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ANALGESIC PROPERTIES OF DEALCOHOLIZED EXTRACT OF *ACORUS CALAMUS* LEAVES

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Opioid and non-narcotic analgesics, non-steroidal anti-inflammatory agents, anesthetics, antidepressants, myorelaxants, combined agents and phytopreparations are widely used for the treatment of pain syndrome. One of the promising phytogenic objects with potential analgesic properties is the Acorus calamus (Sweet Flag).
The aim: *the purpose of the study is to determine the analgesic effect of the dealcoholized extract of Acorus calamus leaves (DEAL) on a model of pain in the "Hot plate" test and in the test of tail heat immersion.*
Materials and methods. *During the experimental study, the pharmacological methods have been used. The analgesic properties of DEAL were studied in mice on the "Hot plate" model using the Hot / Cold Plate (Bioseb, France) and in the test of the heat immersion in rats.*
The results. *On the models of pain in the "Hot plate" and tail heat immersion tests, the analgesic effect of the dealcoholized extract of Acorus calamus leaves (DEAL) is determined. On the "Hot plate" model, the use of DEAL probably increased the duration of the latency period. According to the analgesic effect of DEAL and metamizol sodium were comparable to each other continues to 1 and 1.5 hours of experiment, but starting with 2 hours of experiment the analgesic action of metamizol sodium statistically exceeded the analgesic effect of DEAL.*
In the test of heat immersion tail in rats, DEAL increased the latency period of shocking of the rats' tail compared to the starting background by 43.13 % as well as metamizol sodium by 66.6 %. The studies have shown the presence of moderate analgesic effects of DEAL in the investigated dose.
Conclusions*. The analgesic effect of a dealcoholized extract of Acorus calamus leaves (DEAL) on a model of pain in the "Hot plate" and heat tail immersion tests has been carried out. Under the "Hot plate" test in mice, DEAL produces a distinct analgesic effect, however, slightly inferior to the severity of metamizol sodium. Presence of moderate analgesic properties of DEAL has been verified in comparison with the metamizel sodium in thetail heat immersion test in rats. The obtained results indicate the influence of DEAL on the central mechanisms of pain formation*
Keywords*: dealcoholized extract of Acorus calamus leaves, DEAL, analgesic action, "Hot plate" test, tail heat immersion test*

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1. Introduction

One of the most common symptoms in medicine is pain. Pain occurs when exposed to nociceptive factors or as a result of suppression of the antinociceptive system [1–3]. According to statistics, 11.3–40 % of visits to the doctor are made by patients with pain syndromes [4]. Chronic pain of various genesis, which affects one in five, is quite common [5, 6].

Non-narcotic analgesics, opioid analgesics, non-steroidal anti-inflammatory drugs, anesthetics, antidepressants, muscle relaxants, combined drugs, etc. are widely used for the treatment of pain syndrome [7].

Herbal medicines are also widely used in the treatment of pain, because according to the "WHO Strategy for Folk Medicine 2014–2023", it is indicated that about 80 % of the world's population uses herbal medicines [8].

This is due to the fact that the rich composition of plant BAS provides polyvalence of pharmacological effects [9]. This allows you to achieve maximum severity of the main therapeutic effects, i. e. gently and safely affect many systems of the body, which are somehow involved in the pathological process [10].

One of the promising phytoobjects with potential analgesic properties is Acorus calamus L.

There are data from experimental studies on the analgesic properties of azalea rhizome extract in thermal and mechanical hyperalgesia and in neuropathic pain caused by sciatic nerve damage [11], vincristine-induced neuropathic pain [12], acetic acid cramps, etc.

Most researchers believe that the analgesic properties of azalea due to the presence of azarone in its composition [11, 14]. However, azarin is associated with a number of toxic effects of Acorus calamus (carcinogenic, mutagenic action), which limits its widespread use in medicine [15, 16].

At the National University of Pharmacy, under the guidance of Professor TM Gontova, a dealcoholized extract of *Acorus calamus* leaves (DEAL) was obtained, the peculiarity of which is the absence of azarone. Phenolic compounds were identified in the dealcoholized extract of Acorus calamus leaves by HPLC: hydroxycinnamic

acids – caffeic acid, p-coumaric acid (content of the sum of hydroxycinnamic acids in terms of rosemary acid - 0.39 mg / 100 ml) and flavonoids, isoniones – 7-glucoside, apigenin and akacetin.

