UDC 615.272/451.16:582.893:547.262:615.214.24

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INTERACTION OF AEGOPODIUM PODAGRARIA L. (GOUTWEED) PREPARATIONS WITH CENTRAL NERVOUS SYSTEM DEPRESSANTS

This study addressed the influence of Aegopodium podagraria L. (goutweed) aerial part extract (100 mg/kg and 1 g/kg) and tincture (1 and 5 ml/kg) on the course of ethanol-induced as well as thiopental sodium induced narcosis. It has been shown that the extract at a dose of 1 g/kg, but not 100 mg/kg, significantly reduces the duration of ethanol-induced narcosis in male mice, the extract at a low dose decreases the onset of the loss of righting reflex. The tincture does not influence on the course of ethanol-induced narcosis, but significantly reduces the duration of sleep caused by thiopental sodium in male mice (at a dose of 1 ml/kg, but not 5 ml/kg), by this effect it is comparable with Hypericum perforatum L. extract at a dose of 100 mg/kg. Goutweed extract does not influence on the duration of thiopental-induced sleep.

Key words: goutweed (Aegopodium podagraria L.); extract; tincture; central nervous system; ethanol; thiopental sodium; mice

INTRODUCTION

The intensive search of the new drugs is being conducted now among the substances of herbal origin, including extracts and tinctures from herbal raw material. Their advantages, namely the polytropic mechanism of action as well as favourable accompanying effects are due to multi-component composition [21]. Still the latter may cause unwanted pharmacological effects so the problem of safety verification is crucial, especially for drugs that are planned to be used in chronic diseases. Besides the investigation of the possible side effects, it is reasonable to estimate the possibility of herbal substances interaction with the commonly used drugs.

Our research is directed to the verification of the pharmacological properties of the preparations obtained from Aegopodium podagraria L. (goutweed, GW). This perennial plant of the Apiaceae family since time immemorial has been used in traditional medicine for the treatment of kidney diseases, gastrointestinal and metabolic disorders including gout and related states, and consumed as vegetable [6]. GW is ubiquitous and the raw material is available for drug manufacturing.

Dry extract and tincture obtained from GW aerial part exert beneficial effects on purine and carbohydrate metabolism, the extract also possesses significant nephroprotective and hepatoprotective activity [5, 11]. Their influence on anxiety, depressive behaviour, locomotor activity, exploratory behaviour and memory of male and female mice has been described recently [25]. The extract exerts dose-dependent and sex specific antidepressive effect with the worsening of the results of the passive avoidance test. At a dose of 100 mg/kg it tends to reduce anxiety signs in the animals of both sexes, in male mice such reduction is also seen under the influence of the extract (1 g/kg) and the tincture (1 and 5 ml/kg). Besides, the favourable metabolic effects of GW preparations are shown in ethanol-treated rats [10].

However, the comparative study of GW preparations interaction with the central nervous system (CNS) depressants has not yet been conducted. The expediency of such research is determined by the possible interaction between the herbal components (including substances present in GW) and psychotropic drugs, that may be realized at the level of pharmacokinetics as well as pharmacodynamics so this research allows to complete the data on the spectrum of GW pharmacological properties. Ethanol and thiopental sodium are commonly used for the studies of herbal drugs interactions [3, 16].

Therefore, the aim of this study was to verify the results of GW preparations interaction with CNS depressants, namely ethanol and thiopental sodium.

MATERIALS AND METHODS

A. podagraria dry extract and tincture were obtained using the standard technology (that was described previously [5, 11) in accordance with the requirements of State Pharmacopoeia of Ukraine.

Adult random-bred male mice (body weight 16-20 grams) were kept in the Central Scientific-Research

Laboratory of National University of Pharmacy under standard conditions. All the experimental protocols were in accordance with "Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes."

In each of the two series of the experiment the mice were randomly divided into 5 groups:

1. Intact control (IC);
2. Animals receiving GW extract, 100 mg/kg;
3. Animals receiving GW extract, 1 g/kg;
4. Animals receiving GW tincture, 1 ml/kg;
5. Animals receiving GW tincture, 5 ml/kg.

GW preparations were administered intragastrically using the prophylactic regimen, the extract was dissolved in distilled water and ethyl alcohol was previously removed from the tincture. The mice of the intact control groups received distilled water by the similar scheme. The interval between the administration of the last dose of GW preparations and CNS depressants equalled 40 min.

