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Toxicological Evaluation of *Commiphora gileadensis* Methanolic-aqueous Extract: An Acute Toxicity Study in Rats

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Abstract: **Background: *Commiphora gileadensis*, a medicinal plant with traditional use, is gaining recognition in scientific research for its potential therapeutic applications. Objective: This study aimed to investigate the acute toxicity profile of *Commiphora gileadensis* methanolic-aqueous extract in rats, focusing on mortality, body weight changes, and hematological parameters. Methods: Rats were orally administered with varying doses of the extract, and acute toxicity was assessed by monitoring mortality and body weight changes. Hematological parameters, including leukocyte populations and red blood cell indices, were analyzed to elucidate potential systemic effects. Results: No deaths were observed in rats treated with the plant extract, and no statistically significant differences were found in body weights between the treated and control groups. In addition, no significant difference was shown in white, red blood cell counts and hemoglobin. However, the extract showed significant changes in some blood parameters, including neutrophils, lymphocytes, monocytes, eosinophils, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and red cell distribution width, platelet count, and mean platelet volume. Conclusion: This research contributes valuable insights into the acute toxicity of *Commiphora gileadensis* methanolic-aqueous extract, providing a foundation for future studies on its safety profile.**

Keywords: ***Commiphora gileadensis*****; Methanolic-aqueous extract; Acute toxicity; LD_{50}; Hematological parameters; Rats.**

I. INTRODUCTION

In recent years, the exploration of natural compounds derived from medicinal plants has gained prominence in scientific research due to their potential therapeutic applications [1, 2]. Among these plants, *Commiphora gileadensis*, a member of the *Burseraceae* family, commonly known as “Besham” or “Balsam”, and has been traditionally employed in various folk medicine practices, attributed to its perceived medicinal properties [3].

This study aims to contribute to the scientific understanding of the toxicological profile of *Commiphora gileadensis* through an acute toxicity study conducted in rats. The methanolic-aqueous extract of the plant has been selected for investigation, given its prevalence in traditional remedies and its potential relevance for pharmaceutical development [3].

Toxicity studies are crucial in assessing the safety of natural extracts before considering them for therapeutic use [4]. The acute toxicity study in rats serves as a pivotal step in comprehensively evaluating potential adverse effects associated with the administration of *Commiphora gileadensis* extract [5]. Understanding the acute toxicity profile is fundamental for establishing a safe dosage range and ensuring the well-being of individuals who may consume products containing this extract [1].

This research not only addresses the scarcity of scientific literature on the toxicological aspects of *Commiphora gileadensis* but also contributes valuable insights that could guide future research endeavors and facilitate evidence-based decisions in the development of therapeutic agents derived from this plant. As the demand for natural remedies continues to rise, a comprehensive understanding of the safety profiles of such botanical extracts becomes imperative for their responsible integration into modern healthcare practices [6].

The aim of our study was to evaluate the acute toxicity of the methanolic aqueous extract of *Commiphora gileadensis* in rats, by determining the oral lethal dose (LD_{50}), and evaluating the effect of this extract on changes in the body weight of experimental rats, in addition to the effect of the extract on various blood parameters. These objectives will facilitate a comprehensive evaluation of the acute toxicity of the methanolic aqueous extract derived from *C. gileadensis*, highlighting its potential pharmaceutical use.

II. MATERIALS AND METHODS

A. Animals

This study was carried out in compliance with 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).

A total of twelve healthy adult male Wistar rats, weighing between 200-220 g, were utilized for this study. 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. Body Weight Determination

The initial and weekly body weights of the rats were assessed using a digital scale at a consistent time each morning. It is important to note that the weight measurements were taken when the rats were in a fasting state. Furthermore, continuous monitoring of the experimental animals was carried out throughout the study to identify any signs of abnormalities or irregularities.

C. Collection and Identification of C. geladeensis

Fresh aerial parts (succulent part) of *C. gileadensis* plant were collected from Makkah Province, 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).

D. Preparation and Extraction of Plant Materials

200 g of dry powder of *C. gileadensis* 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 *C. gileadensis* powder was 31 g. The extracts were kept at 4°C for further use.

E. Experimental Design

Twelve adult male Wistar rats, measuring 200 - 220 grams, were randomly grouped into two experimental groups, each group included six rats:

- The rats of the **first group (G1),** which served as the **Control Group**, received 0.9% sodium chloride (normal saline solution).
- The **second group (G2)** was given a dose of **2000 mg/kg of *C. gileadensis***, using a gavage, ensuring the food was held for 3-4 hours after dosing.

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

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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.

