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PROSPECTS FOR THE USE OF ACORUS CALAMUS EXTRACTS IN MEDICINE: GENERALIZED DATA FROM OWN RESEARCH

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Abstract

Despite significant advances in the treatment of inflammatory diseases of the gastrointestinal tract, the problem of pharmacological correction of this pathology remains quite relevant.

Thus, following the purpose of the research, we conducted a comprehensive experimental study of extracts of Acorus calamus leaves. It has been established that extracts, and especially DEAL, have pronounced antioxidant, membrane-stabilizing, hepatoprotective, antiulcer, gastroprotective effects. The presence of other pharmacological effects in the studied extract, in particular, actoprotective, antidepressant, and antimicrobial activity was also established. In our opinion, the multifactorial action of the studied extract is due to its powerful phytochemical composition, and primarily the presence of a significant amount of flavonoids, oxycinnamic acids, etc., which have a high antioxidant effect. Summarizing the results of pharmacological, toxicological, and physiological studies on the effectiveness of extracts of Acorus calamus leaves, we can conclude on the prospects of their further in-depth study for a new domestic anti-inflammatory, antiulcer, hepatoprotective, and actoprotective agent for prevention and treatment of human diseases.

Keywords: *Acorus calamus, anti-inflammatory, antiulcer, hepatoprotective, actoprotective* activity.

Despite significant advances in the treatment of inflammatory diseases of the gastrointestinal tract, the problem of pharmacological correction of this pathology remains quite relevant. Many drugs used in the pharmacotherapy of inflammatory pathology are characterized by several adverse reactions, which limits their use in polymorbid patients [21, 83, 73, 65, 62].

A promising way to solve this problem is the use of herbal remedies that af-fect some inflammatory patterns, reduce manifestations of oxidative stress, have a polymodal effect, have a sufficient raw material base, and are characterized by efficiency and safety [5, 4, 63, 6, 74, 75, 60].

After analyzing the phytochemical composition of Acorus calamus leaves and the obtained liquid hydroalcoholic extract of Acorus leaves (LHAEAL) and dealco-holized extract of Acorus leaves (DEAL), it can be stated of the presence of a pro-nounced antioxidant, anti-inflammatory, antiulcer, hepatoprotective capacity of BAS in their compositions, and the creation of drugs based on these extracts will expand and optimize phytotherapy of inflammatory diseases of the gastrointestinal tract and other diseases caused by oxidative stress [60, 4, 5].

Therefore, this work aimed to experimentally substantiate the possibility of using extracts of Acorus calamus leaf and to determine the points of therapeutic effect for pharmacocorrection of inflammatory diseases of the gastrointestinal tract.

At the first stage of our experiments, comparative in vivo and in vitro studies of the toxicological properties of hydroalcoholic extracts of Acorus leaves, Acorus rhizomes (extractant - 70% ethyl alcohol), and dealcoholized extracts were conduct-ed.

In modern toxicology, a significant role belongs to in vitro studies. This type of research can reduce the cost and time of previous studies, has good reproducibil-ity, allows accurate dosing of the substances entering the cell, and most importantly: in some cases is an alternative to animal studies [15, 70].

Among such studies, an important role belongs to the study of basic cytotoxi-city, which can manifest itself in the form of cell membrane disorders and mito-chondrial dysfunction.

In our experiments with the MTT assay on rat bone marrow cells and research on the HepG2-line human hepatocellular carcinoma cells using extracts of Acorus rhizome the presence of cytotoxicity caused by the destruction of cell membranes and mitochondrial dysfunction (increase in the cytotoxicity, with an increase in the contact time of cells with the rhizome extract from 15 to 90 minutes) and lack of cy-totoxic effect when using extracts of Acorus leaves have been found. A similar trend was observed when studying the effect of extracts on HepG2 cell culture.

The use of Acorus rhizome extract led to an increase in the number of dead cells, reducing the viability, and the cytotoxicity of this extract increased with in-creasing contact time with HepG2 cells to 72 hours. Acorus leaf extract showed no toxicity in the MTT test and HepG2 cell assay.

