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Estimation of LD50 and Acute Toxicity of *Zygophyllum fabago* in Mice

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Abstract *Zygophyllum fabago*, a member of the genus Zygophyllum, has lonely used in traditional medicine in Iraq for the treatment of many diseases. However, no studies have done to evaluate the acute toxicity of its extract. Thus, we designed this study to examine its acute toxicity and LD-50 value in mice depending on OECD 423 guidelines for testing of chemicals. Twenty-one mice were given *Zygophyllum fabago* extract in doses of 2000 or 5000 mg/kg. The results showed that both doses of *Zygophyllum fabago* extract did not show any toxic signs or mortality. Moreover, body weights and relative organs weight were not affected. Furthermore, it did not induce kidney and liver injuries. Therefore, results of this study suggested that *Zygophyllum fabago* extract is safe within the tested doses and did not produce acute toxicity when administered orally as a single dose.

Keywords: *Acute oral toxicity, Zygophyllum fabago, LD-50, OECD423*

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1. Introduction

Zygophyllum fabago L. (also called Syrian beancaper) is one of the members of the genus Zygophyllum, a genus usually contains tropical shrubs with ill-smelling flower buds [1,2]. It is distributed in Turkey, Mediterranean region, southern, northern and north-eastern Africa and central Asia [2,3]. *Zygophyllum fabago* crude material has been used in folk medicine in many countries for its numerous biological effects [4]. For instance, it is used as antitussive, expectorant, anti-inflammatory agent, pain reliever, anthelmintic, anti-rheumatic, and antiasthmatic, and as a part of a drug for rheumatism and gout, as well as a stupe for the treatment of skin conditions and wounds [5,6]. In Iraq, *Zygophyllum fabago* grows individually or as groups in many districts. It is widely spread on the roadsides and humid areas of middle, east, and west of Iraq [7]. This plant has longley used in traditional medicine in Iraq, it is used to control some skin diseases and its fruits used to relief colic pain [8]. The whole plant extract is used by herbalists to cure some infectious diseases [8] and hemorrhoids. Despite the extensive use of *Zygophyllum fabago* in traditional medicine, no safety data for its use is available. This plant was cytotoxic when used at high concentrations of the whole extract on *Candida albicans* and brine shrimp cells [9]. However, no in vivo studies have done to evaluate its acute toxicity in animals. Thus, this study was carried out to evaluate the acute toxicity of *Zygophyllum fabago* extract on mice. Moreover, the lethal dose-50 (LD-50) was also estimated based on OECD Guidelines for the Testing of Chemicals.

2. Materials and Methods

2.1. Plant Material

The whole plant was blended in fine powder and extracted by methanol using maceration method [10]. The extract was concentrated and kept at room temperature in the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad until use.

2.2. Animals

A total of 21 mice (25 to 33 g body weight, 5 weeks old) were used in this study. The animals were breaded and housed in the Animal House, College of Pharmacy, and University of Baghdad. Mice were kept in polypropylene cages at controlled temperature (25±3°C), a 12 h: 12 h light: dark cycle, and allowed to access to food and tap water *ad libitum*. The Local Research Ethics Committee of the College of Pharmacy, University of Baghdad, approved the research protocol. Mice were divided in to three groups (7 mice in each).

2.3. Experimental Design

OECD 423 guideline was used to investigate the acute oral toxicity of *Zygophyllum fabago* extract [11]. The animals in the two test groups received a single dose of 2000 or 5000 mg extract/kg body weight, while the control group received distilled water (vehicle). Animals were superintend closely by cage side observation the first 30 minutes, periodically during the first 24 hours, and then

daily for 14 days. Moreover, the change in body weight was measured on days 0,1,4,7,10, and 14 post treatment [12]. Mice then sacrificed on day 14 and liver, kidney, heart, and spleen were obtained, and their relative weight was calculated. Additionally, blood samples were taken and the serum was isolated for measurement of some of biomarkers of kidney and liver functions, including blood urea nitrogen (BUN), creatinine (sCr), alkaline phosphatase (ALP), glutamic-oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) [13,14] were measured for all studied groups. The results of were analyzed with mixed-factorial 2-way analysis of variances (ANOVAs) test, with time as the repeated measure. One-way ANOVA followed by Dunnett's test was used to compare relative organ weights of treatment groups compared with the respective control group. Serum measured parameters results were analyzed by independent-sample *t*-tests. $p<0.05$ was considered as statistically significant difference.

