RESEARCH ARTICLE

# Blood Hemolytic Activity and Acute Toxicity of Saponins extract from *Lepudium aucheri* Boiss.

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**ABSTRACT**

The hemolytic activity process was applied to erythrocytes by an analyst, as well as the lysis of erythrocytes resulted in a positive test result. The *L. aucheri* Boiss. extracts are high in triterpenoids saponins, according to the findings.

Both extracts were used in acute toxicity *in vivo* research on four groups of rats (six rats in each group). After 72 hours of treatment with various concentrations of terpenoids saponins extracts (25, 50, and 100 mg/kg BW), no mortality was detected in the rats of the trials. This demonstrated that both extracts are harmless when taken orally.

**Keywords:** Acute toxicity, Blood Hemolytic, Extraction, *L. aucheri* Boiss.

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## INTRODUCTION

Hemolysis occurs when the structure of erythrocyte cell membranes is damaged or disrupted, causing hemoglobin to be released. Hemolysis of red blood cells happens during the assembly of the components, storage, distribution, and delivery to the patient. The percentage of free hemoglobin in the medium used to suspend red blood cells, such as a plasma or added solutions., indicates hemolysis in blood products. Storage of erythrocytes leads to a high level of hemolysis. Various procedures, most popular, which is visual assessment, can be used to quantify the amount of hemolysis. Spectrophotometric tests, photometric methods, and microplate technology are among the others.[1]

Two different methods cause hemolysis. Intravascular hemolysis occurs when red blood cells are destroyed in the circulation, and their contents are released into the plasma. Mechanical damage from a weakened endothelium can trigger direct membrane breakage and cell death, Infectious microorganisms, as well as complement attachment and activation on the cell surface Extravascular hemolysis is the removal and destruction of erythrocytes by spleen and liver macrophages with cell wall alterations.[2]

## MATERIAL AND METHOD

### Plant Collection

Plant collected in March 2013 from local markets in Nasiriyah city at, Iraq.

### Extraction of Crude Saponin from a Plant

Air-dried powdered plants of Lepidium aucheri boiss (100 g) were separated with 70% ethanol (24 hrs x 1000 mL) at room temperature. By low pressure evaporated at 45°C, the organic phase was concentrated to a small amount (300 mL) and isolated in three steps with chloroform (24 hrs x 100 mL x 3) and n-Butanol (24 hrs x 100 mL x 3). Given the saponins extract, the n-Butanol layer was reduced to dried (10 g).

### Saponins Compounds are Tested using the Following Method

- Aqueous saponin extract solution was made in a test tube and shaken, after which the soapsuds remained for a long period.
- 5 mL saponin extract aqueous solution in test tube, 5 mL silver nitrate, then placed in a water bath at 100°C for 5 minutes, resulting in a silver mirror on the inner surface of the test tube 15.[3]

### Acute Toxicity Study for Saponins Extracts

Laboratory animals were separated into four groups, each with six rats, according to the following:

Group 1: normal controls who were given 0.2 mL of DMSO
Group 2: Rats were given Saponins extract (25 mg/kg).
Group 3: Rats were given Saponins extract (50 mg/kg).
Group 4: Rats were given Saponins extract (100 mg/kg).

In acute toxicity testing, only water was available to the animals throughout their four-hour fast. Saponins extract was

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administered orally at the doses stated above for 3 days, and any mortality was recorded. The identical groups of animals used in the Saponins extract toxicity study were treated with Saponins extract in the same procedures and amounts as before after two weeks without treatment.

### Investigation of Hemolytic Activity of Saponins Extracts

The hemolytic activities of both saponin extracts were assessed using the technique Pipetting 0, 0.25, 0.5, 0.75, and 1-mL of each Extract (1.5 mg/mL) into clean dried test tubes was used for the experiment. With distilled water, the volume was reduced to 1-mL. After that, 2.5 mL of normal saline was added to each tube, followed by 2 percent (v/v) erythrocytes that had been freshly produced. For 1 hour, the reaction mixture was incubated at 37°C. After that, centrifuge for fifteen min at 3500 rpm. The supernatant was collected, as well as the absorption at 630 nm was measured. The lysis delay erythrocytes served as a control and indicated 100% lysis. The formula was used to calculate the percentage of hemolysis.[4]

$$Hemolysis\ \% = \frac{Absorbance\ of\ sample - Absorbance\ of\ blank}{absorbance\ of\ control}\ x\ 100\%$$