Previous studies for DEAL have shown anti-inflammatory, membrane-stabilizing effects, as well as no cytotoxic effect in in vitro studies on the human hepatocellular carcinoma line HepG2 and by MTT-test [17, 18].

The aim of the research was to determine the analgesic effect of DEAL on the model of pain in the test "hot plate" and in the test of thermal immersion of the tail.

2. Materials and methods

The experiments were conducted on the basis of the Training and Research Training Center for Medical and Biological Research of the Central Research Laboratory of the National University of Pharmacy (NUPh) in compliance with European Union Directive 2010/63/EU on the protection of animals used for scientific purposes (Directive 2010/63/EU of the European Parliament) [19] with the approval of the NUPh commission on bioethics (protocol No. 2 of 04.11.2019).

During the experiment, the animals were in the vivarium of the NUPh training center at an air temperature of 20–22 °C, natural light regime "day and night", in standard ventilated cages, on a standard diet [20].

The study used 24 outbred male mice aged 3.5–4.0 months, body weight 22.0±2.0 g and 24 white outbred male rats weighing 180–210 g.

DEAL was administered intragastrically at a dose of 1 ml/kg (conditional therapeutic dose for anti-inflammatory activity). The comparison drug was the traditional analgesic metamizole sodium (tablets "Analgin" produced by "Darnitsa" 500 mg) (at a dose of 50 mg/kg, which was administered intragastrically [21–23]. volume.

The central mechanisms of analgesic action of DEAL were studied in mice on the model "hot plate" [22–24] using the device Hot / Cold Plate (Bioseb, France). Randomization of animals was performed at baseline of latency period (LP), and animals in which LP exceeded 8 s were not included in the experiment [23]. The response to stimulation (bouncing, licking or shaking the hind paw) of experimental animals on a hot plate (+ 51 °C) was recorded in seconds [22]. In order to minimize tissue damage, the residence time of the animals on the hot plate was limited to 30 s. The test evaluated the changes in the pain threshold after administration of the test compounds after 30, 60, 120 and 180 minutes.

In rats, a study of the analgesic activity of DEAL in the test of thermal immersion of the tail was performed by immersing the tip of the tail of rats (5 cm) in hot water with a temperature of 55 °C [22, 25]. The duration (sec) of LP tail pulling was recorded. The criterion for the presence of analgesic action was considered a statistically significant increase in the duration of the latent period relative to the control group of animals.

The results of the experiment were analyzed using the program Statistica-6 (StatSoft, Inc., USA), determined the normality of the distribution using the W Shapiro-Wilk test. Under normal distribution, ANOVA analysis of variance was used, data expressed as M±m.

3. Results

In the “hot plate” test, it was found that in control animals, responses to thermal irritation did not change significantly throughout the observation period (Table 1), while the use of DEAL and metamizole sodium caused an analgesic effect.

Table 1

Analgesic effect of DEAL and metamizole sodium on the “hot plate” model in mice (M±m; n=8)

Group of animals	Duration of the latent period, p				
	Baseline	1.0 h	1.5 h	2.0 h	3.0 h
Control animals	4.38±0.42	3.5± 0.42	3.88±0.39	3.75±0.37	3.88±0.30
DEAL, 1 ml/kg	4.13±0.35	4.48±0.41	5.75±0.59*/**	7.13±0.55*/**	9.13± 0.51*/**
Metamizole sodium, 50 mg/kg	3.88±0.23	4.88±0.30*/**	6.25±0.37*/**	8.63±0.37*/**/#	15.38±0.71*/**/#

*Note: * – deviations are significant relative to the values of the original background of the group, p<0.05; ** - deviations are significant for the values of control animals for the relevant study time, p<0.05; # deviations are significant for the values of the group of animals treated with DEAL for the relevant study hour, p<0.05; n is the number of animals in the group*

Thus, compared with both the original background of the group and the group of untreated animals, the use of DEAL probably increased the duration of the latent period, starting with 1.5 h of the experiment (Table 1).

When using metamizole sodium, a statistically significant experiment was recorded during the increase of the latency period during all 3 hours, which indicates a pronounced analgesic effect. It should be noted that the analgesic effect of DEAL and metamizole sodium were comparable during the 1st and 1.5 hours of the experiment, but starting from 2 hours of the experiment, the analgesic effect of metamizole sodium was statistically higher than the analgesic effect of DEAL (Table 1). These results are consistent with data from other researchers who observed an increase in the analgesic effect of metamizole sodium from 2 hours of the research [23].

