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ACUTE TOXICITY STUDY OF THICK EXTRACTS OF LEAVES OF COLEWORT HEART-LEAVED (*CRAMBE CORDIFOLIA* STEV.) AND COLEWORT KOKTEBELICA (*CRAMBE KOKTEBELICA* (JUNGE N. BUSCH.)

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Abstract

Crambe cordifolia and *Crambe Koktebelica* are promising but uncommon and understudied crops. Phytochemical studies have shown that they contain a number of important biologically active substances with different pharmacological properties. A mandatory characteristic of medicinal plant substances, along with high pharmacological activity, should be their safety. Thus, the aim of our study was to study the acute toxicity of thick extracts obtained from the leaves of heart-leaved colewort and colewort Koktebelica. Studies of acute toxicity of thick extracts from the leaves of *Crambe cordifolia* and *Crambe koktebelica* were performed V. B. Prozorovskyi method on white nonlinear mice of both sexes weighing 19–22 g. Animals were injected intragastrically with test extracts in the dose range of 1000 mg/kg, 3000 mg/kg, and 5000 mg/kg. As a result of determining the acute toxicity, thick extracts obtained from the leaves of colewort Koktebelica and colewort heart-leaved, according to the classification by K.K, Sidorov can be determined in toxicity class VI – almost harmless substances, LD50> 5000 mg/kg.

Keywords: *Crambe cordifolia* STEV., *Crambe koktebelica* (JUNGE N. BUSCH.), colewort Koktebelica, colewort heart-leaved, leaves, thick extracts, acute toxicity

Introduction

Today, plants are acknowledged as a form of folk Modern pharmacotherapy increasingly considers the centuries-old experience of folk medicine with the use of herbal drugs as monotherapy and in combination with synthetic drugs [1]. Today, interest in the use of medicinal plants in medical practice is growing. However, the irrational use of natural resources leads to their reduction or destruction [2, 3]. Therefore, there is a growing demand for the use of plants of cultivated flora, such as colewort Koktebelica and colewort heart-leaved. Colewort heart-leaved and colewort Koktebelica belong to the genus *Crambe* L., a family of cabbage (*Brassicaceae*). The genus *Crambe* L. is represented by annual and perennial plants of the cabbage family (*Brassicaceae*), which grow naturally in Europe, eastern Africa and southeastern Asia [4].

Previous phytochemical studies of the aboveground parts of some species of the genus *Crambe* L. have revealed the presence of glucosinolates [5, 6] and such flavonoids as luteolin, apigein and hercetin; kaempferol 3- (*p*-coumaroyl) glucoside-7, 4'-diglucoside and quercetin 3-feruloylglucoside-7, 4'diglucoside, kaempferol, quercetin, kaempferol 4-glucoside, kaempferol-7-glucoside, quercetin 4'-glucoside and quercetin 7-glucoside [7, 8]. Scientific literature has information that *Crambe cordifolia* and *Crambe koktebelica* contain amino acids [9], organic and fatty acids [10], as well as important flavonoids such as acylated glycoside kaempferol or quercetin.

In folk medicine, colewort has long been used to improve appetite, as well as a phytoncide. Traditionally, the leaves of plants of the genus *Crambe* L. are used as a tonic for fatigue and nervous tension. Decoction of crushed roots, mixed with honey, taken orally to remove salts from the body. Seed oil is used to heal ulcers and treat gastritis [11].

Muhammad Abid Rashid and other Pakistan scientists [12, 13] established the antioxidant and antimicrobial activity of the methanolic extract of *Crambe cordifolia* Steven. roots for most of the studied microbial strains (*Escherichia coli*, *Bacillus subtilis*, *Pasteurella multocida*, *Staphylococcus aureus*, *Aspergillus niger* and *Fusarium solani*).

Crambe cordifolia and *Crambe Koktebelica* are promising but uncommon and understudied crops. Phytochemical studies have shown that they contain a number of important biologically active substances with different pharmacological properties. A mandatory characteristic of medicinal plant substances, along with high pharmacological activity, should be their safety. Therefore, the aim of our work was to study the acute toxicity of thick extracts obtained from the leaves of heart-leaved colewort and colewort Koktebelica.

Materials and Methods

Plant Materials

Leaves of the *Crambe cordifolia* and *Crambe Koktebelica* were selected as the objects of the study. The raw materials were provided by the Department of cultural flora of M. Gryshko National Botanic Garden of the National Academy of Sciences of Ukraine. The leaves were collected in the summer of 2018. The leaves were dried using a conventional method and stored in paper bags in a dry place [14-16]. The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine) [17-20]. Samples of herbal raw materials have been deposited in Departmental Herbarium for future record [21-23].

Animal models

42 white nonlinear mice of both sexes weighing 19–22 g were used as the experimental animals. The animals were kept in a room with a temperature (22±2) ° C, and relative humidity of 44–55 % under 12/12 hour light and dark cycle with standard laboratory diet and water were given ad libitum [25, 26].