F. Acute toxicity study

The acute toxicity assessment of the methanolic-aqueous extracts of *C. gileadensis* plant was conducted using the Oral Lethal Dose (LD_{50}) method. The LD_{50} was determined using the "limit dose test" approach, following the guidelines provided by the Organization of Economic Cooperation and Development (OECD). A prior investigation on the instructions and guidelines for evaluating chemicals in animal testing recommended a maximum prescribed dose of 2000 mg/kg when administering substances orally [1]

It should be noted that if three or more rats survive, the LD_{50} value is determined to be greater than 2000 mg/kg. Conversely, if three or more animals perish, the LD_{50} value is considered to be less than 2000 mg/kg.

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

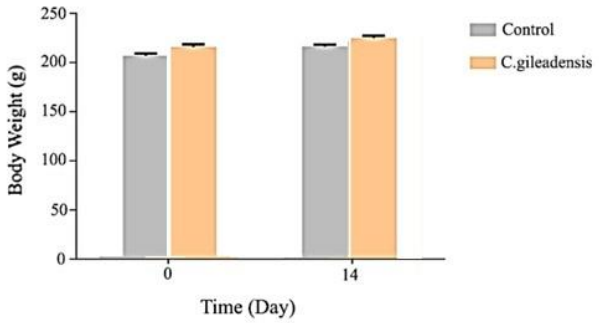
A. Observation and Mortality

The findings from administering an oral dose of 2000 mg/kg of *C. gileadensis* to group 2 indicated that none of the animals experienced mortality during the experimental period. Additionally, the animals did not exhibit any signs of acute toxicity. An intriguing observation was made as the animals abstained from food and drink intake on the initial day of extract administration. Furthermore, they exhibited signs of sedation and displayed a diminished response to external stimuli, such as sounds or physical sensations, within the first 4 hours. However, apart from these effects, all the rats that received the treatment appeared to be in a normal state as compared to the control during the entire study (14 days).

B. Body Weight

During the 14-day study period, there was no statistically significant distinction found between the average body weights of the rats treated with the plant and those in the control group, Figure 1.

Figure 1. Changes in body weight in control and *C. gileadensis* groups after 14 days.



Values are expressed as mean ± SD.

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C. Hematological Analysis

The objective of this study is to assess the acute toxicity of *C. gileadensis* on hematological characteristics. Table 1 indicates that the methanolic-aqueous extract of *C. gileadensis* did not notably impact the white blood cell count (WBC). However, the extract exhibited a notable rise in neutrophils ($P< 0.001$), lymphocytes ($P< 0.01$), and monocytes ($P< 0.01$) alongside a significant reduction in eosinophils ($P< 0.05$).

Table 1. Effect of Methanolic-aqueous Extract of *C. gileadensis* on white blood cells and their different types.

Variables	Control (G1) (n= 6)	*C. gileadensis* Extract (G2) (n= 6)	*P*-value
WBC ($x10^6/\mu L$)	2.85 ± 0.49	2.97 ± 0.23	0.635
Neutrophils ($x10^6/\mu L$)	0.19 ± 0.01	1.16 ± 0.035	0.0001^{***}
Lymphocytes ($x10^6/\mu L$)	0.008 ± 0.004	0.015±0.005	0.005^{**}
Monocytes ($x10^6/\mu L$)	0.005 ± 0.005	0.015 ± 0.005	0.010^{**}
Eosinophils ($x10^6/\mu L$)	2.64 ± 0.50	1.77 ± 0.2	0.040^{*}
Basophiles ($x10^6/\mu L$)	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 provides insight into the effect of the methanolic aqueous extract of the plant on red blood cells (RBC) and their corresponding indices as well as platelet count and size. While the extract had no discernible effect on RBC count and hemoglobin levels, it notably influenced the following parameters: hematocrit (HCT, $P< 0.01$), mean corpuscular volume (MCV, $P< 0.01$), mean corpuscular hemoglobin (MCH, $P< 0.01$), and red cell distribution width (RDW, $P< 0.001$). Furthermore, the extract significantly affected platelet count (PLT, $P< 0.001$) and mean platelet volume (MPV, $P< 0.01$).

