

## RESEARCH ON THE ACUTE TOXICITY OF THE GRAIN OF INDIGOFERA TINCTORIA LINN

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**Abstract.** Some scientific sources provide information about the presence of toxic compounds for living organisms in the content of plants belonging to the *Indigofera* family. The aim of this research is to examine the overall impact and acute toxicity of the *Indigofera tinctoria* plant's grain, belonging to the *Fabaceae* family, which were grown in the meadow-oasis soils in the "Abdurazzok-Mukhtarjon Yuksalish" farm, in the Uchkoprik district of the Fergana region. Experiments were performed on male non-purebred white laboratory mice with a body weight of  $20 \pm 2.0$  g. As a result of the research on the acute toxicity properties of the grain, it is determined that the grain of *Indigofera tinctoria* belongs to the class of non-toxic compounds.

**Keywords:** grain of the plant *Indigofera tinctoria*, acute toxicity, non-purebred white laboratory mice, medium-lethal dose ( $LD_{50}$ ), toxicity class.

### Introduction.

The chemical content of the plants belonging to the family *Fabaceae* is rich in many complex compounds such as protein, oil, carbohydrates, vitamins, flavonoids, indigoids, macro and microelements, and the plants belonging to the order *Indigofera* are particularly important among them.

Some scientific sources provide information about the presence of toxic compounds for living organisms in plants belonging to the order *Indigofera*. Our aim is to determine the toxicity level of the *Indigofera tinctoria* plant grown in the climatic conditions of Fergana region to animals and to use this plant as fodder for livestock.

The decree of the President of the Republic of Uzbekistan №DP-120 "On approval of the 2022-2026 program for the development of the livestock industry and its branches in the Republic of Uzbekistan" dated February 8, 2022, outlines several tasks, including the development of modern technologies for fodder production and preparation, the use of organic materials, the production of appropriate equipment by local manufacturers, development of the production of appropriate types of equipment by local manufacturers through the storage and delivery of livestock products, and increasing the yield from 8 centners to 12 centners by planting drought-resistant fodder plants in mountain and sub-mountain pastures.

Studying and applying the chemical composition of plants belonging to the order *Indigofera* can help solve many important problems. For example, using natural compounds such as *flavonoids*, *terpenoids*, *steroids*, *rotenoids*, and *alkaloids* found in *Indigofera* plants in the pharmaceutical industry can lead to the creation of medicinal natural preparations. Additionally, cultivating these plants in agriculture can increase the productivity of infertile and low-yielding lands, enrich the soil with nitrogen, and using the obtained fodder products in animal husbandry can increase the productivity of animals.

Literature review.

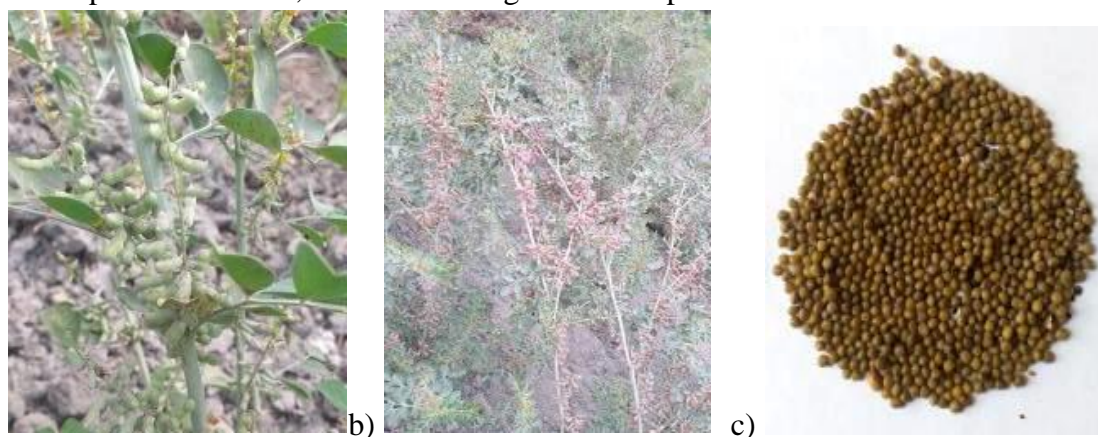
Scientists have been interested in studying the chemical composition of plants belonging to the order *Indigofera* for a long time, and this problem has been continuously researched for years, and these scientific researches are still being continued today. In particular, the most studied species, *Indigofera spicata*, was proven to be hepatotoxic to chickens [1] and cattle when grazed [2] or toxic when eaten by rabbits, mice and rats [3]. Free non-protein amino acid analogue of Arginine “*Indospisin*” [4] was identified in the grain and leaf of *Indigofera spicata*. *Indigofera hirsute*, *indigofera linifolia*, and other species of *Indigofera spicata* among Ethiopian species have not been given enough information about palatability and toxicity [5].

3-nitropropionic acid (3-NPK) has been identified as a toxic constituent for some species of the order *Indigofera* [5]. Britten and other scientists (1963) found that chicks are especially sensitive to pure 3-NPK in leaves, but the stem and grain of *Indigofera spicata* are not affected because these parts do not contain 3-NPK. An experiment by Strickland and others (1987) found no signs of 3-NPK poisoning without alveolar emphysema or locomotor disturbances in any of the rats observed in the experiment [6]. Plant species containing less than 2.5% dry matter of 3-NPK are suggested to be only mildly toxic (Williams and Davis 1982). The highest 0.34% dry matter and 3-NPK [7] reported by Strickland and others was for *Indigofera* species, therefore, a significant toxic effect of 3-NPK is observed with such minimal amounts. However, Williams (1981) found 3-NPK to be present in 64 out of 250 species of *INDIGOFERA* at a concentration of 0.5–3% dry matter and it is recommended that *indigofera* species be screened for 3-NPK before using as fodder.

