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ORIGINAL ARTICLE

Study of acute and chronic toxicity of ginkgo biloba samples

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The article presents the results of acute and chronic toxicity of Ginkgo Biloba samples. In assessing the general condition of animals after 30 minutes and 1 hour single extract administration at doses of 1,000, 2,500 and 5,000 mg/kg, it has been established that the motility and mobility of mice were reduced. After third hour of observation these phenomena was not observed. Other signs of intoxication, in particular the central and autonomic nervous system (lateral position, reduction of muscle tone, violation of coordination of movements, blepharoptosis, hyperalsalivation, etc.) were not noted. Based on the results of the studies, Ginkgo Biloba extract with subcutaneous administration to white mice for 14 days in a therapeutic dose did not cause significant changes in the general condition, body weight, morphological and biochemical blood parameters.

Key words: Ginkgo biloba; Acute toxicity; Chronic toxicity; Physiological state; Blood morphological indices; Extracts

Introduction

Ginkgo biloba (Ginkgo biloba) - (ginkgo dicotyledonous, ginkgo biloba) is a leafy tree, also known as the adiantuum, or a stone tree used centuries in oriental medicine. Ginkgo (Ginkgo biloba) is one of the oldest living tree species. Most ginkgo products are made with extract prepared from its fan-shaped leaves. Ginkgo biloba - a representative of the Ginkgo family (Ginkgoaceae) (G. Leontyac, 2015). As medicinal raw materials, leaves, seeds and fruits of Ginkgo biloba are used. Regarding Ginkgo biloba, a great clinical experience has been accumulated, which has no doubt about its efficacy in the treatment of cerebral insufficiency, neurosensory disorders and peripheral vascular disease (S. G. Burchinsky et al., 2011).

Ginkgo biloba contains specific substances - terpelactones (terpene lactones - ginkgolides, white halides) and flavonglicosides (flavonoids - quercetin, campsferol, isozaremetine, proanthocyanids) that affect metabolic processes, normalize metabolic processes in cells, rheological properties of blood and microcirculation. Ginkgo supplements are associated with several health claims and uses, most of which focus on brain function and blood circulation. Ginkgo has the ability to reduce inflammation caused by various conditions. This may be one of the reasons it has such broad health applications. Ginkgo's apparent ability to increase blood flow to various parts of the body may be the origin of many of its supposed benefits (Mishenko, 2015)

One study in people with heart disease who supplemented with ginkgo revealed an immediate increase in blood flow to multiple parts of the body. This was attributed to a 12% increase in levels of circulating nitric oxide, a compound responsible for dilating blood vessels (S. M. Kuznetsova et al., 2016). Similarly, another study showed the same effect in older adults who were treated with ginkgo extract (G. Singh et al., 2013). Ginkgo biloba has strong anti-inflammatory and antioxidant capacities and an ability to improve circulation. Together, these characteristics have the potential to affect numerous body systems and diseases, although the science behind it still has some catching up to do. (Ansley Hill, 2018). Additional research also points to ginkgo's protective effects on heart health, brain health and stroke prevention. There are multiple potential explanations for this, one of which may be the anti-inflammatory compounds present in the plant (A. Mdzinarishvili et al. 2012). Ginkgo can increase blood flow by promoting the dilation of blood vessels. This may have applications for the treatment of diseases related to poor circulation. There is some speculation that ginkgo may enhance brain function in healthy individuals. Some research shows that ginkgo may help treat anxiety, though this is likely due to its antioxidant content. A handful of small studies support the notion that supplementing with ginkgo may increase mental performance and perceived well-being Very little research has investigated how ginkgo relates to vision and eye health. However, early results are promising. The most helpful components of ginkgo are believed to be flavonoids, which have powerful antioxidant qualities, and terpenoids, which help improve circulation by dilating blood vessels and reducing the "stickiness" of platelets. Ginkgo biloba is a popular supplement and one of the top-selling herbal medicines. Ginkgo biloba extract is collected from the dried green leaves of the plant and is available as liquid extracts, capsules, and tablets. People use it for a variety of reasons. The therapeutic properties of the ginkgo plant are said to include treatment for blood disorders and memory problems, enhancement of cardiovascular function and to improve eye health. Gingko contains high levels of flavonoids and terpenoids, antioxidants that provide protection against oxidative cell damage from harmful free radicals. In this way, antioxidants are believed to help reduce the risk of cancer.