3. Results

All mice treated with *Zygophyllum fabago* extract (2000 or 5000 mg/kg) did not show any abnormal behavior or sings of toxicity after 30 minutes and during the 14 days post treatment. Moreover, no mortality observed in all groups during the entire experiment. There was no significant change in body weights (Figure 1) in treatment groups compared to control group ($P= 0.9844$). Furthermore, Dunnett's test revealed no significant changes in relative organ weights (Figure 2) in the treatment groups compared to control group ($p>0.05$). Meanwhile, treatment with *Zygophyllum fabago* extract did not induce changes in the serum biomarkers of liver and kidney functions, and also no differences between the two utilized doses, where *t*-test exhibited no significant changes in BUN, sCr (Figure 3), GOT, GPT, and ALP (Figure 4) between tested groups ($p>0.05$).

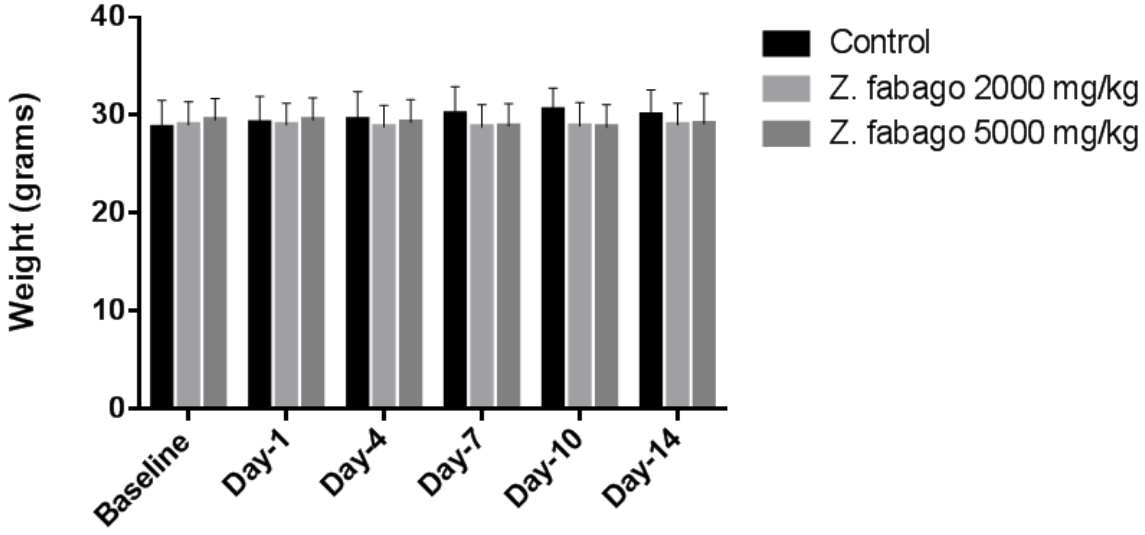

Figure 1. Effect of *Zygophyllum fabago* extract on body weight changes in mice. Value = mean ± S.D; n=7. Analyzed using mixed-factorial 2-way analysis of variances (ANOVAs) test