In the study of GW drugs influence on ethanol narcosis, the duration time of sleep was registered as time between the loss and recovery of righting reflex after intraperitoneal administration of ethanol as a 12.5 % solution at a dose which caused narcotic sleep lasting 1-2 hours in intact mice [9]. The preliminary course of GW preparations administration lasted 7 days (similar to the previous experiments) [10].

The influence of GW drugs on thiopental sodium induced narcosis was studied using the protocol similar to the described above. After course administration (12 days) of the investigated preparations, the mice received thiopental sodium intraperitoneally at a dose of 65 mg/kg that caused sleep in the most of intact mice [16]. The quantity of mice in which the sleep developed, the onset and duration of loss of righting reflex were recorded. Besides the groups of the mice mentioned above, in this study two additional groups were used to compare GW preparations effects with that of reference drugs, namely Hypericum perforatum L. (St. John's wort, Clusiaceae) and Passiflora incarnata L. (Passifloraceae) extracts. H. perforatum extract ("Deprivit" from Kyiv Vitamin Factory, Ukraine) was used as a drug with verified ability to stimulate CNS and a dose of 100 mg/kg intragastrically which was effective in experiments [19] was used. P. incarnata extract ("Alora" syrup from NOBEL ILAC Sanayii ve Ticaret A. S., Turkey) was administered at a dose of 300 mg/kg that was effective in the study [15]. This preparation is used as a reference drug, as there was similarity in the reduction of anxiety signs in animals receiving P. incarnata and GW extracts [25]; In addition, in the study of the sodium thiopental interactions it is expedient to use well studied and clinically significant herbal drug which main effects are anxiolytic and sedative, along with St. John's wort extract, which mainly exerts activatory effect on the CNS [19].

Taking into consideration the modern recommendations on biological and medical data analysis [28], medians, 25 % and 75 % percentiles (upper and lower quartiles) were calculated. Traditionally used means ± standard errors of the mean (SEM) were also shown (M ± m). Statistical differences between groups were analysed using the Mann-Whitney U test (taking into account a problematical character of multiple comparisons in pharmacology and toxicology) and the Fisher angular transformation. The level of significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

The results show that GW preparations do not change the onset of loss of righting reflex in ethanol-treated mice, except for extract at a dose of 100 mg/kg, which significantly reduced this value (Fig. 1). GW extract at a dose of 1 g/kg, but not 100 mg/kg, exerts a anti-alcohol effect significantly reducing the duration of ethanol-induced narcosis in mice (Fig. 2, data novelty has been confirmed [8]). As described in [8], under the conditions of single ethanol administration to rats the extract at both doses does not have a negative influence on CNS and decreases the number of grooming acts as an indicator of emotional reactions. In contrast to the extract, the tincture does not influence on the course of ethanol-induced narcosis.

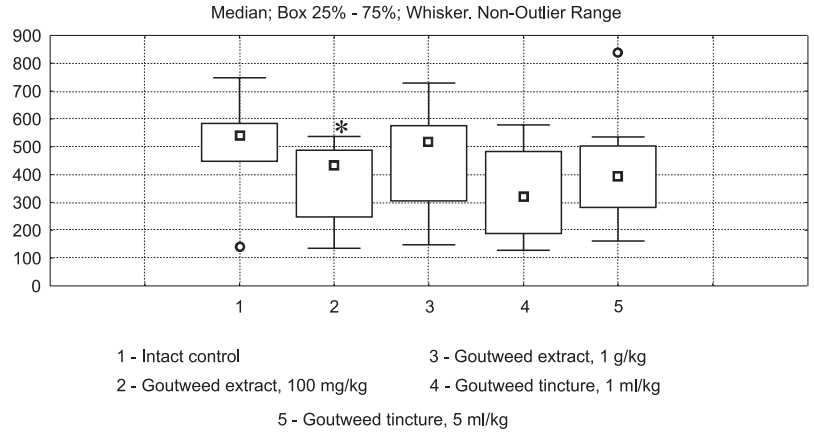

Fig. 1. *The influence of goutweed preparations on ethanol-induced sleep onset in mice, s; * – Significant at p < 0.05 compared with intact control group.*

