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# Hepatoprotective properties and acute toxicity of *Lagotis korolkowii* (Regel & Schmalh.) Maxim.

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**Abstract**

**Introduction.** The genus *Lagotis* (*Plantaginaceae*) comprises species traditionally used for treating liver disorders. *Lagotis korolkowii* (Regel & Schmalh.) Maxim., despite its potential, remains phytochemically and pharmacologically underexplored, particularly regarding its safety and hepatoprotective properties.

**Aim.** This study aimed to evaluate the acute oral toxicity and hepatoprotective efficacy of an extract from *Lagotis korolkowii* (Regel & Schmalh.) Maxim. in experimental models.

**Materials and methods.** Acute toxicity was assessed in albino mice following GOST 32644-2014 and Stefanov's guidelines, administering single oral doses up to 10,000 mg/kg. Hepatoprotective activity was investigated in male albino rats with paracetamol-induced acute hepatitis (1000 mg/kg for 2 days). Treatment groups received the plant extract orally at doses of 50, 100, and 200 mg/kg for 7 consecutive days post-intoxication. Evaluations included observation of general condition, mortality, hematological analysis (complete blood count), and blood biochemical parameters (ALT, AST, bilirubin, total protein, etc.).

**Results and discussion.** The median lethal dose ($LD_{50}$) of *Lagotis korolkowii* (Regel & Schmalh.) Maxim. extract exceeded 10,000 mg/kg, classifying it as practically non-toxic (Class V). In the hepatotoxicity model, the extract dose-dependently ameliorated paracetamol-induced alterations. The 50 mg/kg dose demonstrated the highest activity, significantly reducing plasma ALT (by 4 %), AST (by 27 %), and direct bilirubin (by 39 %) levels while increasing total protein (by 17 %) compared to the intoxicated control group. Positive normalization of erythrocyte, hemoglobin, and leukocyte counts was also observed.

**Conclusion.** *Lagotis korolkowii* (Regel & Schmalh.) Maxim. extract possesses a high safety profile and exhibits significant, dose-dependent hepatoprotective effects against paracetamol-induced liver damage, with the 50 mg/kg dose showing optimal efficacy. These findings support its potential as a source for developing safe and effective hepatoprotective phytomedicines.

**Keywords:** median lethal dose ($LD_{50}$), erythrocyte, hemoglobin, leukocyte, paracetamol-induced hepatitis, biochemical markers

**Conflict of interests.** The authors declare that they have no obvious or potential conflicts of interest related to the publication of this article.

**Contribution of the authors.** Visolaxon M. Nosirova – conceived the experiment, developed the methodology, and provided scientific supervision. Vakhobjon U. Khujaev, Obidjon Turdiboev – reviewed the literature, collected data, and conducted the experiments.

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# Гепатопротекторные свойства и острая токсичность *Lagotis korolkowii* (Regel & Schmalh.) Maxim.

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**Резюме**

**Введение.** Род *Lagotis* (*Plantaginaceae*) включает виды, традиционно применяемые для лечения заболеваний печени. Несмотря на свой потенциал, лаготис Королькова (*Lagotis korolkowii* (Regel & Schmalh.) Maxim.) остается недостаточно изученным в фитохимическом и фармакологическом отношении, особенно в части его безопасности и гепатопротекторных свойств.

**Цель.** Оценка острой пероральной токсичности и гепатопротекторной эффективности экстракта *Lagotis korolkowii* (Regel & Schmalh.) Maxim. на экспериментальных моделях.

**Материалы и методы.** Острую токсичность оценивали на мышах-альбиносах в соответствии с ГОСТ 32644-2014 и руководством А. В. Стефанова, применяя разовые пероральные дозы до 10 000 мг/кг. Гепатопротекторную активность исследовали на крысах-самцах с моделью острого парацетамолиндуцированного гепатита (1000 мг/кг в течение 2 дней). Группы лечения получали экстракт растения перорально в дозах 50, 100 и 200 мг/кг в течение 7 дней после интоксикации. Проводили оценку общего состояния, показателей гематологического (общий анализ крови) и биохимического (АЛТ, АСТ, билирубин, общий белок и др.) анализов крови.

**Результаты и обсуждение.** Средняя летальная доза ($ЛД_{50}$) экстракта *Lagotis korolkowii* (Regel & Schmalh.) Maxim. превысила 10 000 мг/кг, что позволяет классифицировать его как практически нетоксичное вещество (V класс). В модели гепатотоксичности экстракт дозозависимо уменьшал вызванные парацетамолом нарушения. Доза 50 мг/кг продемонстрировала наибольшую активность, достоверно снижая уровни АЛТ (на 4 %), АСТ (на 27 %) и прямого билирубина (на 39 %) в плазме, а также повышая уровень общего белка (на 17 %) по сравнению с группой интоксикации. Также наблюдалась положительная динамика в нормализации количества эритроцитов, уровня гемоглобина и лейкоцитов.

