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Assessment of Acute Toxicity of *Euphorbia taifensis* Methanolic-aqueous Extract in Rats

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Abstract: **The acute toxicity profile of *Euphorbia taifensis* Methanolic-aqueous Extract in rats was investigated in this study. The objectives were to determine its oral median lethal dose (LD_{50}), assess its effects on body weight changes, and evaluate its impact on hematological parameters. The LD_{50} was determined through oral administration of the extract at a concentration of 2000 mg/kg. No mortality occurred, and no signs of acute toxicity were observed throughout the experimental period. Body weight changes were monitored for 14 days, and no statistically significant differences were observed between the *E.taifensis*-treated rats and the control rats. The study evaluated the impact of *E. taifensis* extract on blood parameters. While white blood cell counts remained stable, neutrophils and monocytes increased significantly, and eosinophils decreased. Red blood cell and hemoglobin levels were unaffected, but red cell indices notably changed. Additionally, the extract significantly affected platelet count and volume. In conclusion, *Euphorbia taifensis* extract showed no acute toxicity at 2000 mg/kg, with no mortality or significant body weight changes observed. Hematological analysis revealed the extract may have diverse effects on blood parameters, warranting further research into its potential therapeutic applications.**

Keywords: ***Euphorbia taifensis*****, Methanolic-aqueous extract, Acute toxicity, LD_{50}, Hematological parameters, Rats.**

I. INTRODUCTION

Euphorbia taifensis is a species belonging to the genus *Euphorbia* L. and the family *Euphorbiaceae*. It is a plant species native to the Taif region in Saudi Arabia, has been traditionally used for its medicinal properties [1, 2]. *Euphorbia taifensis* reaches a height of 10 meters, is a spiny tree and has many succulent branches with bright green branches. These plants grow from October to February at altitudes between 1,700 and 2,100 meters on the rocky sides of valleys and near farms. This plant also flowers and produces fruits [1, 3]. Several studies have reported the potential therapeutic benefits of this plant, including anti-inflammatory, antioxidant, and antimicrobial effects [4]. However, despite its long history of use, limited information is available on the acute toxicity profile of *Euphorbia taifensis* and its potential effects on hematological parameters.

In recent years, the assessment of acute toxicity has become an essential part of preclinical safety evaluation for the development of new drugs and herbal extracts [5]. Acute toxicity studies provide valuable information about the potential risks and safety of a substance when administered at high doses within a short period of time. Therefore, understanding the acute toxicity profile of *Euphorbia taifensis* is crucial for its further development as a potential therapeutic agent.

The objective of our study was to assess the acute toxicity of *Euphorbia taifensis* Methanolic-aqueous Extract in rats. We aimed to:

1. To determine the oral median lethal dose (LD_{50}) of *Euphorbia taifensis* Methanolic-aqueous Extract in rats.

2. To assess the effect of *Euphorbia taifensis* Methanolic-aqueous Extract on body weight changes in rats.

3. To evaluate the impact of *Euphorbia taifensis* Methanolic-aqueous Extract on hematological parameters.

These objectives will enable a comprehensive assessment of the acute toxicity of *Euphorbia taifensis* Methanolic-aqueous Extract, shedding light on its potential risks and benefits for future pharmacological applications.

II. MATERIALS AND METHODS

A. Animals

The study was conducted under the Animal Care and Use Committee (ACUC) of King Fahd Medical Research Center and was assigned the reference number Acuc-22-08-12 of Animal Use Protocol (AUP).

Twelve healthy adult male Wistar rats, weighing 200-220 g were used for this investigation. The experimental rats were sourced from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The animals were kept in standard plastic enclosures and sustained under controlled laboratory environments at 60% humidity, temperature (20±2°C), and 12:12 hr light: dark cycle with ad libitum access to nutrients and water. The rats were accustomed to the laboratory environments for 7 days before the experimental treatments were initiated. This experimental work was officially endorsed by the Unit of Biomedical Ethics of King Abdulaziz University (Reference No 93-23).