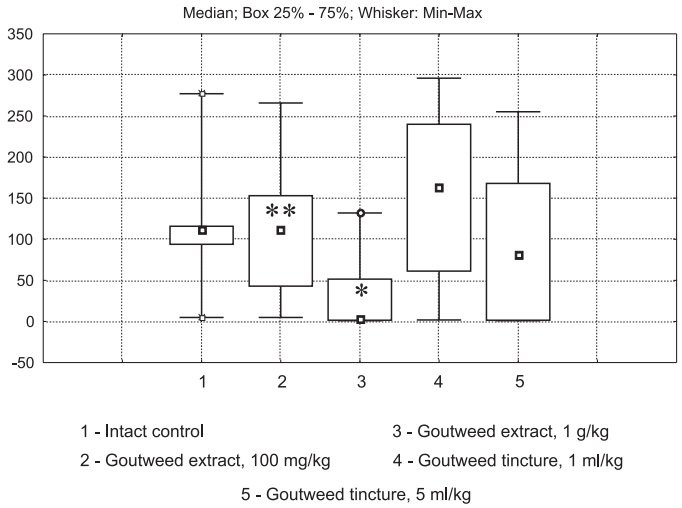

Fig. 2. *The influence of goutweed preparations on ethanol-induced sleep duration in mice, min;*
** – Significant at p < 0.02 compared with intact control;*
*** – Significant at p < 0.02 compared with the group receiving the extract at a dose of 1 g/kg.*

This test is recommended for anti-alcohol drugs screening and it is an integral one as narcotic sleep course depends both on ethanol concentration in the blood and CNS functional state [9]. The data in the literature evidence that herbal biologically active substances (BAS) can favourably change ethanol pharmacokinetics with a decrease in its content in the blood (namely, the extracts of Inula sp., Asteraceae, which also reduce depressant effects of ethanol on CNS, and the activity is associated with flavonoids [7]; numerous saponins as well as the extract of Hovenia dulcis Thunb. (Rhamnaceae), which reduce ethanol adsorption in the stomach, the latter preparation also activates alcohol dehydrogenase and decreases acetaldehyde content in the liver and blood [13, 26].

However, the herbal preparations mentioned above are not comparable in the main constituents with GW drugs. At the same time, information on the influence of the substances present in GW on the course of ethanol-induced narcosis (in contrast with information on their protective action against hepatotoxic and gastrotoxic effects of ethanol) is limited. Phenolic compounds are believed to determine the efficacy of Sorbus commixta Hedl. (Rosaceae) extract, which mainly maintains the activity of catalase and reduces ethanol adsorption [20]. Data on the protective effect of flavonoids are also available. Increased survival rate was shown in rats treated with these compounds at a dose of 50 mg/kg on the model of single ethanol administration [1]. Dihydromyricetin (1 mg/kg intraperitoneally) decreases the duration of ethanol-induced narcosis and counteracts negative neurotropic effects associated with withdrawal syndrome through the influence on the benzodiazepine site of GABA-A receptors [23]. The important flavonoids of GW are quercetin, kaempferol and its glucoside trifolin. Organoprotective activity of the latter has been verified at a dose of 50 mg/kg that is comparable with the dose of GW extract 1 g/kg [7], so it seems probable that flavonoids contribute to the anti-alcohol activity of GW extract. In addition, hydroxycinnamic acids are among the important constituents of GW extract and the protective effect of sodium ferulate against ethanol-induced hepatotoxicity is registered at a dose of 100 mg/kg [27]. As mentioned in [10], animals receive 50 mg/kg of hydroxycinnamic acids with GW extract at a dose of 1 g/kg, indicating a possible role of these compounds in the protective activity of the extract. Since ethanol was administered intraperitoneally and GW preparations – intragastrically, nonspecific influence on ethanol adsorption is hardly involved into anti-alcohol effect of the extract. Pharmacokinetic interaction is possible at the level of the liver, and it is a dose of 1 g/kg of the extract that has shown hepatoprotective effect [5]. At the same time the influence on the CNS with the reduction in duration of immobility as well as anxiety signs was seen in mice receiving the extract at a dose of 100 mg/kg [25]. Therefore, a mechanism of the extract anti-alcohol effect is more likely to be realized through favourable influence on metabolic processes in the liver (still further research it needed).