Most publications on the toxic effects of Acorus concern the effects of aza-rone [9, 32, 50]. Given the fact that in the dealcoholized extract of Acorus rhizome it is practically absent, we can assume that the toxic effect of this extract is realized by other BAS.

The next stage of our research was to study the anti-inflammatory activity of Acorus leaf extracts under conditions of carrageenan and zymosan edema in rats. These models allow assessing the impact of potential anti-inflammatory drugs on the main modulators of the inflammatory response [24, 57].

As a result of experiments, it was found that in the degree of inhibition of ex-udative processes in carrageenan edema throughout the study period, LHAEAL and DEAL have an antiexudative effect, but are somewhat inferior to diclofenac sodium.

The presence of anti-exudative action of the studied extracts, in our opinion, is due to the composition and synergistic effect of BAS (most of them are strong an-tioxidants) of the extracts. Based on own and literature data, we can assume that the anti-inflammatory effect of Acorus leaf extracts is mainly due to the inhibitory effect of polyphenols on the release of inflammatory mediators: histamine, kinins, and prostaglandins [41, 79]. This is confirmed by the significant antiexudative activity of the extracts from 2nd hour of the experiment. In leukotriene-induced edema caused by zymosan administration, DEAL activity

was higher than LHAEAL. The mem-brane-stabilizing properties of Acorus leaf extracts, the effect of which exceeded the effect of comparison drug quercetin, were also experimentally proved.

In our opinion, due to flavonoids, flavonols, and oxycinnamic acids that are part of the extracts, the process of adhesion and migration of leukocytes, the for-mation of prostaglandins E1, F2, and thromboxane A2 is reduced; also reduced is capillary permeability induced by inflammatory mediators and microtraumas.

These same BAS contribute to reducing the intensity of the processes of pro-teolysis, lipolysis in the inflammatory focus. Polyphenolic compounds that are part of the leaf extract can inhibit the activity of hyaluronidase, reduce the intensity of free radical oxidation. It is known that the receptor site of COX-2 (as opposed to physiological COX-1) has an additional lateral hydrophilic cavity, through which the interaction of COX-2 with flavonoids and similar compounds can take place [40]. All this explains the anti-exudative properties of Acorus leaf extracts.

Also, anti-inflammatory and membrane-stabilizing activities are inherent in oxycinnamic acids, primarily rosmarinic, ferulic, caffeic, and p-coumaric acids, for which antioxidant, membrane-stabilizing, anti-inflammatory, cerebroprotective ac-tivities have been established [5, 4].

The universality of the action of the components of Acorus leaf extract was proved in the model of vascular permeability caused by the introduction of various phlogogens (formalin, zymosan, histamine, and undiluted egg white).

It is known that the phlogogenic effect of formalin is associated with its direct effect on the endothelium of capillaries, and as a result of endothelial dysfunction increases the permeability of plasma proteins, water, electrolytes through the mem-branes. Histamine and egg white promote the release of inflammatory mediators, which indirectly involve microcirculation disorders. According to the degree of re-duction of vascular permeability, the effectiveness of the drugs varied according to the quantitative content of DEAL, 1 mL/kg; > LHAEAL, 1 mL/kg > DEAL, 0.5 mL/kg> LHAEAL, 0.5 mL/kg> quercetin, 11 mg/kg> diclofenac sodium, 8 mg/kg. A similar trend was maintained under the action of extracts in McClure-Aldrich tests and the effect on spontaneous hemolysis by the method of F. C. Jager.

This is probably due to the following. It is known that one of the factors dam-aging the membrane structures of cells, mitochondria, endoplasmic reticulum, etc. is the excessive activation of FRO processes [69, 34, 61, 64]. The degree and severity of inflammatory and destructive processes depend on the depth of damage to the membrane apparatus of cells [12]. Due to the high content of flavonoids, hy-droxycinnamic acids, and other compounds with proven antioxidant activity in the extracts of Acorus leaves [5], the intensity of FRO processes decreases, cytodestruc-tion decreases, which leads to a decrease in vascular permeability.

