Possibility of correction of functional disorders with pectin substances central nervous system under conditions of methanol intoxication. Experimental research

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Possibility of correction of functional disorders of the central nervous system by pectin substances in conditions of methanol intoxication. Experimental study

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SUMMARY

The article presents the results of a study on the effect of pectin substances obtained from the inflorescences of Gaillardia pulchella Foug; inflorescences of Tagetes patula L; herbs Sorbaria sorbifolia L; leaves Ribes nigrum L; leaves of Grossularia reclinata L; leaves of Lysimachia punctata L., on the change in neurological and sensorimotor deficits, as well as the functional activity of brain mitochondria in rats against the background of acute methanol intoxication. The results obtained indicate that the introduction of all studied pectin substances at a dose of 100 mg / kg (per os) reduced neurological and sensorimotor disorders in animals and restored the reactions of energy metabolism in the brain tissue,

Key words: poisoning, methyl alcohol, pectin substances, mitochondria,plant objects.

RESUME

The article presents the results of a study of the effect of pectin's obtained from Gaillardia pulchella Foug inflorescences; Tagetes patula L inflorescences; Sorbaria sorbifolia L herb; Ribes nigrum L leaves; Grossularia reclinata L leaves; Lysimachia punctata L leaves at a dose of 100 mg / kg (per os) on changes in neurological and sensorimotor deficits, as well as functional activity of brain mitochondria in rats against the background of acute methanol intoxication. The results show that the administration of all the studied pectin substances reduced neurological and sensorimotor disorders in animals, and, in addition, restored the energy exchange reactions in the brain tissue, which makes these compounds promising objects for further study for use as adjuvant therapy for methyl alcohol poisoning.

Keywords: intoxication, methyl alcohol, pectin substances, mitochondria, plant objects.

INTRODUCTION

Methyl alcohol is a well-known surrogate for illegal spirits. The problem of consuming low-quality alcoholic beverages is especially acute, since cheap sources of surrogate alcohol, in particular methanol, are becoming more and more available. The result of falsification of alcoholic beverages is a growing number of poisonings, including fatalities.

Methanol is a clear, colorless, volatile liquid that is infinitely miscible with water and belongs to the homologous series of monohydric alcohols. Methyl alcohol is widely used in industry as a solvent, in the oil and gas sector and in chemical synthesis. Due to its low cost, simple synthesis and wide availability, methanol is increasingly used in the production of alcohol substitutes, which can lead to acute or chronic methanol intoxication [1]. In Russian federation

Methanol poisoning occurs in more than 60% of cases in a severe form, while methanol intoxication is most often observed in people of working age (36.7 ± 4.8 years) [2].

Methyl alcohol is known as a neurotoxic agent, which causes the clinical picture of intoxication. Signs and symptoms of acute methanol poisoning include weakness, blurred vision, nausea, vomiting, headache, epigastric pain, shortness of breath, and cyanosis. Methyl alcohol intoxication can be caused by accidental or deliberate use, including suicidal attempts, abuse, or misuse [4].

The toxicity of methanol is primarily due to its metabolites, formaldehyde and formic acid. Formic acid causes severe metabolic acidosis that can be fatal. Formaldehyde, in turn, causes dissociation of oxidation and phosphorylation reactions, which leads to a lack of ATP and contributes to the development of severe encephalopathy [3].

For the treatment of methanol poisoning, the following are most often used: a competitive metabolic agent - ethyl alcohol; alcohol dehydrogenase inhibitors - fomepizole; oral adsorbents [17]. Methanol poisoning therapy also involves the use of enterosorbents [5].

In connection with the previously established adsorptive and detoxifying properties of fruit and beet pectin, we suggested that a number of pectin substances obtained from medicinal plant materials would also have a positive effect on the functional state of the central nervous system (in rats). It seemed to us expedient to conduct a study of pectin substances obtained from six types of medicinal plant raw materials (sources of pectin) for the possibility of their use in the treatment of methanol intoxication [6].