B. Collection and Identification of E. taifensis

Fresh aerial parts (succulent part) of *E. taifensis* plants were collected from Al-Taif, Makkah Province, Saudi Arabia, in January 2022. The plant was identified by the Department of Biological Sciences of King Abdulaziz University, Jeddah, Saudi Arabia.

The collected plants were cleaned and washed under tap water to eliminate grime. The washed plants were air-dried at room temperature and sliced into small pieces to speed up drying time with daily checking and flipping for 14 days under disinfectant conditions. Finally, the dry samples were pulverized to fine powder using a mechanical grinder (Christy & Norris 8" Lab Mill, England).

C. Preparation and Extraction of Plant Materials

200 g of dry powder of *E. taifensis* was thoroughly soaked and stirred in 2 L of 80% methanol for 48 h in a sterile Florence beaker and filtered through 750 mm filter paper (Whatman, England). These extraction procedures were repeated three times to collect the entire extract. The methanolic aqueous extract was collected, evaporated, and concentrated at 40°C under low pressure in a rotary evaporator (IKA RV 10 digital V Rotary Evaporators, Germany).

Finally, the solvent was completely removed by evaporation in a heated oven at 40 °C (Memmert™ Universal Oven, UF55plus, Germany), forming a waxy green residue. The final yield of aqueous methanolic extracts (waxy green residue) of 200 g of *E. taifensis* powder was 36 g. The extracts were kept at 4°C for further use.

D. Body Weight Determination

The body weights of the rats were determined at the beginning of the experimental period and every week during the study period using a digital scale, at the same time every morning, noting that the weight of the rats was taken while the rats were fasting. In addition, the experimental animals were monitored throughout the study for signs of abnormalities.

E. Acute Toxicity Study

The acute toxicity study was assessed by *Oral Lethal Dose (LD_{50})*. The oral lethal dose of the methanolic-aqueous extracts of *E. taifensis* plant was estimated using method of the "limit dose test" by following the guidelines of "Organization of Economic Cooperation and Development (OECD). According to a previous study on protocols and recommendations for evaluating chemicals in animals, it was proposed that the maximum recommended oral dose should not exceed 2000 mg/kg [6].

However, if three or more rats survived then LD_{50} is recognized as > 2000 mg/kg while if three or more animals will die then LD_{50} is considered as < 2000 mg/kg.

F. Experimental Design

Twelve adult male Wistar rats were randomly grouped into two experimental groups (number of rats per group = 6):

Rats of Group 1 **(G1)**, which served as the **control group**, received 0.9% NaCl (normal saline). Rats of the second group **(G2)** was given a dose of **2000 mg/kg of *E. taifensis***, using a gavage, ensuring food retention for 3-4 hours after dosing.

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Rats were fasted for 12 hours with unrestricted access to water. The body weight of each fasting rat was taken before dose administration. After administration, experimental animals were observed for the first 2 to 4 hours for immediate signs of toxicity. Food was provided to the animals 4 hours after ingestion of the substance. The deaths observed in each group were recorded. The animals were monitored for an additional 14 days for signs of poisoning. The observations included mortality and changes in skin and fur, eyes, and mucous membranes. In addition, toxic symptoms of piloerection, lachrymatory, locomotor, and respiratory activities were observed.

G. Hematological Investigation

The blood samples were analyzed for white blood cell count (WBC or Leukocyte count), WBC differential count (neutrophils, lymphocytes, basophils, eosinophils, and monocytes), red blood cell count (RBC or erythrocyte count), hematocrit (HCT), hemoglobin (Hbg), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count and mean platelet volume (MPV) with the aid of an automated hematology analyzer (XN-9000 Series™, Sysmex Asia Pacific Pte Ltd, Singapore).

H. Statistical analysis

The mean values of all data and deviations obtained from experiments were taken and expressed as mean ± standard deviation (SD). The comparisons between the experimental groups were made using t-Test: Two-Sample Assuming Unequal Variances, using Microsoft Excel Worksheet, Office 2021 and Microsoft 365. The significant difference was considered less than 0.05 (P-values<0.05).