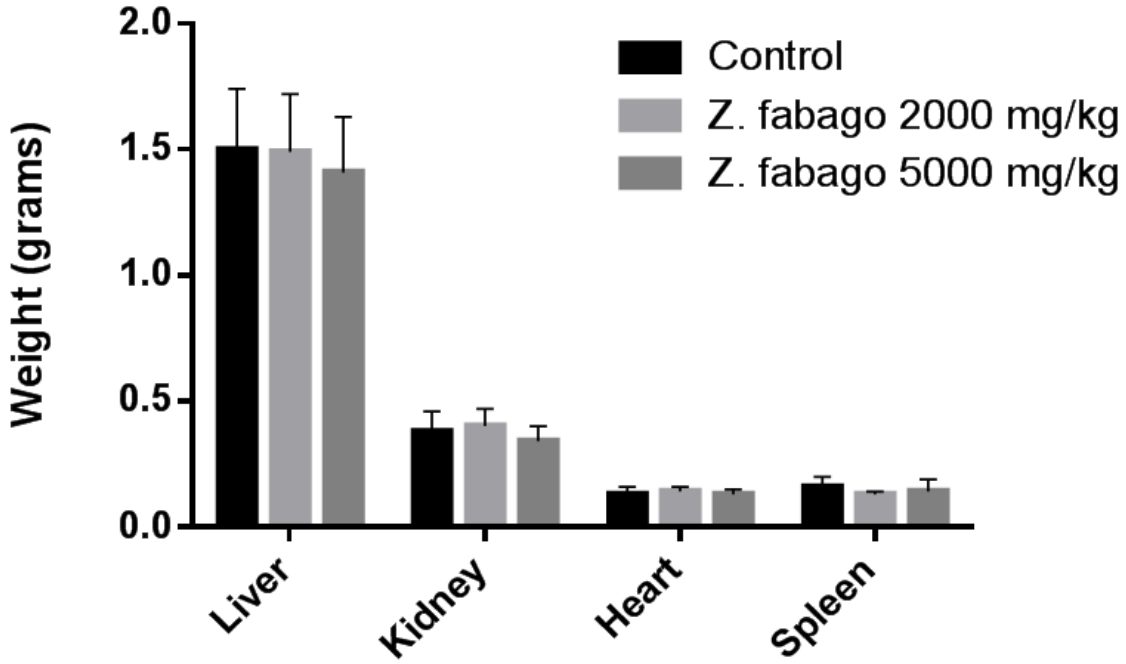

Figure 2. Effect of *Zygophyllum fabago* extract on relative organ weights in mice. Value = mean ± S.D; n=7. Analyzed using Dunnett's test

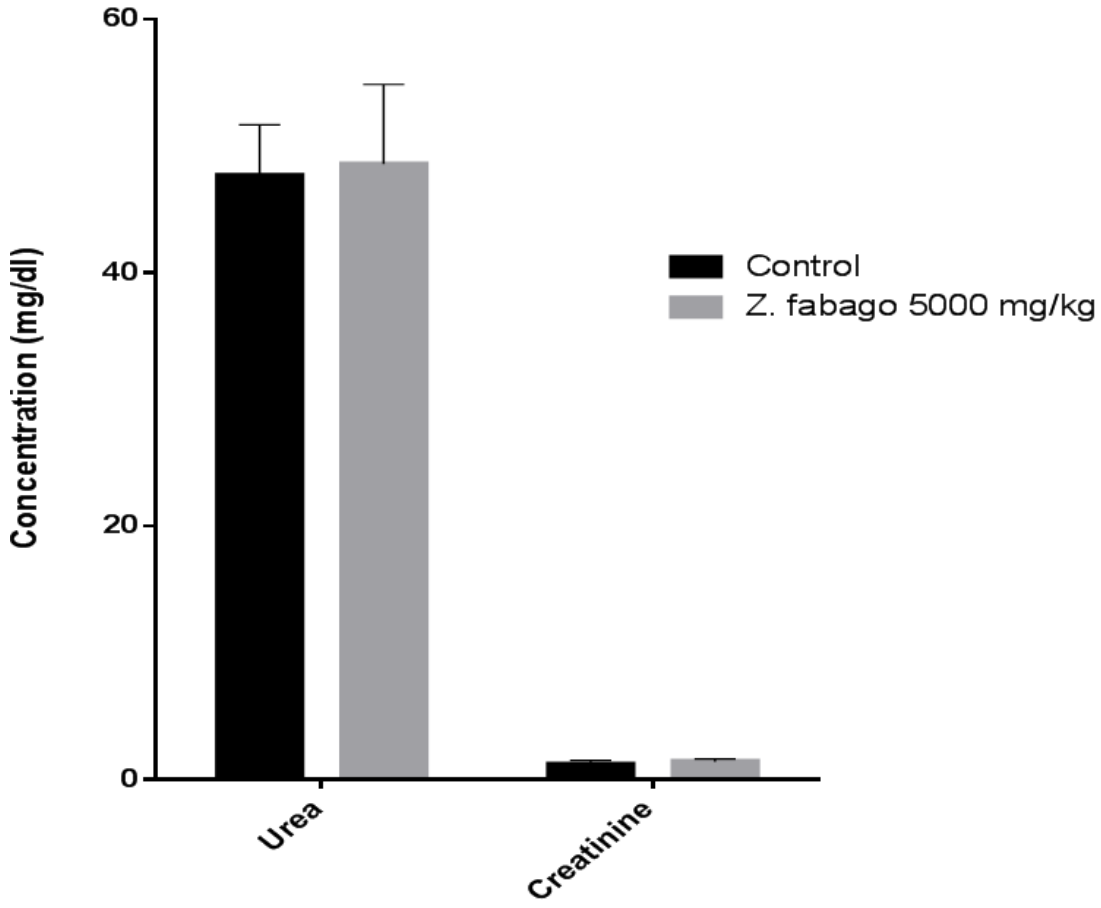

Figure 3. Effect of *Zygophyllum fabago* extract on serum concentration of Urea and Creatinine in mice. Value = mean ± S.D; n=6. Analyzed using t-test