The rationale for the choice of plant objects was the data indicating the presence of pectin substances in them, as well as their widespread use in folk medicine in case of intoxication and the absence of poisonous and potent components in them (Table 1).

Table 1

The use of research objects in traditional medicine (in alphabetical order)

Russian and Latin name plants and families	Morphological group of raw materials	Actions and applications	Bibliography a source
Small-flowered marigolds -Tagetes patula L. Fam. Asteraceae	Flowers (inflorescences)	Antimicrobial, antiseptic, hypotensive, diuretic and anti-inflammatory	[7]
Spotted verbeynik - Lysimachia punctata L. Fam. Primulaceae	Leaves	Used for articular rheumatism, gastrointestinal upset	[eight]
Gaillardia beautiful - Gaillardia pulchella Foug, Fam. Asteraceae	Flowers (inflorescences)	Anti-inflammatory and hepatoprotective	[nine]
Common gooseberry - Grossularia reclinata L. Fam. Grossulariaceae	Leaves	It is used for pulmonary tuberculosis, pneumonia, arthritis and osteochondrosis	[7]
Fieldfare mountain ash -Sorbaria sorbifolia L. Fam. Rosaceae	Grass	Used when gastrointestinal disorders, skin inflammations, lung diseases and migraines	[ten]

Black currant - Ribes nigrum L. Fam. Grossulariaceae	Leaves	Disinfectant and anti-inflammatory	[7]

The purpose of the study was to assess the effect of pectin substances isolated from raw materials Tagetes patula L .; Gaillardia pulchella Foug .; Sorbaria sorbifolia L .; Grossularia reclinata L .; Ribes nigrum L .; Lysimachia punctata L., on changes in the functional state of the central nervous system (CNS) in rats against the background of acute methyl alcohol intoxication.

MATERIALS AND METHODS

The study was carried out on 80 male Wistar rats (mature, weighing 220–250 grams). The animals were obtained from the Rappolovo laboratory animal nursery (Leningrad region) and were quarantined for 14 days before being included in the study. At the time of the experiment, the rats were kept in macrolon cages, five animals each, with free access to food and water. Wood pellets were used as bedding material and were changed daily. The conditions for keeping experimental animals met the requirements of Directive 2010/63 / EC of the European Parliament and of the Council for the protection of animals used for scientific purposes (dated 23.09.2010): ambient temperature 18-22 ° C, relative humidity 60-70 %, with a daily cycle of 12 hours - day, 12 hours - night.

Pectin substances were isolated from dried medicinal plant materials of 6 plant species (Table 1) gravimetrically using the method of N.K. Kochetkova and M. Sinner. The meal remaining after the isolation of water-soluble polysaccharides was further extracted in a water bath at 100 ° C and constant stirring for an hour with a mixture of 0.5% ammonium oxalate solution and 0.5% oxalic acid solution (1: 1). The resulting extract was filtered and an equal volume of ethyl alcohol 95% was added to it to precipitate pectin substances. A precipitate formed, which, depending on the starting material, was colored from dark pink to dark brown [11].

Acute methanol intoxication in rats was simulated by oral administration of methyl alcohol (Vekton, Russia) at a dose of 3 g / kg [12]. The studied pectin substances were administered to animals per os through an atraumatic probe at a dose of 100 mg / kg 4 hours after the introduction of methyl alcohol [13].

According to the study design, the following experimental groups were formed: IU - intact animals; NK - a group of negative control rats that did not receive pharmacological support; groups of animals that were injected with the studied pectin substances. The number of rats in each experimental group was equal to 10 individuals.

The effect of the studied pectin substances on the state of the central nervous system in rats was assessed 24 hours after methanol administration by determining the magnitude of the sensorimotor and neurological deficits, as well as by assessing the functional state of the brain mitochondria [14–16].

