22, 2018. P.111–5.
8. Andronati S A, Semenishyna E ., Pavlovsky V I, et al. Synthesis, structure and affinity of novel 3-alkoxy-1,2-dihydro-3H-1,4-benzodiazepin-2-ones for CNS central and peripheral benzodiazepine receptors. Eur J Med Chem 2010;45: 1346-51.

9. Organisation for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals (TG 407). Repeated Dose 28-Day Oral Toxicity Study in Rodents. OECD/OEDC.2008.
10. OECD. Harmonized integrated hazard classification system for human health and environmental effects of chemical substances. 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Part 2. Paris, France, 1998.
11. Turner R A. Screening methods in pharmacology, New York and London,Academic Press,1965: 64–9.
12. Fujimori H. Potentiation of barbital hypnosis as an evaluation method for central nervous system depressants. Psychopharmacologia 1965; 7: 374-81.
13. Bernhard Jänicke, Helmut Coper. Tests in rodents for assessing sensorimotor performance during aging. Advances in Psychology 1996;114: 201-33.
14. Aartsma-Rus A, van Putten M. Assessing functional performance in the mdx mouse model. Journal of Visualized Experiments 2014;e51303-e51303.
15. Dunham N W., Miya T S. A note on a simple apparatus for detecting neurological deficit in rats and mice, J. Am. Pharm. Assoc 1957;46 208–10.
16. Forde G. Adjuvant analgesics for the treatment of neuropathic pain: evaluating efficacy and safety profiles, Journal of Family Practice 2007;56:3–12,
17. Saganuwan S A. Toxicity studies of drugs and chemicals in animals: An overview. Bulgarian Journal of Veterinary Medicine 2017; 20, 4: 291–318.
18. Koella W P. Animal Experimental Methods in the Study of Antiepileptic Drugs. In: Antiepileptic drugs. Berlin, Heidelberg: Springer-Verlag, 1985; 283-340.
19. Charney DS, Mihic SJ, Harris RA. Hypnotic and sedatives. In:Hardman JG, Limbird LE, Gilman AG eds.Goodman &Gilman's, The pharmacological basis of therapeutics. 10th ed, Mac Graw-Hill, New York, 2001; 399-427.
20. Möhler H. A new benzodiazepine pharmacology. J Pharmacol Exp Ther 2002;300:2–8.
21. Golovenko M., Reder A., Andronati S., Larionov V. Evidence for the involvement of the GABA-ergic pathway in the anticonvulsant and antinociception activity of Propoxazepam in mice and rats. J Pre-Clin Clin Res 2019; 13: 99–105.
22. Datta S. Cellular and chemical neuroscience of mammaliansleep. Sleep Medicine 2010;11(5): 431–40.
23. Gottesmann C. GABA mechanisms and sleep. Neuroscience 2002;111: 231–239.
24. Sigel E. Mapping of the benzodiazepine recognition site on $GABA_A$ receptors. Curr Top MedChem 2002; 2:833–9.
25. Kun Xiang, Damien E. Earl, Kathleen M. Davis, et al. . Chronic benzodiazepine administration potentiates high voltage-activated calcium currents in hippocampal CA1 neurons. JPET 2008; 327:872–83.