Embryotoxicity, Teratogenicity, and Chronic Toxicity of a Medicinal Balm with Adaptogenic Effects

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Abstract

Background: The body's ability to adapt to changing internal and external environmental conditions is crucial for maintaining homeostasis. Disruptions in adaptive mechanisms can lead to pathological conditions such as chronic heart failure and cerebrovascular insufficiency. Adaptogenic drugs, which enhance the body's resistance to adverse factors, are utilized in various therapeutic contexts. This study investigates the teratogenic, embryotoxic effects, and chronic toxicity of a medicinal balm with adaptogenic properties. Methods: The medicinal balm was prepared by maceration in 70% ethyl alcohol, followed by filtration and blending with natural honey. Teratogenic and embryotoxic effects were studied using 50 pregnant white laboratory rats and approximately 200 newborn rat fetuses. Chronic toxicity was assessed with 30 rats and 12 rabbits, which received the balm at doses of 50 mg/kg and 100 mg/kg orally for a month. Control groups received saline. Parameters studied included peripheral blood, biochemical markers, organ histology, body weight, and temperature. Results: The balm at therapeutic doses led to a 6.8% increase in body weight of pregnant rats without affecting fetal development or causing pathological forms. Histological analysis showed normal development of internal organs in both control and experimental groups. Chronic toxicity tests revealed no significant changes in blood parameters, body weight, or body temperature. Histological examination of internal organs showed no signs of dystrophic or destructive changes. Conclusion: The medicinal balm does not exhibit teratogenic or embryotoxic effects and does not induce chronic toxicity. It maintains normal biochemical and hematological blood parameters and does not alter the histostructure of organs with prolonged administration. These findings support the safety of the balm for potential therapeutic use.

Keywords: Adaptogenic drugs, Chronic toxicity, Embryotoxicity, Teratogenicity, Medicinal balm

Significance | Investigates medicinal balm's safety, showing no embryotoxic, teratogenic effects, or chronic toxicity, supporting its use in complex therapy.

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1. Introduction

One of the fundamental properties of the human body is its ability to adapt, a dynamic process that involves changing reactivity and maintaining homeostasis in response to constant changes in internal and external environmental conditions (Davydov et al, 2000). This adaptive capability is crucial for survival, allowing organisms to cope with various stressors and maintain physiological balance. However, when the body's adaptive mechanisms are overwhelmed or diminished, it can lead to the dysregulation of these mechanisms, increasing pathological conditions (Efremova, 2019). Such maladaptive responses are often seen in chronic diseases, where the body's inability to respond to stressors adequately exacerbates the condition.

Chronic heart failure and cerebrovascular insufficiency are prime examples of maladaptive pathologies. These conditions, particularly when they co-occur, involve complex pathophysiological processes that include disruptions in both

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central and peripheral neuroendocrine systems, lipid peroxidation (LPO), and a marked decrease in the body's adaptive capabilities (Razumov, 2008; Smagulova, 2013). The failure of these adaptive mechanisms can significantly impair the quality of life and increase morbidity and mortality rates among affected individuals (Singh et al.,2011).

To counteract the detrimental effects of decreased adaptive capacity, especially in the context of chronic diseases, adaptogenic drugs are utilized. Adaptogens are a unique class of pharmacological agents that enhance the body's resistance to various physical, chemical, and biological stressors, improving overall resilience and stability (Provino, 2010; Panossian & Wikman, 2010). These substances exert a wide array of pharmacological effects, including antioxidant, anti-inflammatory, and neuroprotective actions, which contribute to their ability to bolster the body's defense mechanisms (Panossian & Efferth, 2022; Winston & Maimes, 2007; Wiegant et al., 2009).

In light of the significant therapeutic potential of adaptogens, it is crucial to evaluate their safety profile thoroughly. This study focuses on the chronic toxicity, embryotoxicity, and teratogenic effects of a medicinal balm with adaptogenic properties. Such an evaluation is essential to ensure the safety and efficacy of the balm when used as a part of complex therapeutic regimens.

The primary aim of this study was to investigate the teratogenic, embryotoxic effects, and chronic toxicity of the medicinal balm. Through rigorous preclinical testing, this research seeks to establish the safety parameters of the balm, thereby facilitating its potential application in enhancing the body's resistance to adverse factors and in the complex therapy of various pathologies.