The severity of neurological symptoms in rats was determined according to the McGraw scale according to the sum of the corresponding points (Table 2). At the same time, the score of 0.5–2.0 corresponded to a mild degree of neurological deficit; 2.5–5.0 - moderate neurological deficit; 5.5–10 severe neurological deficits [14].

table 2

Indicators of the McGraw scale in determining neurological deficits (according to [14])

The investigated parameter	Score
Lethargy	0.5
Tremor	1.0
Unilateral half-tosis	1.0
Bilateral half-tosis	1.5
Inability to withdraw a limb while holding it	1.5
Unilateral ptosis	1.5
Bilateral ptosis	1.5
Manege movements	2.0
Paresis of 1-4 limbs	2.0-5.0
Paralysis of 1-4 limbs	3.0-6.0
Coma	7.0
Death	10.0

Sensomotor deficit in animals was assessed using the "Beam walking" test. The setup consisted of a tapering track 165 cm long with sides for fixing the fall of the animal's limbs and a dark chamber at the end of the track. The starting point of the animals was illuminated with bright light, motivating the animal to move towards the final goal - a dark chamber. Preliminarily (before the introduction of methanol and the development of intoxication), the animals were trained in the testing procedure for 4 days. 24 hours after methanol administration, the rats were retested to determine the degree of sensorimotor deficit, while the number of complete sets of limbs on board and the number of slippages were recorded. Sensomotor deficit was calculated using the formula [15]:

Sensomotor deficit,% = number of complete limbs positioning - 1 +
the number of slips - 0.5 / the total number of steps - 100% The functional

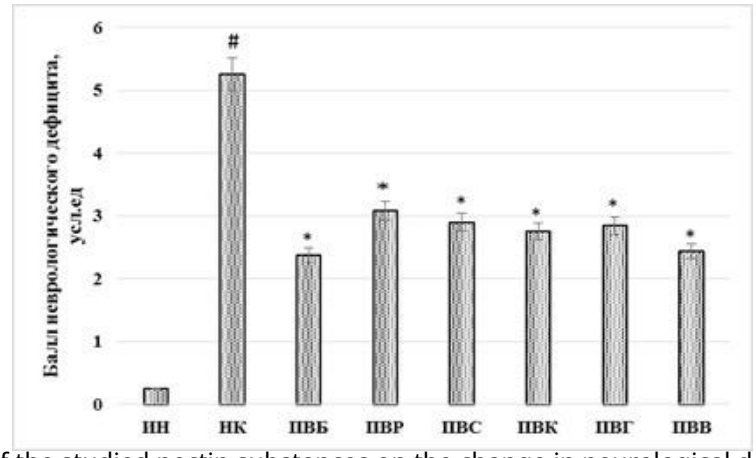
activity of the mitochondria of the brain was assessed by the method of respirometry using a laboratory respirometer AKPM-01L (Alfa Bassenas, RF) with the introduction of cellular respiration uncouplers into the analyzed medium: oligomycin 1 μg / ml; 4- (trifluoromethoxy) phenyl) hydrazono) malononitrile (FCCP) 1μM; rotenone – 1μM; sodiumazide –20mmol. During the analysis, ATP-generating capacity was determined (by the difference in oxygen consumption after the addition of FCCP and oligomycin); the maximum level of consumption and the difference in respiration capacity after the addition of FCCP and oligomycin); oxygen after the addition of FCCP and the base level of oxygen consumption). Biomaterial for respirometry was obtained by centrifugation of the PBS-homogenate of the head of the animals in the mode of 1,400g → 3 min at 4 ° C. The resulting supernatant was re-centrifuged at 13000g → 10 min. The secondary supernatant was removed for analysis [16].

Statistical processing of the data obtained was carried out using the STATISTICA 6.0 software (StatSoft). Data were expressed as M (mean) ± SEM (standard error of the mean). Comparison of groups was carried out by the ANOVA method with the Newman-Keisle post-test at $p < 0.05$.