In addition to ethanol, barbiturates such as thiopental sodium are widely used to study the possible interactions of herbal drugs [3, 16]. As can be seen from the table, GW ext-ract as well as reference drug passionflower extract does not change the duration of thiopental-induced sleep and even a tendency towards its prolongation was seen against the background of high dose of the former. In contrast to these preparations, GW tincture at a dose of 1 ml/kg, but not 5 ml/kg, as well as St. John's wort extract significantly reduce sleep duration. Besides, all investigates drugs, except for GW extract and tincture at high doses, substantially decreased the number of animals, in which thiopental sodium was able to cause sleep at the used dose, but did not change the onset of the loss of righting reflex.

Table

THE EFFECT OF GOUTWEEED AND REFERENCE DRUGS ON THIOPENTAL-INDUCED SLEEPING TIME IN MICE, Q50 (Q25-Q75), M ± m

Group	Onset of sleep, seconds	Duration of sleep, minutes	Number of mice in which the sleep developed, % (absolute quantity)
Intact control, n = 8	220 (166-274) 251 ± 52	13 (9-100) 60 ± 27	88 (7/8)
Goutweed extract, 100 mg/kg, n = 8	234 (223-281) 270 ± 46	19 (4-64) 49 ± 36	50 (4/8)*
Goutweed extract, 1 g/kg, n = 6	224 (178-269) 223 ± 27	39 (16-92)& 70 ± 44	67 (4/6)
Goutweed tincture, 1 ml/kg, n = 8	294 (265-329) 300 ± 21	1.4 (0.7-2.3)**# 1.6±0.6	50 (4/8)*##
Goutweed tincture, 5 ml/kg, n = 6	201 (181-255) 215 ± 37	33 (21-116)& 66 ± 29	100 (6/6)
Passiflora extract 300 mg/kg, n = 6	309 (227-325) 265 ± 61	13 (8-41) 28 ± 21	50 (3/6)##
Hypericum extract, 100 mg/kg, n = 7	194 (138-270) 205 ± 35	1.5 (1.0-4.2)*** 2.9 ± 1,1	71 (5/7)##

* – Significant at $p < 0.05$ compared with intact control group; ** – Significant at $p < 0.01$ compared with intact control group; *** – Significant at $p < 0.005$ compared with intact control group; # – Significant at $p < 0.05$ compared with the group of animals receiving goutweed tincture at a dose of 5 ml/kg; ## – Significant at $p < 0.01$ compared with the group of animals receiving goutweed tincture at a dose of 5 ml/kg; & – Significant at $p < 0.05$ compared with the group of animals receiving Hypericum extract.

The vast majority of the published research interpret the prolongation of thiopental-induced sleep as as a marker of sedative and hypnotic activity [16, 18] and the opposite effect – as an evidence of CNS stimulation. The latter is described for the extracts of Operculina turpethum (L.) Silva Manso (Convolvulaceae) and Rosmarinus officinalis L. (Lamiaceae), and the activity is partially associated with phenolic compounds [14], [12] (it is not expedient to address the numerous data on herbal drugs containing alkaloids as they are not comparable with GW).

At the same time, the influence of the herbal compounds on thiopental sodium pharmacokinetics seems to be more probable than the pharmacodynamic interactions mentioned above. It is known that thiopental is metabolised through the formation of inactive substances involving NADH, intermediate carriers of electrons and cytochrome P-450 as well as by desulfurization with the formation of phenobarbital, which can contribute to post-narcosis sleep prolongation [4]. The duration of barbiturate-induced sleep is commonly used as a marker of liver detoxification function status [2]. Since many herbal BAS are able to exert a significant influence on the activity of liver enzymes, including cytochromes [29], the result of interaction crude herbal medicines is difficult to predict. The reference drug – St. John's wort extract – is a well-known cytochromes inducer [29] and in our study it significantly reduced sleep duration as well as the number of animals in which thiopental sodium caused sleep (Table). The latter value was also affected by passionflower extract, however, it did not change the duration of sleep. In the study [24] augmentation in thiopental-induced sleep duration was seen after intraperitoneal administration of Passiflora incarnata L. extract, so the difference with our results may be determined by pharmacokinetic factors.

The influence of the certain herbal BAS on thiopental-induced narcosis course is not sufficiently studied (as it is with ethanol-induced narcosis), complicating analysis of GW phytopharmacological properties. It is unlikely that flavonoids are involved in counteraction of GW to hypnotic effect, as some of flavonoid glycosides (at intraperitoneal administration) are, on the contrary, able to potentiate barbiturate-induced sleep [17], and in rats with flavonoid-deficiency its duration decreases [22].