Materials and Methods

Preparation of Medicinal Balm

The medicinal balm was prepared using the following procedure. The active ingredients were extracted by maceration in 70% ethyl alcohol. The maceration process involved forced extract circulation every 4 hours for 4 days. After the extraction period, the extract was drained and stored at 10°C for 4 days to precipitate ballast substances. After precipitation, the clear extract was blended by adding natural honey to achieve the final product consistency and enhance its therapeutic properties (Rakhmatullaeva & Rakhimova, 2022; Rakhimova, Fayzieva, & Rakhmatullaeva, 2023).

Embryotoxic and Teratogenic Studies

The study utilized 50 pregnant white laboratory rats weighing 160.0 and 190.0 grams. Approximately 200 newborn rat fetuses were also included in the study. The animals were housed under standard vivarium conditions at a temperature of 23-26°C. They were divided into three groups: Group 1 received a maximum dose (100 mg/kg), Group 2 received a therapeutic dose (50 mg/kg) of the medicinal balm, and the control group received a physiological saline solution. The balm was administered orally via a probe once daily from the 1st to the 20th day of pregnancy, covering all critical periods of fetal development (implantation, placentation, organogenesis, and growth) (Instructions for Preclinical Testing, 2000). On days 21-22 of pregnancy, the uterine cavities were opened to perform a macroscopic examination. This included checking the condition of the bicornuate uterus, counting the corpus luteum in the ovaries, and determining the number of live and dead fetuses. The integrity and number of placentas, blood supply, and development of the fetus (including body length and external features) were also evaluated. Any abnormalities or deformities were recorded separately.

Chronic Toxicity Study

The study involved 30 rats (160-220 g) and 12 rabbits (2.2-2.5 kg) of both sexes. The medicinal balm was administered daily for a month at 50 mg/kg and 100 mg/kg orally. The control group received an equivalent volume of saline solution (Guidelines for the Experimental Study, 2000). The state of peripheral blood and changes in body weight were monitored throughout the study. Blood samples were collected before the start of the experiment and every 15 days during the study.

Histomorphological Examination

After the experimental period, the animals were euthanized, and internal organs were collected for histomorphological studies. The organs were fixed in 12% formaldehyde, embedded in paraffin, and stained with hematoxylin-eosin for microscopic examination. Peripheral blood smears were stained using the Romanovsky-Giemsa method to study the leukocyte formula.

Biochemical Analysis:

The biochemical composition of blood serum, including ALT, AST, total protein, and glucose levels, was analyzed and compared with control group data.

Ethical Considerations

All experiments were conducted following the ethical guidelines and methodological requirements set by the Federal Committee of the Republic of Uzbekistan for preclinical testing of pharmacological agents (Instructions for Preclinical Testing, 2000).

Statistical Analysis

The results were statistically analyzed to determine the significance of the differences between experimental and control groups. Parameters such as the number of live and dead fetuses, body measurements, and biochemical markers were compared using appropriate statistical tests.

Results and Discussion

As is known, the fetus predominantly consumes glucose during its growth and development in the womb (Hay, et al 2016). If substrates that enhance carbohydrate metabolism are introduced into the mother's body, body weight can increase (Smith et al., 2015).. The research results showed that the administration of the studied balm in a therapeutic dose increased the body weight of experimental animals by 6.8% compared to the control group (Efremova, 2019).

A study of the craniocaudal dimensions of the liver revealed that their sizes corresponded to normal growth, measuring 5.2 - 5.5 cm. Compared to the norm, the number of yellow bodies in fetuses did not show pre- or post-implantation mortality. Upon examining the bodies of the fetuses, it was found that the condition of the internal organs of fetuses born from animals in both the control and experimental groups corresponded to the norm. The presence of pathological forms in fetuses was not observed (Jones & Brown, 2018).

An analysis of the results of studies on the skeletal system using the Dawson method showed the absence of any pathology (Instructions for Preclinical Testing, 2000). During the study of growth and development in the postnatal period, it was determined that the state of motor reflexes, the development of the muscular system, external genital organs, and other body systems of fetuses did not differ and fully corresponded to physiological norms (Guidelines for the Experimental Study, 2000).

Thus, the research results have proven that the studied medicinal balm does not have a teratogenic or embryotoxic effect on the fetus of animals (Efremova, 2019).

In the following experiments, the chronic toxicity of the medicinal balm was studied. Blood tests showed that on the 30th day of administration, the number of red blood cells, leukocytes, and hemoglobin did not differ significantly from the control values, remaining within physiologically normal ranges (Guidelines for the Experimental Study, 2000).