III. RESULTS

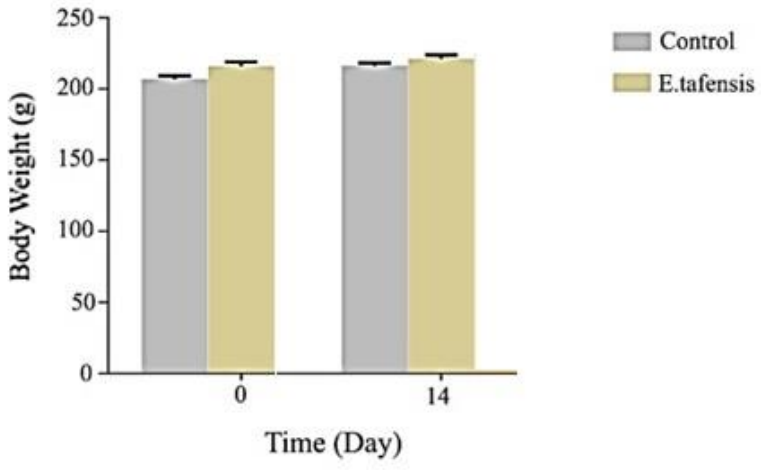
A. Observation and Mortality

The result of the acute toxicity study of *E. taifensis* using an oral dose at a concentration of 2000 mg/kg (Group 2) showed that no mortality occurred in any of the animals throughout the experimental period. The animals also did not show signs of acute toxicity. Interestingly, the animals refused food and drink during the first day of taking the extract and were observed to show signs of sedation with a weak response to stimuli such as sounds or thrusts for the first four hours. Apart from that, all rats treated with the plant appeared normal during the study period (14 days) compared to the control group.

B. Body Weight

A statistically non-significant difference was observed between the average body weights of the plant-treated rats compared to the control rats throughout the 14-day study period (Fig. 1).

Figure 1. Changes in body weight in control and *E. taifensis* groups after 14 days.



Values are expressed as mean ± SD.

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C. Hematological Analysis

This study aims to evaluate the acute toxicity of *E. taifensis* on hematological parameters. Table 1 shows that there was no significant effect of the methanolic-aqueous extract of *E. taifensis* on the white blood cell count (WBC), while the extract had a significant increase in neutrophils ($P< 0.001$) and monocytes ($P< 0.01$) and a significant decrease in eosinophils ($P< 0.05$).

Table 1. Effect of Methanolic-aqueous Extract of *E. taifensis* on white blood cells count and their different types.

Variables	Control (G1) (n= 6)	*E. taifensis* Extract (G2) (n= 6)	*P*-value
WBC ($x10^6$/µL)	2.85 ± 0.49	3.31 ± 0.00	0.058
Neutrophils ($x10^6$/µL)	0.19 ± 0.01	0.72 ± 0.02	0.0001***
Lymphocytes ($x10^6$/µL)	0.008 ± 0.004	0.001 ± 0.004	0.841
Monocytes ($x10^6$/µL)	0.005 ± 0.005	0.01 ± 0.005	0.003**
Eosinophils ($x10^6$/µL)	2.64 ± 0.50	2.60 ± 0.21	0.0179*
Basophiles ($x10^6$/µL)	0.005 ±0 .005	0.005 ± 0.005	1.000

Data is presented as Mean ± SD (n = 6). *Statistically significant at $P< 0.05$. **Statistically significant at $P< 0.01$. ***Statistically significant at $P< 0.001$.

Table 2 shows the effect of the methanolic aqueous extract of the studied plant on red blood cells (RBC) and their various indicators, as well as platelets. The extract did not affect RBC counts and hemoglobin, but it significantly affected the following parameters as follows: HCT ($P< 0.001$), MCV ($P< 0.001$), MCH ($P< 0.01$), and RDW ($P< 0.001$). The results also showed a highly significant effect of the extract on platelet count PLT ($P< 0.001$) and average platelet size MPV ($P< 0.05$).