Also known as the maidenhair tree, ginkgo is one of the oldest species of tree in the world. The trees can grow more than 130 feet tall and can live for over 1,000 years. Some trees in China are said to be over 2,500 years old. The tree is considered to be a "living

fossil," meaning that it has continued to survive even after major extinction events. The extract can be taken as a supplement, and the dried leaves of the plant can be used to make tea. (Joseph Nordqvist, 2017). When taken inside the extract Ginkgo biloba is well absorbed from the gastrointestinal tract. The maximum concentration in blood plasma is reached in 1-2 hours. The half-life is 4-5 hours. Determination of toxicity parameters is a mandatory step in the preclinical study of promising drug compounds (P. Crane, 2013). The research is part of comprehensive scientific research of two departments of the Sumy National Agrarian University. Chair of vetsan examination, microbiology, zoohygiene and safety and quality of animals products, Veterinary medicine Department and Chair of Ecology, botany and gardening and forestry, Agronomy Department according with the thematic plan of the research work "Biological and ecological features of the cultivation of Ginkgo biloba L ., as organic raw materials, for pharmaceutical purposes by creating plantations in the conditions of the North-Eastern forest-steppe of Ukraine "(state registration number: 0117U006533).

The Purpose of Research

Determination of acute and chronic toxicity of various Ginkgo biloba samples.

Materials and Methods

The researches were carried out on the basis of the laboratory of "Innovative technologies and safety and quality of animals` products" and "Veterinary pharmacy" lab. and at the Chair of vetsanexamination, microbiology, zoohygiene and safety and quality of animals products, Veterinary Medicine Department, Sumy National Agrarian University. Pharmacotoxicological studies were carried out in accordance with Guidance "Preclinical studies of Veterinary Medicinal Products" (2006). White rats and white mice were used in accordance the guidelines.

Ginkgo Biloba leaves were collected in September 2018 from four locations: sample №. 1 - Kianyanitsa Park; sample №. 2- Greenhouse; sample №. 3 - Box; sample №. 4 - Main park. Intra-gastric administration of the tested extracts at doses 10, 25 and 50 times higher than therapeutic (1000, 2500 and 5000 mg/kg, respectively) did not lead to the death of animals in any of the groups within 14 days (Figure 1).

