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A Screening Study of Hepatoprotective Activity of Liquid Extract from Common Tansy *Tanacetum vulgare* L. Herb in the setting of Subchronic Hepatitis in Rats

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ABSTRACT:
The number of patients with pathology of the hepatobiliary system is growing steadily every year, and the main etiological factors that cause the disease are action of drugs, organic solvents salts of heavy metals, and environmental pollution. Regardless of etiology of hepatobiliary system diseases, given common features of their pathogenesis, the leading role in treatment belongs t hepatoprotectors. *The aim of the study* is to substantiate the existence of the hepatoprotective properties of liquid extract from common tansy (Tanacetum vulgare) herb. The task set is solved b studying hepatoprotective activity of liquid extract from common tansy herb on the model subchronic liver damage. A pathology model was reproduced by introduction of carbon tetrachlorid in combination with alcohol. The liquid extract of common tansy herb tested was administered in the therapeutic mode at doses of 25, 50, 75, 100, and 150 mg/kg. Hepatoprotective activity wa assessed by extract' effect on regulation of prooxidant-antioxidant imbalance and activity of transminases, cytolysis markers. A beneficial effect of liquid extract of common tansy herb on th course of subchronic toxic hepatitis at a dose-leader – 75 mg/kg has been determined, as evidenced by increased content of reduced glutathione (1.4-fold) accompanied by significant decrease o transminases (1.3-fold) and decreased content of TBA-AP (1.5-fold). The mechanism of hepatoprotective action of liquid extract of common tansy herb is probably conditioned by binding o toxic free radicals and stabilizing of cell membranes by phenolic compounds present in its composition (luteolin, rutin, apigenin, apigenin-7-glucoside, hyperoside, chlorogenic, caffeic, ferulic and gallic acids). Further studies of liquid extract of common tansy herb at a dose of 75 mg/kg aimed at development of a hepatoprotective agent is recommended.

KEYWORDS: liver, hepatoprotectors, common tansy, herb, liquid extract.

INTRODUCTION:

In spite of progress in hepatology and medicine in general, diseases of the hepatobiliary system take the lead among the diseases of gastrointestinal tract organs [1,2]. Hepatoprotectors are widel used in treatment of liver and biliary tract diseases; their integrated pharmacological effect is a complex of antioxidant, membrane-stabilizing, choleretic, anti-inflammatory and othe activities[3,4,5]. Today, there is a fast-growing interest in phytotherapy in modern medicine, as herbal drug products are characterized by multifaceted pharmacological activity and usually hav low toxicity[6,7]. According to the WHO data, almost 75% of patients prefer phytopreparations[8]. Common tansy (*Tanacetum vulgare* L.) is especially noteworthy among medicinal plants; it is perennial herbaceous plant of Compositae family (*Asteraceae*), which is widespread almost all over Ukraine with significant reserves of raw materials. Officinal raw material of this plant i flowers of common tansy, which are used as an agent that improves digestive tract activity by stimulating biliary excretion and increased secretion of gastric juice[9,10]. However, according t many scientists[9,10,11], tansy herb is no less important medical raw material of this plant, which is characterized by possible hepatoprotective activity[12,13], however, this type of action has nc been studied experimentally yet. This prompted to carry out studies of the substance from common tansy herb, liquid extract, for the presence of its hepatoprotective activity. The aim of th study: to substantiate presence of hepatoprotective properties of liquid extract of common tansy herb in the setting of experimental subchronic hepatitis.

MATERIALS AND METHODS:

Liquid extract of common tansy herb (LECTH) under conventional name of "Tanahol" is a liquid dark brown substance with a distinct camphor odor, obtained at Botany Department of th NPhaU by postgraduate student M. Yu. Zolotaikina under the guidance of Professor T. M. Gontova. The extract studied was obtained by the method of fractional maceration; the extractant use was ethanol 70 %; the extraction was carried out for 14 hours. Purification was carried out by staged filtration, and evaporation was carried out under vacuum until the ratio of 1:1. LECTH wa standardized by the method of spectrophotometry in terms of phenolic compounds' content, namely, the sum of flavonoids equivalent to luteolin and the sum of hydroxycinnamic acid equivalent to chlorogenic acid[14]. In addition, phytochemical composition was investigated by HPLC method, and it was found that LECTH contains luteolin, rutin, apigenin, apigenin-7 glucoside, hyperoside, chlorogenic, caffeic, ferulic, and gallic acids[15].