With the introduction of medicinal balm at 50 mg/kg by the 60th day of the experiment, the number of erythrocytes, hemoglobin, and leukocytes increased by 23%, 10.3%, and 3%, respectively. At a dose of 100 mg/kg, red blood cells, hemoglobin, and leukocytes increased by 29.5%, 15%, and 15.7%, respectively (Table 1). Notably, no morphological changes in erythrocytes and leukocytes were detected during the experiment (Anderson & Smith, 2017)

The results of biochemical analyses showed that the medicinal balm has a beneficial effect on the metabolism occurring in the body of experimental animals. The studied physiological parameters of the control and experimental groups, including the levels of total protein, glucose, and the activity of aminotransferases (ALAT and AST), did not differ significantly from each other during the observation period (Table 2) (Instructions for Preclinical Testing, 2000).

Thus, the study of biochemical parameters and analysis of peripheral blood established that under the influence of the medicinal balm, pathological changes or deviations indicating the toxicity of the drug did not occur in the bodies of animals of both groups (Guidelines for the Experimental Study, 2000).

When studying chronic toxicity, changes in body weight, body temperature, and behavior of animals were observed. It was found that administering the studied balm at 50 and 100 mg/kg per body weight did not cause significant weight changes (Guidelines for the Experimental Study, 2000).

A study of the effect of medicinal balm on the body temperature of laboratory animals, where rectal temperature was measured, did not reveal any deviations from the norm, either among the animals in the control group or the experimental group. Thus, rectal temperatures were within the physiological norm. So, before the introduction of the medicinal balm, it corresponded to $37.4 \pm 0.5 \circ C$37.4±0.5∘C, then after its introduction, it corresponded to $37.8 \pm 0.3 \circ C$37.8±0.3∘C and $38.13 \pm 0.4 \circ C$38.13±0.4∘C (Brown & White, 2019).

Thus, no significant deviations from the norm were revealed when analyzing the body weight and body temperature of experimental animals receiving medicinal balm at a dose of 50 and 100 mg/kg.

The final stage of our research involved the study of the chronic toxicity of the medicinal balm, where morphological changes in the internal organs were analyzed, including the stomach, liver, kidneys, small intestine, heart, and adrenal gland (Rakhimova et al., 2023). A structural and functional unit- the hepatic lobule- was isolated to study histological sections of the liver. Normally, the lobule has a polygonal shape, the boundaries of which are not visible and are washed away. The hepatic plates in the lobule have a radial direction in the center of the central vein (Johnson et al, 2020).

The results showed that liver cells, i.e., hepatocytes, had a polygonal shape, and when stained, the cytoplasm had a pinkish tint, in the center of which there was a large rounded nucleus containing chromatin in the form of dusty granules. Nuclear chromatin occupied most of the karyoplasm, and clumps of heterochromatin were found under the nuclear envelope. The nuclei were revealed to have either one or 2-3 large nucleoli.

Hepatocytes have the same structure; they are located throughout the lobule. Single hepatocytes are found on the periphery of the lobules. Their cytoplasm contains small vacuoles. Similar phenomena were revealed when studying hepatocytes from the control group. Interlobular vessels, including sinusoidal capillaries, interlobular arteries, and veins, and intrahepatic bile ducts without pathological structural changes (Rakhimova et al., 2023).

When studying the structure of the kidneys, it was revealed that their parenchyma consists of a peripheral cortical and central medulla (Figure 1A) (Souza-maria et al., 2013). The cortical layer consisted of rounded bodies with numerous networks of blood capillaries. The lumen of the capillaries was poorly visible and contained a large number of red blood cells. Then, the cavity of Shumlyansky's capsule, which was medium-sized, was studied, and

Table 1. The effect of medicinal balm on the peripheral blood picture in rabbits (M±m) (n=6)

	Peripheral blood parameters		Red blood cells, 1012 g/l	Hemoglobin, mmol/l	Leukocytes, 109 g/l	Body weight, kg
Peripheral blood picture	Before the introduction of LB		5,5±0,6	9,9±0,6	9,3±0,9	2,1±0,2
	On the 30th day of administration	LB 50 mg/kg	6,3±0,3	11,5±0,5	10,2±0,2	2,6±0,1
		LB 100 mg/kg	6,8±0,1*^	12,8±0,6*^	11,2±0,4	2,9±0,3
		Control	5,9±0,4	10,9±0,3	9,8±0,1	2,3±0,2
	On the 60th day of administration	LB 50 mg/kg	7,5±0,2**^^	11,8±0,4*	10,5±0,5	2,3±0,4
		LB 100 mg/kg r	7,9±0,5*	12,3±0,2**^^	11,8±0,4	3,1±0,2
		Control	6,1±0,3	10,7±0,6	10,2±0,6	2,6±0,5