**Заключение.** Экстракт *Lagotis korolkowii* (Regel & Schmalh.) Maxim. обладает высоким профилем безопасности и проявляет значительные дозозависимые гепатопротекторные эффекты против повреждения печени, индуцированного парацетамолом, при этом доза 50 мг/кг показала оптимальную эффективность. Полученные данные подтверждают его потенциал в качестве источника для разработки безопасных и эффективных гепатопротекторных фитопрепаратов.

**Ключевые слова:** средняя летальная доза ($ЛД_{50}$), эритроцит, гемоглобин, лейкоцит, парацетамолиндуцированный гепатит, биохимические маркеры

**Конфликт интересов.** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

**Вклад авторов.** В. М. Носирова – идея проведения эксперимента, методология и научное руководство. В. У. Хужаев, О. Турдибоев – обзор литературных данных, сбор материалов, проведение экспериментов.

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## *Introduction*

The genus *Lagotis* (*Plantaginaceae*) comprises approximately[1] 30 species and is primarily distributed in mountainous regions of Asia, particularly in Central Asia, the Himalayas, and parts of China [1–3]. Representatives of this genus are notable for their rich content of biologically active compounds, especially flavonoids, phenolic compounds, and other secondary metabolites [1, 2].

In recent years, several species within the genus *Lagotis* have become subjects of comprehensive scientific investigation. For instance, the chemical composition and biological activity of species such as *Lagotis brevituba* Maxim., *Lagotis integrifolia* (Willd.) Schischk.,

[1] POWO (2024). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Available at: https://powo.science.kew.org/ Accessed: 06.11.2024.

*Lagotis yunnanensis* W.W.Sm., *Lagotis alutacea* W.W.Sm., *Lagotis integra* W.W.Sm., *Lagotis cashmeriana* (Royle ex Benth.) Rupr., and *Lagotis brachystachya* Maxim. have been extensively studied. Numerous compounds have been isolated from these species, and their antioxidant, anti-inflammatory, and hepatoprotective properties have been confirmed [3–6]. Flavonoids and phenolic compounds [7, 8] identified in these species have been reported to play a significant role in protecting liver cells from oxidative stress and toxic agents [9, 10]. Preclinical studies on certain phytocompositions further demonstrated antifibrotic activity and safety in animal models [11].

Despite the remarkable biological activity exhibited by many representatives of the genus *Lagotis*, the chemical composition of *Lagotis korolkowii* (Regel & Schmalh.) Maxim. remained largely unexplored for a long time. To address this gap, we conducted the first comprehensive investigation into the chemical composition of *Lagotis korolkowii* (Regel & Schmalh.) Maxim. [12, 13]. This study confirmed the presence of biologically active compounds in the plant, demonstrating its pharmacological potential.

The findings on the chemical composition highlighted the need for deeper investigation into the biological activity of *Lagotis korolkowii* (Regel & Schmalh.) Maxim. Accordingly, it was decided to proceed with experimental studies aimed at assessing the safety of extracts from this plant and determining its hepatoprotective activity.

**Aim.** The aim of this study was to evaluate the acute toxicity and hepatoprotective activity of *Lagotis korolkowii* (Regel & Schmalh.) Maxim. extract under experimental conditions.

## Materials and Methods

For the study, we used the dried herb of *Lagotis korolkowii* (Regel & Schmalh.) Maxim., which was collected in the Namangan region in 2025 and dried to a residual moisture content not exceeding 14 %.

The acute toxicity of *Lagotis korolkowii* (Regel & Schmalh.) Maxim. plant extract was evaluated according to GOST 32644-2014 and the methodological guidelines of A. V. Stefanov [14] using male albino mice with a body weight of 19–22 g. The extract was dissolved in distilled water to prepare 5 %, 10 %, and 50 % solutions. It was administered orally via a metal probe at doses of 500, 1000, 5000, and 10,000 mg/kg. The animals were observed closely for the first 3–4 hours, then over 24 hours, and subsequently once daily for 14 days. During the observation period, the following parameters were assessed: the condition of the fur, motor activity, food and water consumption, body weight, respiration, heart rate, and any observable mortality.

### *Assessment of hepatoprotective activity of Lagotis korolkowii (Regel & Schmalh.) Maxim. extract in albino rats*

#### *Experimental Animals*

The study was conducted on 25 adult male albino rats (Wistar strain) weighing 200–270 g. The animals were obtained from a certified laboratory animal breeding center. Before the experiment, the animals were acclimatized for 7 days under standard laboratory conditions.