Acknowledgements. The author would like to acknowledge Central Scientific-Research Laboratory of National University of Pharmacy and researcher T. K. Yudkevich for hosting the research as well as postgraduates K. O. Kalko and V. V. Tsyvunin for the support and helpful discussions.

CONCLUSIONS

Aegopodium podagraria L. extract at a dose of 1 g/kg, but not 100 mg/kg, significantly reduces the duration of ethanol-induced narcosis in male mice, the extract at a dose of 100 mg/kg decreases the onset of the loss of righting reflex, Aegopodium podagraria L. at doses of 1 and 5 ml/kg does not change the course of ethanol-induced narcosis.

Aegopodium podagraria L. tincture at a dose of 1 ml/kg, but not 5 ml/kg, significantly reduces the duration of sleep caused by thiopental sodium in male mice, by this effect it is comparable with Hypericum perforatum L. extract at a dose of 100 mg/kg. Aegopodium podagraria L. extract does not influence on the duration of thiopental-induced sleep.

The extract and the tincture of Aegopodium podagraria L., despite their in principle antagonising effect to the CNS depressants, show differences in these effects that may be due to the distinct mechanisms of narcosis development as well as biotransformation of ethanol and sodium thiopental.

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УДК 615.272/451.16:582.893:547.262:615.214.24

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ВЗАЄМОДІЯ ПРЕПАРАТІВ ЯГЛИЦІ ЗВИЧАЙНОЇ (AEGOPODIUM PODAGRARIA L.) З РЕЧОВИНАМИ, ЩО ПРИГНІЧУЮТЬ ЦЕНТРАЛЬНУ НЕРВОВУ СИСТЕМУ

Досліджено взаємодію екстракту (100 мг/кг та 1 г/кг) та настойки (1 та 5 мл/кг) надземної частини яглиці звичайної з етанолом та тіопенталом натрію. Показано, що екстракт у дозі 1 г/кг, але не 100 мг/кг вірогідно зменшує тривалість наркозу, індукованого етанолом у мишей-самців, екстракт у меншій дозі зменшує латентний період наркозу (за критерієм настання бічного положення). Настойка не впливає на перебіг етанолового наркозу, але значимо зменшує тривалість сну, спричиненого тіопенталом натрію у мишей-самців (у дозі 1 мл/кг, але не 5 мл/кг), за вираженістю цього ефекту настойка є порівнюваною з екстрактом звіробою Hypericum perforatum L. у дозі 100 мг/кг. Екстракт яглиці не змінює тривалість тіопенталового сну.

Ключові слова: яглиця звичайна (Aegopodium podagraria L.); екстракт; настойка; центральна нервова система; етанол; тіопентал натрію; миші

УДК 615.272/451.16:582.893:547.262:615.214.24

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ВЗАИМОДЕЙСТВИЕ ПРЕПАРАТОВ СНЫТИ ОБЫКНОВЕННОЙ (AEGOPODIUM PODAGRARIA L.) С ВЕЩЕСТВАМИ, УГНЕТАЮЩИМИ ЦЕНТРАЛЬНУЮ НЕРВНУЮ СИСТЕМУ

Изучено взаимодействие экстракта (100 мг/кг и 1 г/кг) и настойки (1 и 5 мл/кг) надземной части сныти обыкновенной с этанолом и тиопенталом натрия. Показано, что экстракт в дозе 1 г/кг, но не 100 мг/кг достоверно снижает длительность наркоза, индуцированного этанолом у мышей-самцов, экстракт в меньшей дозе уменьшает латентный период наркоза (по критерию перехода в боковое положение). Настойка не влияет на течение этанолового наркоза, но значимо снижает длительность сна, вызванного тиопенталом натрия у мышей-самцов (в дозе 1 мл/кг, но не 5 мл/кг), по выраженности этого эффекта настойка сравнима с экстрактом зверобоя Hypericum perforatum L. в дозе 100 мг/кг. Экстракт сныти не изменяет длительность тиопенталового сна.

Ключевые слова: сныть обыкновенная (Aegopodium podagraria L.); экстракт; настойка; центральная нервная система; этанол; тиопентал натрия; мыши

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Надійшла до редакції 18.12.2015 р.