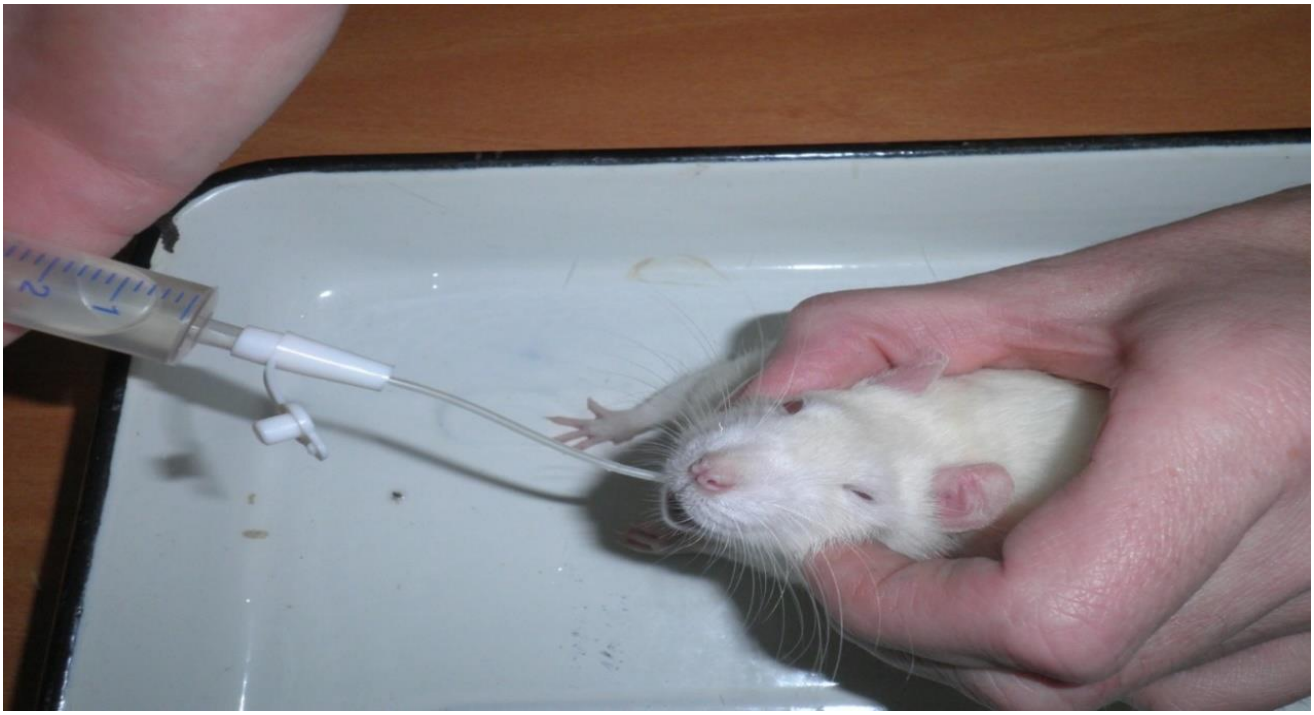

Figure 1. Intra-gastric drug's administration.

Results and Discussion

At assessing the general condition of animals after 30 min. and at 1 h after single administration of the extract at doses of 1000, 2500, and 5000 mg/kg, a decrease in the mobility of both mice and rats was observed in a third hour of observation. This effect could be due to the large volume of phyto-drug solution that was administrated. Other signs of intoxication, including effects on the central and autonomic nervous systems (lateral position, decrease in muscle tone, impaired movement coordination, blepharoptosis, hypersalivation, etc.) were not noted. The same results were obtained in study with other samples. But sample №.4 reduced the mobility of the animals during four hours (Table 1). According to the data obtained, Ginkgo Biloba extract belongs to the V toxicity class according to the Hodge and Sterner classification - practically non-toxic substances (LD50> 5000 mg/kg).

To detect possible toxic effects of the drug on the animal body at 21 and 31 days from the beginning of drug administration we have studied the antitoxic function of the liver using a hexenal sample. We have used 10 mice per group. And other 10 mice in each group were placed on a swimming test. Chronic toxicity parameters were determined on white mice. For the experiment on the principle of paired analogues were selected 80 white mice 8-9 - week of age, weighing 18-20 g, which was divided into four groups - 20 animals in each (three experimental and one control). Mice from I, II, III and IV groups had extract injection subcutaneously.

Table 1. Determination of acute Ginkgo Biloba toxicity in animals by sample № 1 single intragastric administration.

Species of animals	Ginkgo biloba dose, mg /kg	Mortality	
		absolute number	%
Mice	1000	0/3	0
Mice	2500	0/3	0
Mice	5000	0/3	0
Rats	1000	0/3	0
Rats	2500	0/3	0
Rats	5000	0/3	0

Notes: In the numerator - the number of animals killed, in the denominator - the number of animals in the group.