RESULTS

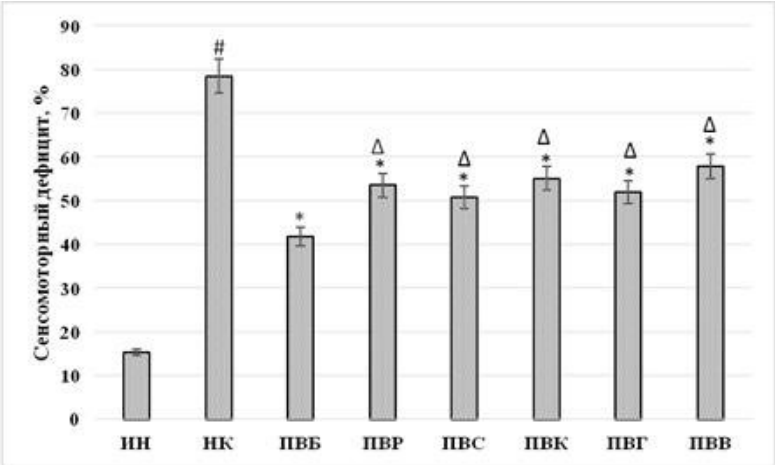
The results of evaluating the effect of pectin substances on the change in neurological deficit in rats under conditions of methanol intoxication are shown in Fig. 1. Thus, in the course of this study, it was found that in the NC group of animals in relation to intact rats, the degree of neurological deficit increased 21 times ($p < 0.05$). At the same time, the administration of the studied pectin substances to animals contributed to a decrease in the severity of neurological symptoms. So, against the background of the use of pectin substances obtained from the studied objects, the neurological deficit decreased relative to the NK of the group of rats by 54.8

% (p <0.05); 46.0% (p <0.05); 41.4% (p <0.05); 47.7% (p <0.05); 44.9% (p <0.05) and 53.6% (p <0.05), respectively (Fig. 1). It should be noted that there were no statistically significant differences between the groups of animals that received the studied pectin substances.



Rice. 1. The effect of the studied pectin substances on the change in neurological deficit in rats in conditions of methanol intoxication: IN - intact animals; NK - a group of negative control rats; PVB - a group of rats that received pectin substancesTagetes patula L .; PVR - a group of rats,receiving pectin Sorbaria sorbifolia L .; PVA - a group of rats that received pectinsubstances Ribes nigrum L .; PVK - a group of rats that received pectin substances from Grossularia reclinata L .; PVH - a group of rats that received pectin substances Gaillardia pulchella Foug .; PVV - a group of rats,receiving pectin Lysimachia punctata L .; # - statistically significant relative to IN groups of rats; * - statistically significant relative to the NC group of animals.

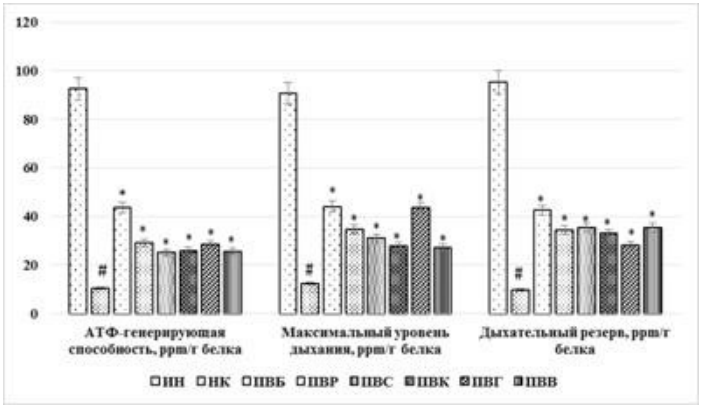
When analyzing the data obtained in the course of assessing the effect of the studied pectin substances on the change in sensorimotor deficit in rats with methanol intoxication, it was found that in the NK group of rats the sensorimotor deficit increased in relation to IN animals by 5.1 times (p <0.05) ... At the same time, in rats that received the studied pectin substances from Tagetes patula L., Gaillardia pulchella Foug., Sorbaria sorbifolia L., Grossularia reclinata L., Ribes nigrum L., and Lysimachia punctata L., the severity of sensorimotor deficiency was lower than that of NC -groups of animals, respectively, by 46.9% (p <0.05); 33.9% (p <0.05); 31.8% (p <0.05); 29.1% (p <0.05); 35.4% (p <0.05) and 26.2% (p <0.05) (Fig. 2).