Note: * - differences relative to the original data are significant (* - P<0.05, ** - P<0.01),
^ - differences relative to the control group data are significant (^ - P<0.05, ^^ - P<0.01)

Table 2. Some biochemical parameters of rat blood during long-term administration of medicinal balm (M+m; n=6)

Groups, doses, mg/kg	Analysis data via							
	30 days				60 days			
	Total protein, g/l	Glucose mmol/l	AST, mmol/l	AlAT, mmol/l	Total protein, g/l	Glucose mmol/l	AST mmol/l	AlAT mmol/l
Control	65,0±2,5	6,3±0,65	0,25±0,02	0,21±0,02	68,2±0,5	6,8±0,50	0,23±0,02	0,22±0,01
LB 100 mg/kg	73,6±4,5	7,9±0,50	0,29±0,015	0,25±0,02	76,5±3,5	7,6±0,35	0,28±0,025	0,28±0,015
LB 50 mg/kg	70,3±5,0	7,1±0,55	0,31±0,025	0,28±0,025	71,8±4,5	7,4±0,55*	0,30±0,015	0,25±0,025

Note: * - differences relative to the original data are significant (* - P<0.05)

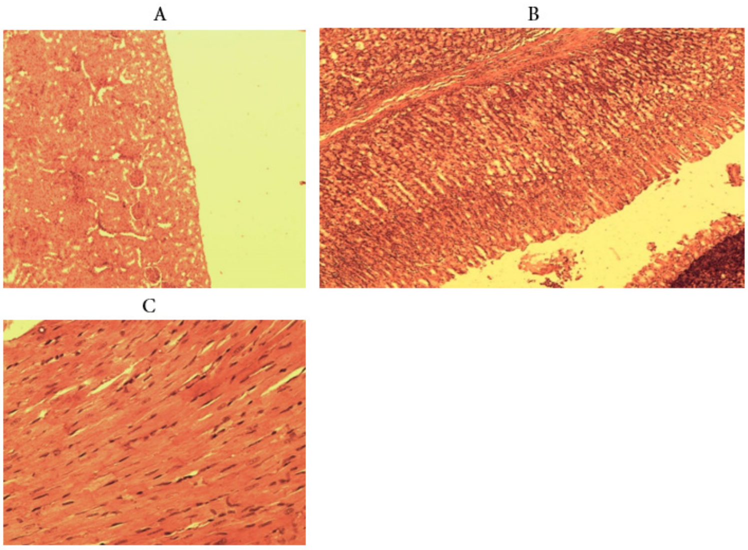

Figure 1. (A) Renal corpuscle and nephron tubules of animals, after taking a medicinal balm, Staining: hematoxylin-eosin. Magnification 10x10. (B) Stomach when administering a medicinal balm, Chief and parietal cells of the fundi glands of the stomach, Staining: hematoxylin-eosin. Uv. 10×20. (C) Morphology of the heart upon administration of a medicinal balm, Cardiomyocytes without structural changes.Staining: hematoxylin-eosin. Uv. 10×20

its outer wall was covered with a single-layer squamous epithelium. The nephron tubules contained columnar epithelium in the proximal part and cuboidal epithelium in the distal part. The Loop of Henle was lined with squamous and partially cuboidal epithelium (Rakhimova et al., 2023). Nephrocytes of all parts of the nephron, having cytoplasm and nucleus, had a similar structure. The interstitial tissue between the tubules consisted of thin, delicate collagen fibers with single connective tissue cells. The morphology of the kidneys of experimental and control animals did not differ significantly.

When familiarizing yourself with the structure of the stomach, it was found that its wall consists of 4 membranes: mucous membrane, submucosa, muscular membrane, and serous membrane (Figure 1B) (Rakhimova et al., 2023). The mucous membrane is covered with a single-layer columnar epithelium, in which the apical and basal parts are distinguished.

When examining the mucous membrane of the stomach under the microscope, it was observed that the apical part of the cytoplasm is colored pale pink, while the basal part contains a basally colored nucleus (Rakhimova et al., 2023). Beneath the epithelial cover in the lamina propria of the mucous membrane, numerous tubular fundic glands of the stomach were observed. Irregularly shaped parietal cells were identified in the body and bottom of the gland, characterized by slightly pink cytoplasm and spherical nuclei.

The main cells of the gland, cylindrical in shape, contained secretory granules in the cytoplasm, although these granules were not clearly visible under the microscope. These cells exhibited slightly basophilic cytoplasm, with a small nucleus in the basal part. Cervical and accessory cells were also observed in the body and neck of the gland, characterized by small cytoplasmic volume and hyperchromatically stained nuclei (Misbah et al., 2013).