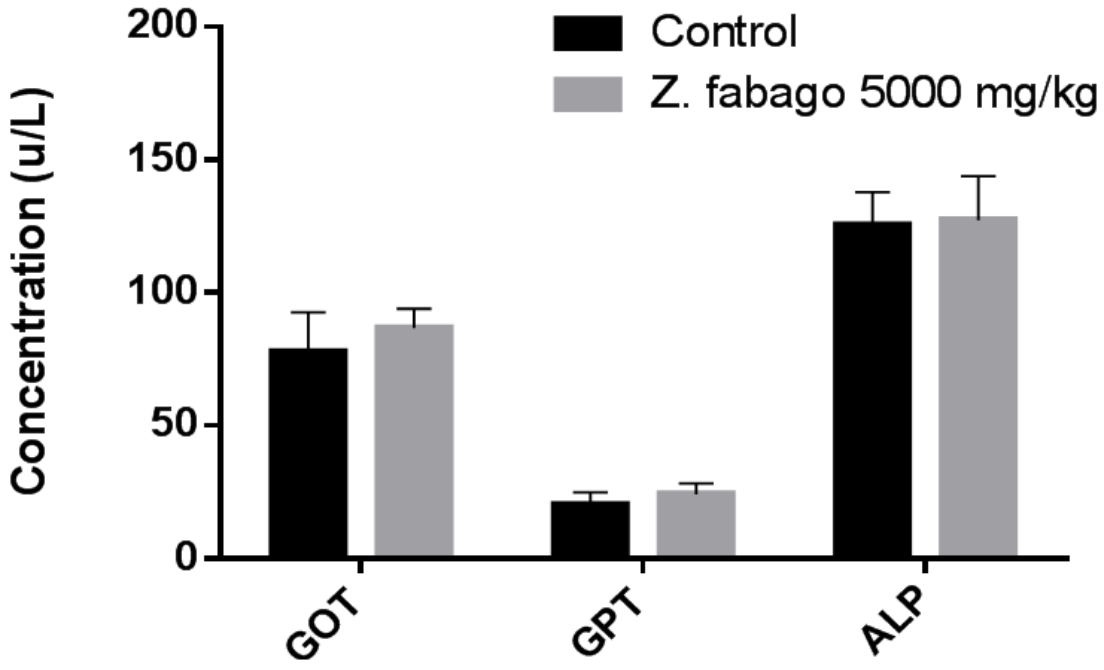

Figure 4. Effect of *Zygophyllum fabago* extract on serum concentration of GOT, GPT, and ALP in mice. Value = mean ± standard deviation; n=6. Analyzed using t-test

4. Discussion

This study was performed in accordance with OECD 423 Guideline, which considered as an alternative to the conventional acute toxicity tests [11], and helps to provide valuable information to identify the target organ toxicities of substances after acute exposure [15]. Even with higher doses of *Zygophyllum fabago* extract, mice did not show any toxic signs and abnormal behavioral, indicating that its active components, when orally administered, did not induce acute toxicity. The results did not show mortality or moribund animals; therefore, the LD50 could not be determined. However, one can expect that the LD50 is higher than 5000 mg/kg body weight in mice. According to OECD 423 Guideline and classification, *Zygophyllum*

fabago can be ranked as closely to category 5, which is of low acute toxicity hazard. Changes in the body weight have been used to assess any suspected pathological changes and/or overlap of treatment with mice activity. Our results revealed that no abnormal changes in body weights, which may indicate that *Zygophyllum fabago* extract does not interfere with energy homeostasis or metabolic dysregulation. On the other hand, the extract showed no deleterious effects on liver and kidney. Elevation in BUN and/or sCr may indicate acute kidney disease, yet, they still considered as usually used markers for kidney diseases [16]. Moreover, ALP, GPT, and GOT are a part of laboratory panel and reliable biomarkers for hepatocellular injury [17], and abnormal values of these biomarkers might refer to liver abnormality [17,18,19]. Our results revealed no significant changes in both kidney and liver biomarkers between all groups. These findings inferred that *Zygophyllum fabago* extract does not induce kidney and liver injuries. According to The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and International Labor Organization (ILO), *Zygophyllum fabago* extract is not need to be classified and does not cause target organ systemic toxicity [11,20]. Based on the obtained results, we can suggest that treatment with *Zygophyllum fabago* extract does not produce acute toxic effect when administered orally as a single dose. Further studies to evaluate the chronic and/or sub-chronic toxicity will be valuable to improve our knowledge about the toxic profile of *Zygophyllum fabago* extract.

5. Conclusion

We deduce from this study that *Zygophyllum fabago* extract is safe when it is administered orally to mice. It did not cause: mortality, abnormal behaviors, abnormal weight changes, as well as injuries to various vital organs like kidney and liver. LD-50 of *Zygophyllum fabago* extract in mice is higher than 5000 mg/kg, referring to the high safety of the extract when is given orally.

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