Table 2. Effect of Methanolic-aqueous Extract of *E. taifensis* on red cell indices and platelets.

Variables	Control (G1) (n= 6)	*E. taifensis* Extract (G2) (n= 6)	*P*-value
RBC ($x10^6$/µL)	8.37± 0.28	8.28 ± 0.16	0.526
Hbg (g/dL)	14.06 ± 0.42	14.60 ± 0.44	0.060
HCT (%)	44.50± 0.93	47.25 ± 1.15	0.001***
MCV (fL)	53.25 ± 1.41	57.01 ± 1.40	0.0001***
MCH (pg)	16.71 ± 0.29	17.71 ± 0.60	0.008**
MCHC (g/dL)	31.36 ± 0.48	31.01 ± 0.58	0.286
RDW (%)	15.76 ± 0.83	20.78 ± 1.74	0.0003***
PLT ($x10^3$/µL)	746 ± 19.68	1491 ± 21.17	0.0001***
MPV (fL)	6.45 ± 0.25	6.766 ± 0.12	0.030*

Data is presented as Mean ± SD (n = 6). *Statistically significant at $P< 0.05$. **Statistically significant at $P< 0.01$. ***Statistically significant at $P< 0.001$.

IV. DISCUSSION

In this study, we aimed to assess the acute toxicity of *Euphorbia taifensis* Methanolic-aqueous Extract in rats through LD_{50} determination, body weight change analysis, and evaluation of hematological parameters.

Regarding the acute toxicity assessment, our results demonstrated that the oral administration of *E. taifensis* extract at a concentration of 2000 mg/kg (Group 2) did not result in any mortality or signs of acute toxicity throughout the experimental period. This indicates that the extract may have a relatively low acute toxicity in rats. These findings are in line with a previous study by Ugwah-Oguejiofor et al. (2019), where no mortality or pronounced signs of toxicity were observed in rats treated with a similar extract derived from the *Euphorbia* genus. However, it is worth noting an intriguing observation during the initial phase of the experiment [7]. The rats in Group 2 showed a temporary aversion to food and drink on the first day of extract administration. Additionally, they exhibited signs of sedation, with a weak response to stimuli such as

sounds or thrusts for the first four hours. These effects gradually subsided, and the animals appeared normal throughout the study period.

In our investigation of the acute toxicity of *E. taifensis* Methanolic-aqueous Extract, we also assessed the effect of the extract on body weight changes in rats. Interestingly, our results revealed a statistically non-significant difference in the average body weights between the *E. taifensis* treated rats and the control rats throughout the 14-day study period. This implies that the administration of the extract did not significantly affect the normal growth and development of the rats. These findings align with a study conducted by Rajeh et al. (2012), who evaluated the acute toxicity of a related *Euphorbia* species. They reported similar results, with no significant impact on the body weight of rats treated with the plant extract compared to the control group. This consistency suggests that *E. taifensis* shares a similar no-toxicity profile regarding body weight changes [8].