V group was control, animals were administered daily 0.9% sodium chloride solution at a dose of 0.1 ml/kg during the experiment. The extract was administered daily for 14 days, in the morning at the same time.
The experimental animals were monitored daily for 30 days, it was determined clinical condition, appetite, condition of the hair and mucous membranes, behavioral responses, locomotor reflex, dynamics of body weight, antitoxic function of the liver and degree of reversibility. It was conducted an "open field" test, during which the mice were planted in the center square of a rectangular field 140 × 70 cm, divided into squares 10 × 10 cm, where in the center of a certain number of them randomly we have made holes in the floor ("minks"), and recorded a latent period of exit from it. The criterion for moving an animal to another square was considered by moving through the dividing line of both pelvic extremities. At placing animals to the field, before fixing indicators, the animals were covered with a dark cap to soothe for 1 min. The number of squares which mice visited (horizontal movement activity), the number of pelvic liftings ("vertical stand"), the number of "minks" which mice sniffed and which mice interested, the number of washes (acts of grooming), urinations and bowel movements (number of boluses) were took into our account. All tests were performed three times . The datas obtained were compared with datas from control group. Indicators of body mass dynamics were determined by comparative evaluating the results of mice weigh before the start and end of the experiment. On the following day after the last drug administration 10 mice were collected from each group, which were decapitated (with light anesthesia), blood samples were taken from them for morphological and biochemical studies.
Blood biochemical studies were performed on an analyzer "COBAS-E-MIRA". After the autopsy, internal organs were selected, weights were calculated and compared with the animals from the control group. When observing the experimental animals, significant changes in the behavior of the mice were not observed (Table 2).

Table 2. Indicators of the physiological state and white mice activity during the 14-day administration of Ginkgo Biloba extracts (M ± m, n=20).

Animal group	Appetite	Behavioral response	Vertical motor activity
I Group	Satisfactory	Mink reflex preserved	5,28 ± 0,21
II Group	Satisfactory	Mink reflex preserved	5,20 ± 0,41
III Group	Satisfactory	Mink reflex preserved	5,42 ± 0,32
IV Group	Satisfactory	Mink reflex preserved	4,60 ± 0,30
V Group	Satisfactory	Mink reflex preserved	5,22 ± 0,31

The appetite of all mice from the experimental groups was satisfy and did not differ from control. Animals from 4 group for 14 days have slightly decreased of motor activity. The study of the emotional and behavioral responses of white mice after 14 days extract administration at a therapeutic dose has not showed significant effect on their nervous system. We have established tentatively experimental (number of sniffs and stares) and emotional (number of bowel movements and boluses) reactions of experimental animals and there was not differ between animals from control groups. The clinical status of the animals from the experimental and control groups remained within the physiological norm during the experiment. There was a slight increase in body weight of the test and control animals during the experiment (Table 3). However, the lowest percentage increase in total weight was in mice from the fourth group.

Table 3. The dynamics of body weight of white mice in a chronic experiment under the influence of Ginkgo Biloba extracts (M ± m, n=20).

Animal group	Before the experiment		At the end of the experiment		
	total by group	body weight, g	total by group	average of one animal	increase in total mass (%)
I	384,4	19,81 ± 0,38	398,0	19,65 ± 0,35	106,20

II	382,2	19,09 ± 0,38	392,2	19,15 ± 0,37	102,16
III	382,4	19,18 ± 0,44	388,4	19,64 ± 0,39	103,15
IV	382,6	19,08 ± 0,42	385,4	19,14 ± 0,39	101,17
V (control)	382,5	19,07 ± 0,39	398,6	19,85 ± 0,34	104,14

The significant changes in in morphological parameters (leukocyte and erythrocyte counts, hemoglobin content, and leukogram) were not found (Table 4).

Analysis of hematologic indices shows a slight decrease in the hemoglobin content of the animals from the fourth group.
The blood content of experimental and control animals of erythrocytes and leukocytes were not differ significantly. There were no significant changes in biochemical parameters compared to control animals. In animals of group IV, there was a tendency for the tight functioning of the excretory system and the liver. There was an increase in urea content by 0.18 and the activity of liver-specific enzymes alanine aminotransferase and aspartate aminotransferase by 2.23 and 10.48, respectively, compared with the control.

Table 4. Morphological parameters of blood of white mice for the determination of chronic toxicity of Ginkgo Biloba extract (M ± m, n=10).