The anti-exudative properties of quercetin are well known. Quercetin is an ac-tive inhibitor of leukotriene formation [39, 2, 46, 53, 16]. Quercetin is also able to bind to serotonin receptors and competitively inhibit the effect of the latter on in-flammatory processes [39]. It has a much weaker effect on the cyclooxygenase sys-tem.

The next step was to study the antiulcer effect of Acorus leaf extracts on mod-els of gastric ulcer in mice caused by acetylsalicylic acid, reserpine ulcer in mice, and alcohol-prednisolone gastric ulcer in rats.

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used drugs in the world. About thirty million people worldwide take NSAIDs every day, with 40% of these patients over the age of 60 [38].

The mechanism of NSAIDs action is due to inhibition of prostaglandin (PG) synthesis [22]. The latter are involved in the formation of inflammatory processes (formed mainly by the enzyme COX-2) and affect the synthesis and formation of protective factors in the stomach, improve microcirculation in the kidneys, etc. (formed mainly by COX-1). A side effect of non-selective NSAIDs is NSAID gas-tropathy, which manifests itself in the form of erosion and gastric ulcers, dyspepsia, and pain syndrome, etc. [11].

Most NSAIDs are weak acids and in the acidic environment of the stomach, they acquire the ability to penetrate the lipid membranes of epithelial cells. In an al-kaline intracellular environment, NSAIDs turn into an ionized form, can accumulate locally in relatively high concentrations, and cause so-called NSAID gastropathy. The local action of NSAIDs

promotes the development of reverse diffusion of hy-drogen ions; the development of cellular acidosis with subsequent changes in epithe-lial cell metabolism is a characteristic manifestation of peptic lesions with the devel-opment of pain and dyspeptic syndromes [1, 27].

Typical of NSAID-induced gastrointestinal lesions is the appearance of small single ulcers or multiple superficial hemorrhagic erosions of the mucous membrane. According to many researchers, the incidence of such lesions in the endoscopic ex-amination can be about 11% at low doses of ASA and reach 30% when using other non-selective NSAIDs. For example, in the United States alone in the 1990s, up to 107,000 patients with ulcerative hemorrhage or perforation caused by NSAIDs were hospitalized each year, with a mortality rate of 10% to 15% [71].

We have found a gastroprotective effect of Acorus leaf extracts and quercetin comparison drug in aspirin ulcers in mice, and antiulcer activity was higher when using DEAL than LHAEAL. The latter is due, in our opinion, to the irritating effect of ethanol, which is part of LHAEAL, on the gastric mucosa. The gastroprotective effect of extracts is due to their antioxidant and cytoprotective effects [5].

Reserpine lesions of the stomach are somewhat isolated from other drug-induced gastric lesions. Reserpine belongs to the group of sympatholytics [44]. It disrupts the uptake and deposition of neurotransmitters (primarily norepinephrine and serotonin) in the presynaptic terminals of neurons with a simultaneous disrup-tion of its transport, which quickly depletes the reserves of mediators. These pro-cesses take place against the background of increased gastric acid secretion and vio-lation of the reverse diffusion of hydrogen ions through the gastric mucosa.

Reserpine promotes the release of catecholamines and serotonin, which affect the vessels of the stomach, causing them to spasm, and reduce the protective proper-ties of the gastric mucosa. According to signs of damage to the mucous membrane of the gastrointestinal tract may appear as early as 12-18 hours after taking reserpine [31].

At alcohol-prednisolone ulcer, the combination of 2 factors of aggression is observed. Ethanol causes microcirculation and permeability disorders; degeneration and superficial desquamation of the epithelium; neutrophilic infiltration and intersti-tial hemorrhage.

Glucocorticosteroids cause ulceration mainly due to COX blockade [58].