Analgesic properties of DEAL were confirmed in the test of thermal immersion of the tail in rats (Table 2).

Table 2

Analgesic effect of DEAL and metamizole sodium in the test of thermal immersion of the tail in rats (M±m; n=8)

Group of animals	LP of tail pulling (s)		
	Baseline	After 120 min	Changes relative to baseline, %
Control animals	6.25±0.37	5.75±0.25	-8 %
DEAL, 1 ml/kg	6.38±6.38	9.13±0.22*/**	+43.13 %
Metamizole sodium, 50 mg/kg	6.75±0.49	11.25±0.70*/**/#	+66.6 %

*Note: * – deviations are significant relative to the values of the original background of the group, p<0.05; ** – deviations are significant relative to the values of control animals for the relevant study time, p<0.05; # – deviations are significant relative to the values of the group of animals treated with DEAL, p<0.05; n is the number of animals in the group*

It was found that during the use of DEAL, latent period of pulling the tail of rats compared to baseline increased by 43.13 %, and with the use of metamizole sodium – by 66.6 %. Therefore, the study confirmed the presence of DEAL in the studied dose of moderate analgesic action.

4. Discussion

The mechanism of pain formation in the test of thermal immersion of the tail is due to the spinal flexor reflex due to the activation of C-fibers, Ad-fibers of polymodal nociceptors and high-threshold mechanoreceptors [22, 26]. In the "hot plate" test, the occurrence of pain is due to the activation of certain parts of the nociceptive system - thermoreceptors and nociceptors [20, 24, 25].

The presence of the analgesic effect of DEAL in these model injuries indicates a central link in the analgesic effect of the extract. Given the absence of azarone in the composition of DEAL, which to some extent has analgesic effects [14], we could assume that analgesia is realized by other mechanisms [18, 27].

Prostaglandin E2, histamine and a number of cytokinins are the main factors responsible for lowering the threshold of neuronal activation and peripheral neuronal sensitization [28].

Flavonoids have antioxidant, anti-inflammatory, analgesic, hypoazotemic, and hepatoprotective effects [29–31]. The realization of these effects is apparently due to their inhibition of NF-κB-dependent proinflammatory cytokines [32] and intercellular adhesion molecule 1 (ICAM-1) [33]. In addition, the COX-2 receptor site (as opposed to physiological COX-1) has an additional lateral hydrophilic cavity through which COX-2 could interact with flavonoids and similar compounds [30]. Due to flavonoids, flavonols and oxycinnamic acids, which are part of DEAL, the formation of prostaglandins E1, F2 and thromboxane A2 is reduced; capillary permeability decreases, which indirectly leads to a decrease in pain [27, 29, 31].

Obviously, the presence of these BAS to some extent explains the analgesic properties of dealcoholized extract of Acorus calamus leaves.

Study limitations. The studies were of a screening nature to establish the presence of an analgesic effect in DEAL in a limited number of male animals. Therefore, it is rational to investigate the dependence of the analgesic effect of Acorus calamus leaf extract on the age and sex of animals in a wide range of doses on other models.

Prospects for further researches. To further investigate the analgesic properties of Acorus calamus extract and to clarify the probable mechanisms of analgesic action of DEAL, it is advisable to conduct a series of pharmacological studies on models of formalin test, capsaicin test, acetic acid cramps, etc., as well as appropriate biochemical, immunohistochemical studies. The analgesic properties revealed in these studies and the anti-inflammatory and capillary-strengthening properties of the dealcoholized extract of Acorus calamus leaves established in previous experiments are the basis for the creation of new drugs of analgesic and anti-inflammatory action.

5. Conclusions

The analgesic effect of dealcoholic extract of Acorus calamus leaves (DEAL) on the model of pain in the tests "hot plate" and thermal immersion of the tail was determined. Under the test conditions, the "hot plate" in DEAL mice has a pronounced analgesic effect, which, however, is somewhat inferior to the severity of metamizole sodium. The presence of moderate analgesic properties of DEAL in comparison with metamizole sodium in the rat tail thermal immersion test was verified.

Evaluating the results of pharmacological studies of the analgesic action of DEAL on the models of "hot plate" and the test of thermal immersion of the tail, we could conclude that its implementation involves the central mechanism of analgesia.

Conflict of interests

The authors declare that they have no conflicts of interest.

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