Pharmacological studies were conducted in accordance with the rules and requirements of the "General Principles for the Work on Animals" approved by the I National Congress on Bioethics (Kyiv, Ukraine, 2001 and agreed with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Council of Europe No. 123, Strasbourg 1985), and the Law of Ukraine "On the Protection of Animals from Cruelty" of February 26, 2006 [27-30]. The removal of animals from the

experiment was carried out under light inhalation (ether) anesthesia by decapitation.

Acute toxicity studies

Studies of acute toxicity of thick extracts from the leaves of *Crambe cordifolia* and *Crambe koktebelica* were performed V. B. Prozorovskyi method [31] on white nonlinear mice of both sexes weighing 19–22 g. Animals were injected intragastrically with test extracts in the dose range of 1000 mg/kg, 3000 mg/kg, and 5000 mg/kg. If the size of the extract exceeded 5 ml, the administration was performed in a fractional manner during the day [32]. At the end of the experiment (14 days), the mortality rate was determined in each group to calculate the average lethal dose (LD50) [33]. Intragastric administration through a metal probe of the studied extracts of *Crambe cordifolia* and *Crambe koktebelica* were performed after night (8–12 h) fasting of animals. During the studies, the animals had free access to water; they were allowed to eat only 4 hours after administration [32, 33].

Throughout the experiment, the survival of animals, consumption of food and water by them, as well as clinical manifestations of intoxication (if any): general condition, changes in body position, skin condition, the color of mucous membranes, and individual symptoms (lacrimation, diarrhea, changes in the color of urine and feces, drowsiness, convulsions) were observed. In case of death of animals, their autopsy was performed and macroscopic analysis of abdominal organs was performed in order to establish that the lethal outcome of the animal did not occur due to manipulation errors, as well as to determine the probable cause of death [34].

Statistical analysis

Statistical processing of the results was performed using Statistica 8.0 program. The obtained values by using the Student's t-test were compared and the non-parametric MannWhitney U-test [35, 36]. The difference of significance was set at *p<0.05 for all statistical analyses [37-41].

Results and Discussion

In experimental animals, body weight was monitored on day 1 (before administration) and 14 days after intraperitoneal administration of the studied extracts (Tables 1, 2). The results show that a single intraperitoneal administration of the studied dry extracts to mice of both sexes at doses of 1000 mg/kg, 3000 mg/kg and 5000 mg/kg did not affect the dynamics of body weight compared to control. Experimental and control animals gained weight according to physiological norms. During the external examination the animals did not show signs of pathological changes in their condition: fur and skin were clean, the subcutaneous layer of adipose tissue was moderate, there were no damage, inflammatory lesions on the mucous membranes and the skin. Visual assessment of the internal organs condition also showed no signs of pathological changes.

The results showed that after a single intraperitoneal oral administration of extracts (at doses of 1000 mg/kg, 3000 mg/kg and 5000 mg/kg of body weight) mice of both sexes during the entire observation period were not registered the death of experimental animals. There were no deviations in appearance, toxic manifestations recorded after the administration of a thick extract of colewort Koktebelica and colewort heart-leaved leaves and until the end of the observation period. All animals were active, had smooth fur, clean skin. The lack of mortality in animals gives reason to believe that the value of LD_{50} at enteral administration of both test extracts exceeds the maximum dose used in the experiment, ie in mice LD_{50}> 5000 mg/kg. This value of LD_{50} makes it possible to determine the studied extracts according to K.K. Sidorov classification to toxicity class VI – almost harmless substances [32].

Conclusions

As a result of determining the acute toxicity, thick extracts obtained from the leaves of colewort Koktebelica and colewort heart-leaved, according to the classification by K.K, Sidorov can be determined in toxicity class VI – almost harmless substances, LD_{50}> 5000 mg/kg. The results of the study are the basis for the creation of effective phytosubstances from colewort Koktebelica and colewort heart-leaved, and further dosage forms based on them.

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Table 1. Dynamics of body weight of mice (g) after a single intra-abdominal administration of a thick extract of colewort Koktebelica leaves (M ± m; n = 3)

Extract dose	Body weight on day 1	Body weight on day 14
Males		
Control	20.33±0.33	21.67±0.33
1000 mg/kg	19.67±0.33	21.67±0.33
3000 mg/kg	21.00±0.58	22.33±0.33
5000 mg/kg	21.33±0.33	22.00±0.58
Females		
Control	20.33±0.67	22.00±0.58
1000 mg/kg	19.0±0.00	20.33±0.33
3000 mg/kg	19.67±0.33	22.67±0.33
5000 mg/kg	21.33±0.33	22.00±0.58

Table 2. Dynamics of body weight of mice (g) after a single intra-abdominal administration of a thick extract of heart-leaved colewort leaves (M ± m; n = 3)

Extract dose	Body weight on day 1	Body weight on day 14
Males		
Control	20,33±0,33	21,67±0,33
1000 mg/kg	20,33±0,88	21,67±0,88
3000 mg/kg	21,00±0,58	22,00±0,58
5000 mg/kg	19,00±0,00	20,33±0,33
Females		
Control	20,33±0,67	22,00±0,58
1000 mg/kg	20,33±0,67	21,67±0,88
3000 mg/kg	21,00±1,00	22,00±1,00
5000 mg/kg	19,33±0,33	21,67±0,33