A study of LECTH hepatoprotective action was conducted on the model of subchronic toxicity of liver damage due to carbon tetrachloride (CTC) in combination with alcohol[16], which wa reproduced by a single s.c. administration of a 50% oil solution of carbon tetrachloride at a dose of 0.4 ml/100 g in rats with subsequent i.c. administration of 40% ethyl alcohol. The above mentioned mode of toxicant administration was repeated for 4 days. To determine hepatoprotective activity, LECTH at doses selected for screening, namely, 25,50,75,100 and 150 mg/kg, wa administered in rats once a day 7 days before modeling of the pathology (preventive mode). During production of the pathology, the extracts were administered 1 h before carbon tetrachlorid administration and in 2 h after it (therapeutic and prophylactic mode). After the last administration of the toxin (day 4 of pathology modeling), LECTH was administered once a day, last time a hour before decapitation. As a comparison drug product, hepatoprotector Carsil (Sopharma, Bulgaria) was selected (the content of silymarin per tablet is 22.5 mg) in a dose of 100 mg/kg[17]. Th choice of the comparison drug, Carsil, is substantiated by its marked hepatoprotective activity of this drug product, which is confirmed by data of evidence-based medicine[3].

Laboratory animals (rats with body weight of 190-230 g) were subdivided into groups, eight rats each: intact control; model pathology; groups of animals which received LECTH at doses of 25 50, 75, 100, and 150 mg/kg. The choice of this range of doses for the extract studied is substantiated by the desire to explore manifestations of pharmacological properties of the extrac depending on the dose administered.

Hepatoprotective activity of the extract studied was evaluated by changes in the activity of enzymatic markers of cytolysis (ALT and AST) and the degree of imbalance in lipid peroxidation antioxidant defense (LPO-AOD) system (RG, TBA-AP). 72 hours after the last administration of toxins, rats were decapitated followed by obtaining serum with further determination of th

indexes studied. The activity of ALT and AST was determined by a standardized dinitrophenylhydrazine Reitman-Frankel method[17]. In liver homogenate, the content of RG (modification of C L. Ellman method) was determined as a component of AOD system and marker of LPO activity–TBA-AP content (in terms of thiobarbituric acid)[18].

Statistical processing of the results obtained was carried out using software "Statistica 8.0". Non-parametric Mann-Whitney test was used; when comparing statistical indicators, significanc level of $p<0.05$ was adopted[19].

The animals were kept in the vivarium of NUPh CSRL, certified by the MOH of Ukraine (certificate No. 058/15 dated 08.12.2015, valid until 07.12.2019). Work with animals was conducted i compliance with GLP regulations, recommendations by the State Expert Center of the MOH of Ukraine[14], Law of Ukraine No. 3447-IV dated 21.02.2006 with amendments "On the protectio of animals from cruel treatment", and decree of the first national Congress on Bioethics.

RESULTS AND DISCUSSION:

Carbon tetrachloride toxicity is a classic model of damage to hepatocyte subcellular structures. Toxic effect of xenobiotic is related to formation of free-radical oxidation (ARO) products as result of liver metabolism. They are LPO-inducers; it results in a disruption of the membrane structure of liver cells, which, in its turn, leads to disruption of organ function in general. Toxi action of ethanol on the liver is conditioned by the fact that it is 80% metabolized in this organ. The major metabolite of ethanol – acetaldehyde – is superior to the action of ethanol as fo hepatotoxicity. Upon ethanol intake by liver cells, there are changes in microsomal enzyme activity, content of cytochrome P-450, primarily ethanol-dependent P-450 2E1 isoform, moreove orientation and intensity of these changes depend on the dose and duration of ethanol intake, which is able to "load" the liver by 3/4 of all its oxidizing power. This is enough to reduce any othe oxidizing processes that occur with the participation of nicotinamide adenine dinucleotide (NAD) (metabolism of triglycerides, fatty acids, hormones, etc.). Damage to hepatocyte membranes i this case happens both, as the result of direct action of ethanol and as the result of activation of lipid peroxidation under the influence of its metabolites. The mechanism of damage to live parenchyma is herein similar, but the degree and depth of cell damage is increased due to synergism of these substances[16].

In the setting of pathology, there was observed an increase in marker index of LPO process activation–TBA-AP content, 1.9-fold compared to intact control animals. At the same time, there wa a 2.5-fold decrease in RG content, which is probably due to depletion of this antioxidant component in the setting of the pathological process (Table 1).

Table 1 The influence of liquid extract of common tansy herb on parameters of LPO-AOD system in the setting of subchronic hepatitis *(n=6, M±SEM)*

Experimental conditions	RG, relative units	TBA-AP, μmol/g
IC	108.12±4.47	33.33±1.58
CP	57.98±7.36*	82.05±9.99*
LECTH, 25 mg/kg	50.69±13.14	79.27±9.70
LECTH, 50 mg/kg	72.20±6.46	69.87±10.04
LECTH, 75 mg/kg	79.31±10.24	55.13±9.73#
LECTH, 100 mg/kg	56,71±6,15	67,73±7,48
LECTH, 150 mg/kg	74.03±9.02	58.76±7.06
Carsil, 100 mg/kg	120.89±18.12**	49.14±4.46**

Notes:
n is the number of animals in one experimental group, in which the content of the indexes studied was determined;
M is the mean value in the sample;
SEM is standard error of the mean in the sample;
*deviation of the value is statistically significant compared to that of intact animals ($p<0.05$);
**deviation of the value is statistically significant compared to that of hepatitis group animals ($p<0.05$);
#deviation of the value is statistically significant compared to that of Carsil group ($p<0.05$).