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

Variables	Control (G1) (n= 6)	*C. gileadensis* Extract (G2) (n= 6)	*P*-value
RBC ($x10^6/\mu L$)	8.37± 0.28	8.33 ± 0.13	0.794
Hbg (g/dL)	14.06 ± 0.42	14.40 ± 0.21	0.132
HCT (%)	44.50± 0.93	46.15 ± 0.83	0.009^{**}
MCV (fL)	53.25 ± 1.41	55.36 ± 0.53	0.014^{**}
MCH (pg)	16.71 ± 0.29	17.35 ± 0.13	0.002^{**}
MCHC (g/dL)	31.36 ± 0.48	31.31± 0.27	0.833
RDW (%)	15.76 ± 0.83	19.58 ± 0.34	0.0001^{***}
PLT ($x10^3/\mu L$)	746 ± 19.68	2087.0±18.40	0.0001^{***}
MPV (fL)	6.45 ± 0.25	6.83 ± 0.08	0.013^{**}

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

IV. DISCUUSION

The present study aimed to assess the acute toxicity of *Commiphora gileadensis* methanolic-aqueous extract, focusing on mortality, body weight changes, and hematological parameters in rats.

Mortality and Body Weight Changes

Rats treated with *C. giladensis* extract initially showed reluctance to ingest food and water upon receiving the extract, along with signs of anesthesia characterized by decreased response to stimuli such as sounds or prodding during the first four hours. Despite this observation in rats, no deaths were observed among expirmental rats treated with *C. giladensis*. This result is consistent with a previous investigation by Ugwah-Oguejiofor et al. (2019), where no deaths or obvious signs of toxicity were observed in rats given an extract originating from an herbaceous plant [7].

Regarding body weight change, there was no statistically significant distinction in body weights between the treated rats and the control group. This finding is consistent with Al-Hazmi's 2024 study [8] which highlights the potential safety of *C. gileadensis* at the administered doses.

Hematological Parameters

Evaluation of hematological parameters highlights the effect of *Commiphora gileadensis* on blood composition. The results indicated that the methanolic aqueous extract of *C. gileadensis* had no significant effect on white blood cell (WBC) count, this implies that the extract did not cause significant disruptions in these aspects of the immune system [9].

Interestingly, our findings revealed a significant increase in the numbers of neutrophils, lymphocytes, and monocytes in animals treated with *C. giladensis* extract. Elevated neutrophil counts usually indicate an acute inflammatory response, implying that such a response may be elicited by the extract [10]. Likewise, increased monocyte counts may serve as an indicator of the body's immune response to physiological stress or inflammation [11]. The study also showed an increase in the number of lymphocytes that help the body's immune system fight cancer, viruses, and foreign bacteria [9]. On the other hand, we observed a significant decrease in the number of eosinophils. These observations may yield valuable insights into the potential immunomodulatory effects of the extract [12].

All these results together indicate a potential immunomodulatory effect of the extract, anti-inflammatory activity, and antioxidant effects [5, 13]. Moreover, *C. giladensis* extract can exert indirect effects on immune cell populations by modulating the production and secretion of various signaling molecules, such as cytokines and chemokines, by other cell types [5, 14, 15].

Concerning the impact of the methanolic aqueous extract of *C. gileadensis* on red blood cells (RBC) and related indices, as well as platelet count and size, the extract did not produce noticeable changes in RBC count and hemoglobin levels. However, it notably altered parameters such as hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and red cell distribution width. Additionally, the extract had a significant effect on platelet count and mean platelet volume.

These results can be explained by the fact that the plant *C. gileadensis* contains bioactive compounds (such as polyphenols and flavonoids) that exert powerful antioxidant action so it can enhance oxygen transport, stimulate red blood cell production, and improve platelet function [16]. These compounds may promote erythropoiesis in the bone marrow, leading to higher levels of RBCs and increased HCT, MCV, MCH and RDW. This observed rise in HCT suggests a potential influence on red blood cell concentration, potentially impacting erythropoiesis [17-19].

In addition, plant extracts can also stimulate platelet production and activation, leading to higher PLT and MPV, which is because plant extracts usually possess anti-inflammatory properties, which indirectly promote blood cell production and improve blood parameters [20]. It is known that elevation of platelet-related parameters indicates stimulation of thrombopoietin, activation of platelets, reduction of inflammation, and enhancement of blood cell production in the bone marrow [8].

Limitations and Future Directions

It is essential to acknowledge the limitations of the present study, including the focus on acute toxicity and the need for further investigations into subchronic and chronic effects. Additionally, a detailed exploration of the molecular mechanisms underlying the observed hematological changes is warranted for a more comprehensive understanding of *Commiphora gileadensis*'s safety profile.

V. CONCLUSION

In conclusion, the acute toxicity evaluation of *Commiphora gileadensis* methanolic-aqueous extract revealed no mortality and no significant changes in body weight. However, notable alterations in some hematological parameters suggest potential immunomodulatory, anti-inflammatory, and antioxidant effects. Further studies are required to elucidate the underlying mechanisms and establish comprehensive safety guidelines for the use of *Commiphora gileadensis*.

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