Indexes	**Groups of animals**			
	I	**II**	**III**	**IV**
Hemoglobin, g/l	168,2 ± 3,6	164,3 ± 4,2	164,7 ± 4,7	160,7 ± 4,7
Erythrocytes T/l	8,2 ± 0,1	8,1 ± 0,1	8,0 ± 0,3	8,0 ± 0,2
Leukocytes G/l	7,1 ± 0,2	7,2 ± 0,3	7,1 ± 0,2	7,3 ± 0,2
Eosinophils,%	1,16 ± 0,01	1,06 ± 0,02	1,02 ± 0,04	1,01 ± 0,05
Neutrophils,%	29,5 ± 2,4	33,5 ± 2,2	32,4 ± 2,1	35,4 ± 2,4
Lymphocytes,%	59,9 ± 3,2	58,2 ± 3,4	58,8 ± 2,6	56,8 ± 2,8
Monocytes,%	0,60 ± 0,1	0,59 ± 0,2	0,59 ± 0,1	0,58 ± 0,1

The blood glucose content of the animals from the fourth group increased by 1.2 compared to the control, which may indicate impaired glycogenesis of the liver or an increase in the oxidation intensity of carbohydrates. The protein content in the serum of experimental animals from all groups were not change significantly (Table 5). Due to the fact that one of the reasons for the increase in the level of activity of transaminase enzymes is their exit from the affected organs and tissues into the bloodstream, we can assume the beginning of the development of destructive processes in the hepatocytes of mice treated with extract No. 4 (higher phenolic content shortcuts). because the liver is place that localized largest amount of alanine aminotransferase.
However, the average of these indicators did not go beyond the upper limit of the norm for this species. In the pathoanatomical study the shape and size of all mice`s groups liver did not look altered.

Table 5. Blood biochemical parameters of white mice for the determination of chronic toxicity of Ginkgo Biloba extract preparation (M ± m, n=10).

Indexes	**Groups of animals**			
	I	**II**	**III**	**IV**
Total protein, g/l	56,34 ± 2,30	52,62 ± 3,24	52,32 ± 1,33	54,32 ± 1,34
Urea, mmol/l	5,27 ± 0,22	5,22 ± 0,18	5,26 ± 0,24	5,36 ± 0,24
Glucose, mmol/l	4,68 ± 0,26	5,18 ± 0,22	5,13 ± 0,16	5,83 ± 0,26
Alkaline phosphatase, units /l	262,5 ± 6,15	266,4 ± 5,19	260,5 ± 5,34	264,5 ± 6,34
AlAT, units /l	84,86 ± 2,44	86,53 ± 2,24	85,51 ± 2,16	88,51 ± 2,26
AcAT, units /l	94,42 ± 2,42	101,34 ± 3,24	98,62 ± 2,29	104,62 ± 3,29

Mice, from the fourth group had in 1.5-2 times enlarged liver. The liver color also differed. In control, first and second experimental groups the liver was homogeneous, painted in light brown color, elastic, the structure was preserved in the section. In animals of other groups the liver was sometimes heterogeneous, colored in dark brown with a dark cherry stripe.
At macroscopic examination of the spleen was founded that in animals from the fourth experimental group these organ was slightly changed in size. In all cases, spleen had an elongated shape. In mice of other groups - the spleen was dark red in color, the edges are sharpened, on the incision of the pulp scraper is negligible.
The spleen in animals of the fourth group - light red color. Macroscopic examination of the kidneys in all groups of animals showed that the shape of the organ was not changed. In this case, the organ consistency in the animals of all groups was elastic, the capsule from the kidneys was easily removed.

Conclusion

1. Based on the results of the studies, Ginkgo Biloba extract, when administered subcutaneously to white mice for 14 days at a therapeutic dose, have not caused significant changes in general condition, body weight, morphological and biochemical parameters of blood.
2. According to the data obtained, Ginkgo Biloba extract belongs to the V toxicity class according to the Hodge and Sterner classification - practically non-toxic substances (LD50>5000 mg/kg).

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