Research Methodology.

The aim of this research is to examine the overall impact and acute toxicity of the *Indigofera tinctoria* plant's grain (Fig. 1), belonging to the *Fabaceae* family, which was grown in the meadow-oasis soils in the “Abdurazzok-Mukhtarjon Yuksalish” farm, in the Uchkoprik district of the Fergana region.

The *Indigofera tinctoria* plant, which is considered the object of research, was planted in the above-mentioned experimental farm field and cultivated with the implementation of necessary agrotechnical measures. The stem and grain of *Indigofera tinctoria* make up 70-80% of the total mass of the plant. Therefore, we studied the grain of this plant.



**Figure 1. The plant *Indigofera tinctoria* L. a) pod stage, b) ripening stage, c) grain**

Studies were conducted to determine the acute toxicity of the plant *Indigofera tinctoria* plant. Experiments were performed on male non-purebred white laboratory mice with a body

weight of  $20 \pm 2.0$  g. The research was conducted on the basis of the generally accepted methods, on the groups with 6 mice in each and a total of 90 animals involved.

Research analysis and results.

All pharmacological tests were carried out in healthy, sexually mature mice kept in quarantine for 10-14 days. The sorbent was injected into the stomach of mice in doses of 1000, 2000, 3000, 4000 and 5000 mg/kg using a special probe in different concentrations. The animals of the control group were given an equal volume of distilled water. On the first day of the experiments in laboratory conditions, the general condition of the animals of the research and control groups was monitored every hour, to determine possible shivering and death cases as an indicator of the functional circumstance. During the next 2 weeks, in the vivarium, the general condition, activity, fur coat, skin condition, rate and depth of breathing, urination, body weight change, and other indicators of the mice in all groups were examined daily. All experimental animals were kept on the same routine diet, water and food were not limited [8, 9].

At the end of the experiment, the average lethal dose ( $LD_{50}$ ) and toxicity class of the tested drug were determined [10]. Statistical calculation of the obtained results was carried out on the basis of the Windows XP (Excel) program.

The results on determining the acute toxicity of the grain of *Indigofera tinctoria*: The general effects and acute toxicity of the grains of *Indigofera tinctoria* were studied in non-purebred white laboratory mice (Figure 2). The generally accepted method was used to determine the parameters of acute toxicity. The studied substance was injected into the stomach using a special probe in doses of 1000, 2000, 3000, 4000 and 5000 mg/kg. The animals of the control group were injected an equal volume of distilled water. On the first day of the experiment, the general condition of the animals was monitored hourly in the laboratory, where the survival rate, general condition, possible shivering and death were observed during the experiment as indicators of their functional status. Then, for 2 weeks in the vivarium conditions, the general condition and activity of the animals of all groups, the specific states of their behavior, the depth and speed of breathing, changes in fur coat, skin cover and body weight were monitored every day. All animals were kept in the same comfortable living conditions on a common diet, water and food were not restricted.



**Figure 2. Non-purebred white experimental mice**

In the studied doses of 1000, 2000, 3000, 4000 and 5000 mg/kg of *Indigofera tinctoria* plant grain, no effects of acute poisoning were observed in the above-mentioned indicators of animals. Only at doses of 4000 and 5000 mg/kg, 5-10 minutes after the introduction of the sorbent, there were cases of acceleration and accumulation of the animals' breathing, this process lasted

15-20 minutes. No animal deaths were observed at the above doses during the whole experiment. Animals in the experimental groups did not show a decrease in body weight when compared to the control group. Based on the obtained results, we can conclude that the average lethal dose of injecting the sorbent into the stomach of mice is (LD<sub>50</sub>)>5000 mg/kg. The obtained results are presented in Table 1 below.

**Table 1**

**Acute toxicity indicators of the grain of *Indigofera tinctoria* plant when injected into the stomach of mice**

Groups	Animal type & sex	Dose mg/kg, ml	Number of animals/ dead animals in the group	Average animal weight (1 day)	Average animal mass (14 kun)	LD <sub>10</sub>	LD <sub>50</sub> with a confidence interval	LD <sub>84</sub>
Grain of <i>Indigofera tinctoria</i> plant	Mouse Male	1000	6/0	21	25		>5000 mg/kg	
		2000	6/0	20	25			
		3000	6/0	21	24			
		4000	6/0	20	22			
		5000	6/0	21	23			
Control		0,5	6/0	20	24			

**Conclusion/Recommendations.**

The adverse effects and toxicity of species, such as *Indigofera spicata*, *Indigofera hirsute*, and *Indigofera linifolia* of the order *Indigofera* for chickens, rabbits, cattle and other domestic animals are claimed in scientific literature.

Thus, the results of the study of the acute toxicity properties of the grain of *Indigofera tinctoria* showed that the average lethal dose (LD<sub>50</sub>) after a single ingestion is higher than 5000 mg/kg and belongs to the group VI class of non-toxic compounds.

Including, cultivation of *Indigofera tinctoria* on agricultural land and its use as fodder for livestock does not cause adverse effects. *Indigofera tinctoria* can be grown as fodder for livestock. The amount of green mass of this plant is the highest, especially in August, that is, during the pod period.

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