The rats were housed in polypropylene cages under controlled environmental conditions: temperature 22 ± 2 °C, relative humidity 55–60 %, and a 12 h light / 12 h dark cycle. The animals had free access to standard pelleted diet and drinking water ad libitum.

All experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals and were approved by the local bioethics committee [15,16].

#### *Experimental Design*

Acute toxic hepatitis was induced by oral administration of paracetamol at a dose of 1000 mg/kg body weight for two consecutive days.

After induction of hepatitis, the animals were randomly divided into five groups ($n = 5$ per group):

***Group I (Intact control):*** healthy animals without treatment.

***Group II (Negative control):*** animals with paracetamol-induced hepatitis without treatment.

***Group III:*** paracetamol + *Lagotis korolkowii* (Regel & Schmalh.) Maxim. extract at a dose of 50 mg/kg for 7 days.

***Group IV:*** paracetamol + *Lagotis korolkowii* (Regel & Schmalh.) Maxim. extract at a dose of 100 mg/kg for 7 days.

***Group V:*** paracetamol + *Lagotis korolkowii* (Regel & Schmalh.) Maxim. extract at a dose of 200 mg/kg for 7 days.

The plant extract was administered orally once daily for 7 days.

Upon completion of the experiments, the animals were anesthetized using ether anesthesia. A comparative study of complete blood count and blood biochemical analysis was conducted among the intact, control, and experimental groups using modern hematological and biochemical analyzers.

Statistical processing of the obtained data was performed in accordance with statistical tables for the accelerated quantitative assessment of pharmacological effect [17].

## Results and discussion

The conducted experiments demonstrated that a single oral administration at doses of 500, 1000, and 5000 mg/kg had virtually no effect on the physical

and functional state of the experimental animals, nor on their water and food intake. The condition of the mice remained active, and no signs of intoxication were recorded. The animals showed adequate responses to pain, tactile, and sound stimuli. The heart rate and respiratory movements remained within normal limits. At a dose of 10,000 mg/kg, a state of reduced mobility and weakness was observed 30 minutes to 3–4 hours after substance administration. Starting from the second day of the experiment, the general condition of the animals returned to normal. Food and water intake was normal, and no signs of intoxication or mortality were observed throughout the 14-day period. It was proven that the median lethal dose ($LD_{50}$) of *Lagotis korolkowii* (Regel & Schmalh.) Maxim. plant extract is above 10,000 mg/kg.

It is known that the liver serves as the central biochemical laboratory of the body. It facilitates intensive metabolic processes, including protein, lipid, and carbohydrate metabolism. An increase in white blood cell count indicates inflammation of liver cells. A decrease in erythrocyte and hemoglobin levels suggests that liver damage has subsequently affected hematopoiesis. Considering that erythrocytes – formed elements of the blood – are produced and degraded in the liver, and that bile formation is based on this process, the prevention of anemia formation in toxic hepatitis is of significant importance. Taking these factors into account, the blood samples collected on the final day of the experiment were analyzed using a hematological analyzer. In the control group of experimental animals with induced toxic hepatitis, we observed a decrease in the count of blood cells – erythrocytes and platelets. This, in turn, led to a reduction in hemoglobin and hematocrit levels, while causing an increase in leukocyte count. Based on the obtained results, it is evident that pathological changes in the formed elements were observed in albino rats with induced toxic hepatitis. Upon administering *Lagotis korolkowii* (Regel & Schmalh.) Maxim. plant extract at doses of 50, 100, and 200 mg/kg for 7 days, positive dynamic changes in the main formed blood elements were observed. The obtained results are presented in Table 1.

As can be seen from the table, administration of the *Lagotis korolkowii* (Regel & Schmalh.) Maxim. plant extract orally at doses of 50 and 100 mg/kg resulted in a positive shift in the pathological changes observed in the blood cells compared to the control and intact groups. Subsequent investigations focused on blood biochemical analysis, evaluating the levels of total protein, ALT, AST, bilirubin, glucose, urea, and creatinine in the blood plasma.

In the paracetamol-induced model of toxic hepatitis, a decrease in total protein and an increase in ALT, AST, and bilirubin levels – indicative of intensified cytolysis in liver cells – were identified in the control group. An increase in direct bilirubin levels in the plasma indicates damage to liver cells.

The *Lagotis korolkowii* (Regel & Schmalh.) Maxim. plant extract at a dose of 50 mg/kg was observed to reduce plasma ALT levels by 4 %, AST by 27 %, and direct bilirubin by 39 %, while increasing total protein by 17 % compared to the control group.

At a dose of 100 mg/kg, it was found that plasma ALT levels decreased by 14 %, AST by 54 %, direct bilirubin by 35 %, while total protein increased by 8 %.