The high effectiveness of the preventive action of DEAL in model lesions of the stomach is apparently due to the presence in its composition of oxycinnamic ac-ids and flavonoids, which stimulate the protective factors of the central nervous sys-tem, and especially the antioxidant compartment system. The latter is confirmed by a decrease in the level of TB-AP in the stomach (almost to the level of intact animals), an increase in GSH content, and an improvement in the energy supply of cells. BAS of the extract can improve microcirculation in the gastric mucosa and stimulate re-generation, which are the main components of the implementation of anti-ulcerogenic action. Some researchers believe that the antioxidant properties of fla-vonoids are related to their ability to penetrate the lipid layer of biomembranes [66].

In addition, there is other evidence of the protective effect of DEAL under ethanol-induced lesions. It is known that ethanol metabolites, and especially acetal-dehyde, form complexes with proteins, disrupt DNA repair, deplete glutathione and other intracellular antioxidants, separate oxidation, and phosphorylation, intensify FRO processes, etc. [73].

The cytochrome P450-dependent microsomal ethanooxidation system (MEOS) plays a significant role in ethanol metabolism. However, with alcohol abuse or other toxicants, the generation of free radicals by microsomal monooxygenases increases significantly, which enhances the process of cytodestruction.

If the functional activity of the components of the antioxidant system is insuf-ficient, then free radical damage to biomolecules and intensification of lipid peroxi-dation causes a cascade of damage to subcellular and cellular structures [67].

To confirm the antioxidant properties of DEAL, we have studied its antioxi-dant, anticytolytic, anticholestatic properties under conditions of paracetamol and ethanol-tetrachloromethane liver damage. Paracetamol (acetaminophen), which is the most common over-the-counter antipyretic

analgesic, is one of the most common hepatotoxic drugs in adult patients [29, 49].

It is known that after the use of paracetamol in therapeutic doses, about 90 % of the drug is converted in the liver into pharmacologically inactive conjugates - glucuronide-acetaminophen and sulfate-acetaminophen [36]. About 5-10% of the drug may undergo an oxidation reaction with the formation of an intermediate active metabolite of N-acetyl-p-benzoquinonymine (NAPQI). The latter, in addition to ac-tivating the processes of free radical oxidation (FRO), causes the release of the apoptosis-inducing factor with subsequent induction of hepatocyte apoptosis [37]. Under conditions of reduced levels of reduced glutathione, the toxic effect of NAPQI increases, which leads to the development of toxic hepatitis [19, 81].

According to K. Tajiri, Y. Shimizu, paracetamol is a notorious "leader" among all drugs that cause DILI. This drug accounts for 16.9 % of deaths from drug-induced liver injury [47, 35].

It has been experimentally proven and confirmed by histomorphological stud-ies that the use of DEAL in paracetamol hepatitis reduced the intensity of FRO and cytolysis and increased the activity of the antioxidant system, as evidenced by the increase in GSH levels. According to histomorphological studies, the use of DEAL reduces the severity of inflammatory reactions in hepatocytes and cell alteration, re-duces the degree of hydropic, and carbohydrate and fatty degeneration of hepato-cytes, and helps normalize the structure of the organ.

Under the conditions of ethanol-tetrachloromethane hepatitis DEAL also showed hepatoprotective activity. It is known that the metabolism of carbon tetra-chloride produces free radicals: active trichloromethyl radical (CCl3-) and highly re-active trichloromethyl peroxyl radical (CCl3OO-), which change the structural and functional properties of membranes. As a result of the accumulation of oxidized li-pids and the formation of low molecular weight toxic products of oxidation of membrane lipids, carbon tetrachloride causes damage to hepatocytes [14, 59].

It is known that any pathological process (regardless of the etiological factors of liver damage) that develops within the liver parenchyma and occurs with damage to hepatocytes and/or bile ducts, may be accompanied by cholestatic syndrome. In the pharmacotherapy of cholestatic syndrome, herbal and synthetic hepatoprotective agents with choleretic activity such as silymarin, allochol, cholosas, ursodeoxycholic acid, and others are often used [10, 8].