In the setting of pathology modeling, a significant increase in enzymatic markers of cytolysis was observed: 2.5-fold in ALT and 2.4-fold in AST, which confirms the occurrence of the above mentioned cytodestructive processes in hepatocytes in modeling of subchronic hepatitis (Table 2).

Table 2 Activity of enzymatic markers of hepatocyte cytolysis ALT and AST under the influence of liquid extract of common tansy herb (LECTH) in the setting of subchronic hepatitis (n=8, M±SEM)

Experimental conditions	ALT, μmol/h×ml	AST, μmol/h×ml
IC	1.49±0.10	1.45±0.06
CP	3.66±0.09*	3.43±0.23*
LECTH, 25 mg/kg	3.02±0.25	3.39±0.18
LECTH, 50 mg/kg	3.53±0.12	2.88±0.17
LECTH, 75 mg/kg	2.85±0.26**	2.52±0.15
LECTH, 100 mg/kg	3.20±0.13**	2.69±0.29
LECTH, 150 mg/kg	3.54±0.19	3.25±0.23
Carsil, 100 mg/kg	2.48±0.17**	2.29±0.14**

Notes:
n is the number of animals in one experimental group;
M is the mean value in the sample;
SEM is standard error of the mean in the sample;
*deviation of the value is statistically significant compared to that of intact animals ($p<0.05$);
**deviation of the value is statistically significant compared to that of hepatitis group animals ($p<0.05$).

Use of LECTH was characterized by a tendency to decreased content of TBA-AP under the influence of all screening doses of this extract. However, the most distinct trend towards reduction o the level of TBA-AP (1.5-fold) was observed with administration of the extract at a dose of 75 mg/kg (Table 1). A similar trend was observed with the change of RG content (1.4-fold) wit administration of the above-mentioned dose of the extract (Table 2). It should be noted that reference drug product Carsil was superior to the activity of the extract studied as for its effect on th regulation of the pro-oxidant-antioxidant imbalance. It is evidenced by higher content (1.5-fold) of endogenous antioxidant RG in the group of animals treated with Carsil compared to that i LECTH group (at a dose of the extract of 75 mg/kg), but the data as for benefit of Carsil compared to LECTH was not statistically significant.

Administration of drug products having antioxidant action in the setting of pathological conditions through the mechanism of regulation of prooxidant-antioxidant imbalance affects the state o cell membranes and their patency[3]. Hepatocyte cytolysis is an indicator of cytodestruction and is consequence of tetrachlorethane- and ethanol-induced activation of LPO[18,19].

Use of the extract studies at certain doses selected for screening contributed to the inhibition of cytodestruction (Table 2). Thus, in particular, introduction of LECTH at a dose of 25 and 5 mg/kg had a positive effect on the course of pathological changes in cells, as evidenced by almost unchanged activity of transminases. Whereas application of the extract at a dose of 75 mg/k promoted a significant 1.3-fold decrease in activity of transminases (Table 2). It should be noted that administration of LECTH at a dose of 100 and 150 mg/kg were also characterized by significant decrease in the activity of cytolysis markers, but it was less pronounced compared to the dose of the extract of 75 mg/kg. The ability of LECTH to reduce the activity of cytolysi markers was somewhat inferior to that of reference drug product Carsil, which contributed to a significant 1.5-fold decrease in the activity of ALT and AST, respectively (no significar differences compared to LECTH group were detected).

The mechanism of hepatoprotective action of liquid extract of common tansy herb is probably conditioned by binding of toxic free radicals and stabilizing of cell membranes by phenoli compounds present in its composition, in particular, luteolin, rutin, apigenin, apigenin-7-glucoside, hyperoside, chlorogenic, caffeic, ferulic, and gallic acids.

CONCLUSION:

Thus, there has been established a beneficial effect of liquid extract of common tansy herb "Tanahol" on the course of subchronic toxic hepatitis at a dose of 75 mg/kg, which is confirmed b regulation of prooxidant-antioxidant imbalance (reduction of TBA-AP content (1.5-fold) and increased concentration of RG (1.4-fold), as well as inhibition of cytodestruction (1.3-fold). Furthe studies of liquid extract of common tansy herb at a dose of 75 mg/kg aimed at development of a hepatoprotective agent is recommended.

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