At a dose of 200 mg/kg, a reduction in ALT by 3 %, AST by 31 %, and direct bilirubin by 48 % was recorded compared to the control group, with total protein increasing by 7 %. The obtained results are presented in Table 2.

## Conclusion

The conducted experimental studies demonstrate that the acute toxicity of the *Lagotis korolkowii* (Regel & Schmalh.) Maxim. plant extract, in accordance with GOST 32644–2014 and the A. V. Stefanov methodology, classifies it as a practically non-toxic substance (Class V).

**Table 1. Results of Complete Blood Count Parameters for the Studied Substances**

| Parameters / Groups | Intact | Control | 50 mg/kg | 100 mg/kg | 200 mg/kg |
|---|---|---|---|---|---|
| Erythrocytes, $10^{12}$/L | 7.80 ± 0.61 | 6.80 ± 3.24 | 9.89 ± 1.21* | 9.13 ± 0.64* | 7.85 ± 0.97* |
| Hemoglobin, g/L | 130.8 ± 10.45 | 120.8 ± 48.95 | 132.75 ± 4.9* | 145.5 ± 9.3* | 136.0 ± 12* |
| Hematocrit, % | 38.8 ± 2.03 | 34.82 ± 14.36 | 37.53 ± 1.16* | 41.25 ± 2.7* | 39.25 ± 3.47 |
| Leukocytes, $10^9$/L | 8.44 ± 1.46 | 10.50 ± 5.23 | 9.71 ± 2.16* | 9.59 ± 3.60* | 8.75 ± 0.62* |
| Platelets, $10^9$/L | 942 ± 56.8 | 822 ± 93.8 | 912 ± 24.8* | 940.5 ± 47.3* | 912 ± 45.8* |

**Note.** * $p \leq 0.05$ compared to the control group.

The table was provided by the authors

**Table 2. Results of Blood Biochemical Analysis Parameters for the Studied Substances**

| Parameteres / Groups | Intact | Control | 50 mg/kg | 100 mg/kg | 200 mg/kg |
|---|---|---|---|---|---|
| Total Protein, g/L | 73.1 ± 8.25 | 84.26 ± 9.35 | 69.74 ± 7.7* | 77.5 ± 9.9* | 90.6 ± 11.55* |
| ALT, E/L | 64.6 ± 6.6 | 72.6 ± 7.15 | 62.6 ± 4.95* | 70.2 ± 8.8* | 70.4 ± 7.15* |
| AST, E/L | 164.8 ± 15.4 | 284.8 ± 17.6 | 132.2 ± 9.35* | 208.4 ± 14.3* | 197.6 ± 12.65* |
| Total bilirubin, μmol/L | 15.8 ± 2.75 | 24.86 ± 4.4 | 16.68 ± 3.85* | 16.54 ± 3.85* | 17.24 ± 4.4* |
| Direct bilirubin, μmol/L | 2.26 ± 0.55 | 7.4 ± 1.1 | 4.52 ± 0.55* | 4.8 ± 0.55* | 3.84 ± 0.55* |
| Indirect bilirubin, μmol/L | 13.58 ± 1.65 | 17.46 ± 2.2 | 12.16 ± 1.65* | 11.74 ± 1.65* | 13.36 ± 2.2* |
| Glucose, mmol/L | 5.38 ± 1.1 | 3.93 ± 0.55 | 4.47 ± 0.55* | 4.66 ± 0.55* | 4.3 ± 0.55* |
| Creatinine, μmol/L | 60.2 ± 6.6 | 54.4 ± 6.05 | 52.4 ± 5.5 | 51.6 ± 5.5 | 53.0 ± 3.85 |
| Urea, mmol/L | 4.56 ± 1.1 | 3.06 ± 0.55 | 3.08 ± 0.55 | 4.08 ± 0.55 | 2.78 ± 0.55 |

**Note.** * $p < 0.05$ — statistically significant difference compared to the control group.

The table was provided by the authors

Its median lethal dose ($LD_{50}$) upon oral administration exceeds 10,000 mg/kg, confirming a high safety profile for potential therapeutic use.

Analysis of hematological and blood biochemical parameters revealed a pronounced hepatoprotective effect of the *Lagotis korolkowii* (Regel & Schmalh.) Maxim. extract in a paracetamol-induced acute toxic hepatitis model. The extract, particularly at a dose of 50 mg/kg, significantly reduced key markers of liver cytolysis (ALT, AST, bilirubin) and contributed to the restoration of total protein levels, thereby protecting the functional integrity of hepatocytes.

Thus, the obtained results justify the potential of the *Lagotis korolkowii* (Regel & Schmalh.) Maxim. extract as a promising natural hepatoprotective agent characterized by low toxicity and high biological activity. The 50 mg/kg dose stands out as the most optimal therapeutic candidate for subsequent preclinical and clinical investigations.

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