Rice. 2. The influence of the studied pectin substances on the change in the sensorimotor deficit in rats in conditions of methanol intoxication (symbols are similar to Fig. 1, - - statistically significant relative to the group of animals that received pectin substances Tagetes patula L.)

In animals that received pectin substances obtained from Tagetes patula L., the sensorimotor deficit decreased in relation to animals that received Sorbaria sorbifolia L. pectin substances by 22.1% (p <0.05); Ribes nigrum L. - by 17.8% (p <0.05); Grossularia reclinata L. - 24.3% (p <0.05); Gaillardia pulchella Foug. - 19.7% (p <0.05) and Lysimachia punctata L. - by 28.0% (p <0.05).

During the assessment of the effect of the studied pectin substances on the functional activity of the brain mitochondria in rats, it was found that in the NK group of animals, the ATP-generating activity, the maximum level of respiration and the respiratory reserve decreased in relation to IN rats, respectively, by 8.8 times (p <0.05); 7.3 times (p <0.05) and 9.7 times (p <0.05). Against the background of the introduction of pectin substances Tagetes patula L., an increase (relative to the NK group of rats) of ATP-generating activity by 4.2 times (p <0.05) was noted; the maximum level of respiration - 3.6 times (p <0.05) and the respiratory reserve - 4.3 times (p <0.05). At the same time, the use of pectin substances obtained from Sorbaria sorbifolia L. promoted an increase in ATP-generating activity, the maximum level of respiration and respiratory reserve by 2.7 times (p <0.05); 2, 8 times (p <0.05) and 3.5 times (p <0.05), respectively. In rats that received pectin substances from Ribes nigrum L., ATP-generating activity, maximum respiration level and respiratory reserve exceeded the analogous indices of the NK-group of animals by 2.4 times (p <0.05); 2.5 times (p <0.05) and 3.6 times (p <0.05), respectively. The introduction to rats of pectin substances from Grossularia reclinata L., Gaillardia pulchella Foug., Lysimachia punctata L. also contributed to an increase in the parameters of cellular respiration, namely, ATP-generating activity in comparison with the NK group of animals increased, respectively, 2.5 times (p <0.05); 2.7 times (p <0.05) and 2.4 times (p <0.05); the maximum level of respiration increased, respectively, by 2.3 times (p <0.05); 3.5 times (p <0.05) and 2.2 times (p <0.05); respiratory reserve - 3, 4 times (p <0.05); 2.9 times (p <0.05) and 3.6 times (p <0.05), respectively (Fig. 3).



Rice. 3. Influence of the studied pectin substances on the change in functional activitybrain mitochondria in rats under conditions of methanol intoxication (legend are similar to Fig. 1)

DISCUSSION

During the experiment, it was found that a single injection of pectin substances obtained from the inflorescences of Gaillardia pulchella Foug; inflorescences of Tagetes patula L; herbs Sorbaria sorbifolia L; leaves Ribes nigrum L; leaves of Grossularia reclinata L; leaves of Lysimachia punctata L., equally reduced the manifestation of neurological deficit in animals, while the greatest effect on the change in sensorimotor deficiency was observed when animals were administered pectin substances obtained from the inflorescences of Tagetes patula L. From our point of view, positive functional changes on the part of the central nervous system in rats that were injected with the studied pectin substances, they can be mediated both by their high sorption activity and by the restoration of metabolic reactions in the brain tissue, which was

confirmed in the course of this study and is consistent with the literature data [18].

CONCLUSION

Thus, the study showed that the use of pectin substances obtained from the inflorescences of Gaillardia pulchella Foug; inflorescences of Tagetes patula L; herbs Sorbaria sorbifolia L; leaves Ribes nigrum L; leaves of Grossularia reclinata L; leaves of Lysimachia punctata L., can reduce the neurotoxic effects of methanol, which suggests further study of these objects for their antitoxic effect and the possibility of their use in methanol intoxication in clinical practice.

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