In our evaluation of the acute toxicity of *E. taifensis* Methanolic-aqueous Extract, we also investigated its effects on white blood cells and their distinct types. The results of our study indicate that administration of the plant extract did not lead to any noticeable changes in white blood cells and some of their different types. No statistically significant differences were observed in the total counts of white blood cells or lymphocytes and basophils when comparing the group treated with *E. taifensis* with the control group. This suggests that the extract did not induce any notable disturbances in these components of the immune system [9]. Intriguingly, our findings revealed a significant increase in the count of neutrophils ($P< 0.001$) and monocytes ($P< 0.01$) in the animals treated with *E. taifensis* extract. Neutrophil elevation generally indicates an acute inflammatory response, suggesting that the extract may have triggered such a response [10]. Meanwhile, the increased count of monocytes could be indicative of the body's immune response to physiological stress or inflammation [11]. Contrarily, we observed a significant decrease in the count of eosinophils ($P< 0.05$). The observed increase in neutrophils and monocytes and the significant decrease in eosinophils following the administration of *E. taifensis* extract could provide valuable insights into its potential immunomodulatory effects. Neutrophilia and monocytosis are recognized as hallmark responses to acute inflammation and infection [12]. These findings suggest that the extract may have elicited an immune response in the treated animals, stimulating the activation and recruitment of neutrophils and monocytes to the site of inflammation or infection. Similar changes in neutrophil and monocyte counts have been reported in studies involving various herbal extracts and plant-based compounds. For instance, a study by Joshua et al. (2020) demonstrated an increase in neutrophil and monocyte counts following the administration of a specific plant extract. The authors attributed these changes to the immunomodulatory properties of the extract [13]. On the other hand, the significant decrease in eosinophil count observed in our study may indicate inhibition of eosinophil activation or migration. Eosinophils are typically associated with allergic responses and parasitic infestations [14].

Regarding the effect of the methanolic aqueous extract of *E. taifensis* on red blood cells and platelets. The extract did not affect the RBC and hemoglobin. The red blood cell (RBC) count is important as an indicator of the body's health, as the results of the RBC count can be used to help diagnose blood-related conditions, such as anemia caused by iron deficiency, or a deficiency of vitamin B6, B12, or folic acid. It may sometimes indicate internal bleeding, kidney disease, or malnutrition [15, 16]. Red blood cells contain a substance called hemoglobin, which transports oxygen throughout the body. The amount of oxygen delivered to the body's tissues depends on the number of red blood cells and hemoglobin [17]. On other side, the methanolic aqueous extract of *E. taifensis* significantly increased most of the red blood cell indices, including hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW) [18]. The observed increase in HCT suggests that the extract may have influenced the concentration of red blood cells. This finding could indicate a potential effect of *E. taifensis* on erythropoiesis, the process of red blood cell production [19]. Further investigation is required to elucidate the mechanism underlying this increase. Similarly, the significant increase in MCV indicates that the extract might have impacted the average size of red blood cells. This alteration in cell size could be related to changes in cell metabolism or membrane properties [18, 20].

Platelets, also known as thrombocytes, are small parts of cells in the blood that play a crucial role in blood clotting and wound healing [21]. The results of this study showed that *E. taifensis* extract had a significant effect on platelet count (PLT) and mean platelet volume (MPV). An increased number of platelets can occur due to several reasons such as an inflammatory response, tissue damage, trauma, bleeding, blood loss, or the use of certain medications or treatments [22]. Platelet count may be a normal response to certain situations or conditions, such as acute infections, severe bleeding or trauma, exercise, or as a reaction to stress [23].

V. CONCLUSION

In conclusion, our study aimed to evaluate the acute toxicity of *Euphorbia taifensis* Methanolic-aqueous Extract in rats, focusing on LD_{50} determination, body weight changes, and hematological parameters. Our results demonstrated that the administration of the extract did not induce any mortality or acute toxicity symptoms in the treated animals. Additionally, we found no significant changes in body weight between the treated and control groups throughout the 14-day study period, indicating that the extract did not affect normal growth and development in rats. Regarding hematological parameters, the extract revealed several notable findings. While the extract didn't affect overall white blood cell count, it led to significant increases in neutrophils and monocytes and a decrease in eosinophils, suggesting potential modulation of inflammatory responses. Additionally, although it didn't alter red blood cell count or hemoglobin levels, it notably enhanced red blood cell indices, potentially improving oxygen delivery. Furthermore, the extract significantly affected platelet count and mean platelet volume, indicating potential implications for blood clotting. These findings suggest that *E. taifensis* extract may have diverse effects on blood parameters, warranting further research into its potential therapeutic applications.

Overall, our study contributes to the understanding of the acute toxicity profile of E*uphorbia taifensis* Methanolic-aqueous Extract. These findings highlight the need for further investigations to explore the underlying mechanisms for the observed outcomes.

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