It should be noted that against the background of the use of DEAL there was an improvement in bile-forming and bile-excreting functions of the liver, which in our opinion is due to the presence in the extract of a wide range of biologically ac-tive substances - hyperoside, oxycinnamic acids and other BAS with this type of ac-tivity [68]. Improvements in the hepatobiliary system and gastric mucosa may be due to the presence of rutin, the content of which, as noted above, in the DEAL is quite high.

Rutin due to its antioxidant activity can increase the activity of endogenous antioxidant enzymes, reduce damage to internal organs in toxic lesions, have a membrane-stabilizing effect, and increase bile secretion [51]. Decreased bilirubin levels, especially indirect, indicate the preservation of the functional activity of hepatocytes with the use of DEAL, silymarin, and ademethionine.

The hepatoprotective properties of ademethionine are due to the fact that it is a precursor of many thiol compounds - glutathione, cysteine, taurine and provides a redox mechanism of detoxification of hepatocytes [80].

Antioxidant and membrane stabilizing properties are also inherent in silymarin [23, 18]. The antioxidant effect of the drug is due to the interaction of silibinin with free radicals in the liver and their conversion into less toxic compounds. This inter-rupts the process of free radical oxidation of lipids, which prevents the destruction of cellular structures. In addition, silymarin stimulates the synthesis of proteins and phospholipids in the affected liver cells, improves lipid metabolism, has a detoxify-ing effect.

Typical markers of cholestasis are alkaline phosphatase and γ-glutamyltranspeptidase [52, 33]. The decrease in the activity of AP and γ-GGTP against the background of the use of DEAL and comparison drugs indicates a de-crease in the cytodestruction of plasma membranes of hepatocytes.

It should be noted that against the background of the use of DEAL there was an improvement in bile-forming and bile-excreting functions of the liver, which in our opinion is due to the presence in the components of a wide range of biologically active substances, including oxycinnamic acids and other BAS.

Improvements in the hepatobiliary system and gastric mucosa may be due to the presence of rutin, the content of which, as noted above, in the DEAL is quite high. Rutin due to its antioxidant activity can increase the activity of endogenous an-tioxidant enzymes, reduce damage to internal organs in toxic lesions, has a mem-brane-stabilizing effect, and increases bile secretion [45, 26].

Many patients with diseases of the gastrointestinal tract usually have astheno-neurotic and astheno-depressive disorders [48].

The WHO epidemiological study "Global Burden of Disease Study 2017" states that mental and neurological disorders occupy one of the leading places in prevalence and morbidity among the working population of the planet [13].

According to the WHO, about 4.4% of the population suffers from depres-sion, and it occurs more often among women than among men (5.1% vs. 3.6%) [76]. In this case, depression may be an independent disease or be secondary to oth-er pathologies.

In studies by Stepanishchev L.A, et al. data on the incidence of anxiety and depression in patients with peptic ulcer disease are presented. The researchers have found that anxiety was present in 48.2% of patients, depressive disorders - in 39.3% of examined patients, of whom two-thirds had a subclinical level of depression. Cal-culation of the odds ratio (OR) for the risk of depression and anxiety development in patients with UD showed a high probability of these psycho-emotional disorders [43].

According to TT Haug et al., 60-85% of chronic diseases of the digestive sys-tem are accompanied by emotional disorders of varying severity [28].

Antidepressants are widely used to treat such patients. However, this group has several side effects, including hepatotoxic reactions, gastropathy, gastrointestinal bleeding, etc. [77]. Therefore, the use of drugs that have gastro- and hepatoprotec-tive effects in such patients is appropriate.

A drug that combines the properties of hepatoprotector and antidepressant is ademethionine, which we have chosen as a comparison drug.

Ademethionine has antioxidant, membrane-stabilizing, and hepatoprotective effects, as well as able to penetrate the blood-brain barrier and participate in the for-mation of neurotransmitters - catecholamines (dopamine, norepinephrine, adrena-line), serotonin, melatonin, histamine.