Additionally, the mucous membrane contained single small lymphoid follicles, blood vessels, and connective tissue cellular elements. The muscular layer comprised smooth muscle cells without any discernible changes (Rakhimova et al., 2023).

Upon analyzing the structure of the small intestine under the microscope, well-defined structures such as villi and crypts were observed (Gilbert-Barness et al, 2010). The villi appeared finger-shaped and were covered on top with a single-layer columnar epithelium. These cells exhibited a clear brush border in their apical part, with elongated nuclei in the basal part. Solitary goblet cells were observed among the cylindrical border cells. Small blood capillaries and single connective tissue cells were visible in the villi's stroma. The crypts of the small intestine were of normal depth, occasionally containing dividing cells at their bottom (Haldi et al, 2011).

In the lamina propria and submucosa, numerous round lymphoid follicles and tiny blood vessels were observed (Halili et al, 2011).

During the histological examination of the hearts of experimental animals, particular attention was paid to the structure of the myocardium and endocardium (Figure 1C) (Nwodo et al., 2011). The endocardium formed a thin layer consisting of endothelial and subendothelial layers. The myocardium was characterized by intertwined cardiomyocytes, cut in different planes. Each cell contained a rounded nucleus centrally, with numerous longitudinally located thin myofibrils in the cytoplasm. Transverse striations were visible in the myofibrils, and numerous small blood capillaries surrounded the cardiomyocytes. No significant morphological changes were detected in the heart's structure (Kimet al., 2014).

Like the control group, the spleen parenchyma consisted of white and red pulp, with the red pulp occupying the largest part (Ashok et al., 2017). Macrophages containing dark brown pigment grains were detected in the sinus walls and their surroundings. The white pulp appeared round and large, consisting of clusters of lymphocytes. Some follicles exhibited a clear light center known as the germinal center. Smaller cells with hyperchromatic nuclei were observed at the periphery of the follicles, along with accumulations of small lymphocytes (Rajagopal et al., 2017).

In histological sections, the adrenal gland comprises a cortex and medulla, with the medulla appearing darker in color than the cortex and covered by a thin connective tissue capsule (Rakhimova et al., 2023). Within the cortex, three zones are typically distinguished. Beneath the capsule, small cells of the zona glomerulosa are found, characterized by a light appearance of the cytoplasm and small, hyperchromatic nuclei. Polygonal cells representing the zona fasciculata exhibit a pale pink cytoplasm, with some cells showing cytoplasmic swelling and round nuclei containing chromatin in the form of small grains. Cells of the reticular zone also display a polygonal shape, small nuclei, and light pink cytoplasm (Rakhimova et al., 2023).

The medulla consists of large polygonal cells with basophilic cytoplasm, interspersed with lighter cells, and wide sinusoidal hemocapillaries between them. Importantly, the structure of the adrenal glands from both control and experimental animals showed no noticeable differences (Singleman et al., 2014).

Thus, the internal organs of animals administered with the medicinal balm exhibited a normal histological structure, devoid of dystrophic, destructive changes, or signs of histomorphological transformations. These findings suggest the absence of chronic toxicity associated with the medicinal balm (Rakhimova et al., 2023).

Conclusion

The study thoroughly examined the chronic toxicity, embryotoxicity, and teratogenic effects of a medicinal balm with adaptogenic properties, crucial for ensuring its safety and efficacy in therapeutic applications. The research on pregnant white laboratory rats and their offspring encompassed meticulous evaluations of various physiological parameters, including body weight, organ development, and biochemical profiles. The administered balm did not demonstrate teratogenic or embryotoxic effects, indicating its safety for fetal development. Moreover, chronic toxicity studies revealed no significant adverse effects on body weight, peripheral blood parameters, or body temperature in experimental animals. Histological examinations of vital organs affirmed the absence of morphological aberrations, reinforcing the balm's benign nature. These comprehensive findings underscore the potential therapeutic utility of the medicinal balm, providing valuable insights into its safety profile and supporting its incorporation into complex therapeutic regimens for managing various pathologies. The study's meticulous methodology adhered to ethical guidelines, ensuring robust scientific integrity and reliability of the results, thus contributing to the advancement of pharmacological research in adaptogenic therapeutics.

Author contributions

Z.T.F., M.M.R., G.O.R., A.J.R., D.M.H. conceptualized, drafted, wrote, reviewed and edited the article.

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Competing financial interests

The authors have no conflict of interest.

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