One of the stages of our work was to study the effect of DEAL on the func-tional state of the CNS in intact animals and under conditions of reserpine-induced lesions, which somewhat expands the scope of work.

Our experimental work was based on the publication of Pandy V. in which the antidepressant activity of methanolic extract of Acorus calamus leaves was in-vestigated in experimentally induced models of depressive states in mice. The re-searcher found that methanolic and acetone extracts of Acorus calamus leaves may contain psychoactive substances, which by their nature are CNS depressants [20]. In studies by Hazra et al., it was established that BAS of Acorus rhizome have a seda-tive effect and cause behavior modification [30].

We have compared the effect of DEAL on the behavioral responses of mice in the open field test, the manifestations of depression in the immobilization test, the course of thiopental anesthesia, and physical endurance in the test "swimming with load".

In contrast to the data of other researchers, we found a dose-dependent stimu-latory effect of DEAL on locomotor, indicative research activity, as well as a moder-ate actoprotective effect [17]. An analeptic effect has been established for DEAL, which may probably be due to antagonism with barbiturates. In terms of neurotropic activity, DEAL exceeded the effect of the Ginkgo biloba drug - "Bilobil".

Ginkgo biloba preparations are powerful activators of the cognitive sphere, they improve the blood supply to the brain and have both nootropic and vasotropic effects [72].

The differences between the action of DEAL and the above-described extracts of Acorus are in the qualitative and quantitative composition of BAS.

DEAL does not contain azarone, associated with the psychosedative effect of sweet flag [30]. At the same time, DEAL has a high content of identified oxycinnamic acids, primarily caffeic acid. Literature has described that the use of the latter restores the volume ve-locity of cerebral blood flow in the simulation of total cerebral ischemia, while it does not significantly affect the performance of systemic hemodynamics [78].

In the thymoleptic effect in the model of reserpine depression in rats DEAL is not inferior to the comparison drug, dry extract of St. John's wort (Due to its com-plex, multi-component composition (hypericin, hyperforin, flavonoids, xanthones, etc.) St. John's wort is widely used not only in psychoneurology, but also in psy-chosomatic disorders) and probably exceeds the effect of ademethionine.

It is known that flavonoids, in most cases flavones, can interact with different zones of GABA-α-receptors and, therefore, affect their functioning.

In our opinion, the neurotropic properties of DEAL under the reserpine-induced depression in rats can be attributed to the presence of apigenin in DEAL.

Due to the high affinity for central benzodiazepine receptors, flavonoid apig-enin has anxiolytic and antidepressant effects [3]. Apigenin also exhibits neuropro-tective properties by activating astrocytes and reducing the production of interleu-kins (IL) - IL-31 and IL-33 [82].

For caffeic acid studies [55] have found the presence of anxiolytic action without changes in locomotor activity in open field tests; for rosmarinic acid, the presence of anxiolytic and (with increasing dose) stimulating effect on the CNS [42] has been found.

Therefore, given the proven gastro- and hepatoprotective effects of DEAL, the use of the extract in patients with astheno-depressive disorders against the back-ground of gastrointestinal pathology is pathogenetically justified and experimentally proven.

Thus, following the purpose of the research, we conducted a comprehensive experimental study of extracts of Acorus calamus leaves.

It has been established that extracts, and especially DEAL, have pronounced antioxidant, membrane-stabilizing, hepatoprotective, antiulcer, gastroprotective effects.

The presence of other pharmacological effects in the studied extract, in particular, actoprotective, antidepressant, and antimicrobial activity was also established.

In our opinion, the multifactorial action of the studied extract is due to its powerful phytochemical composition, and primarily the presence of a significant amount of flavonoids, oxycinnamic acids, etc., which have a high antioxidant effect.

Summarizing the results of pharmacological, toxicological, and physiological studies on the effectiveness of extracts of Acorus calamus leaves, we can conclude on the prospects of their further in-depth study for a new domestic anti-inflammatory, antiulcer, hepatoprotective, and actoprotective agent for prevention and treatment of human diseases.

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