## RESULTS AND DISCUSSION

These findings showed that the concentrations of Saponins extracted used during oral dose toxic trials (25, 50, and 100 mg/kg) were non-toxic, with no aberrant symptoms or death in the rats, as seen in the Table 1. This finding is comparable to that of the OECD (2001), which concluded that saponin extracts are harmless because the acute oral toxicity limit is typically believed to be 5.0 mg/kg BW.[5] Following 14 days of daily therapy (150 mg/kg and 300 mg/kg), saponin extract had no negative impacts on behavior reaction, normal reflexes, and so forth. An LD50 dose of 2000 mg/kg or more is categorized as unclassified by the OECD criteria for acute oral toxicity, and so the medicine is deemed safe.[6]

### Hemolytic activity of Saponins Extract

Hemolytic activity is one of the main health impacts of saponins, and it can be utilized to measure the efficacy of saponin compounds crude extract in studies.[7] Saponins' hemolytic nature is advantageous in the lab.[8] Saponins extract can hemolyze human RBCs by forming pores in the cell membranes due to the sensitivity of the aglycon moiety for lipid sterols, notably cholesterols, resulting in the formation of complexing agents.[9-12] Hemolysis is defined as the disruption of erythrocytes and the release of hemoglobin from blood cells to plasma (Table 2 and Figure 1).

When Erythrocytes are submerged in a hypotonic solution, water penetrates the cells, causing them to inflate, become spherical, and eventually rupture, releasing Hb into the plasma. This condition is known as hemolysis, or a loss of blood, or erythrocyte instability.[13-15] The instability of erythrocytes in blood stream is higher than in arterial blood. The fragility of blood increases with acidosis due to the acidic PH of the blood.

Suggested of blood can be hemolyzed *in vitro* in the following ways:

- By using a fat solvent such as ether, chloroform, or benzene, some solvents cause hemolysis by dissolving the lipid red cellular membranes.
- By producing an osmotic disruption, adding distilled water or a hypotonic salt solution to the cell promotes endosmosis, elevating cell size and ultimately causing cell lysis.
- Disrupting RBCs surface tension: Bill salts or saponin elevate the surface tension of RBCs, causing hemolysis.
- Physical methods: Physical methods like alternate freezing and thawing of blood breakdown the erythrocyte.
- Hemolysis is caused by mechanical methods such as forceful churning and shaking.
- Hemolysin produces hemolysis when bacteria hemolysin is added.
- The addition of snake venom or viper venom causes hemolysis.

Erythrocytes become brittle and hemolyzed in the human body under various anemic conditions. Hemoglobin is released into the bloodstream after hemolysis and is broken down into heme and globin. Amino acids are extracted and reused from globin. In producing new hemoglobin, iron is isolated, and steroid in different forms is employed. The remainder is changed to bilirubin, which is then oxidation to produce regulated control.

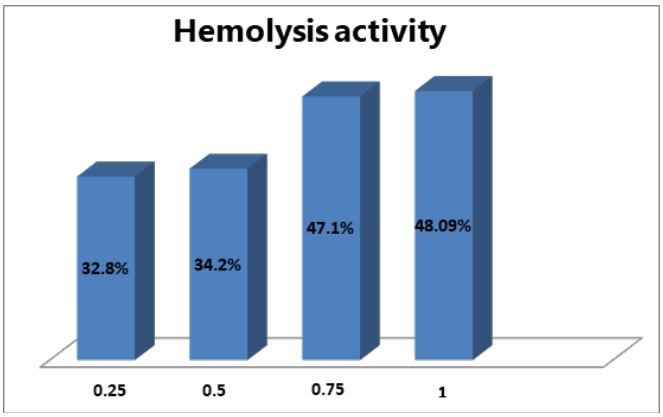


**Figure 1:** Show Saponins extract, the human blood hemolysis activity percentage.

**Table 1:** Shows the acute toxicity of Saponins extract.

| *Groups* | *No. of Rats* | *Number of deaths after 72 hours* |
|---|---|---|
| Group 1 | 6 | 0 |
| Group 2 | 6 | 0 |
| Group 3 | 6 | 0 |
| Group 4 | 6 | 0 |

**Table 2:** Show Saponins extract hemolysis activity as a proportion of human blood.

| *Concentration (mg/mL)* | *Percentage of hemolysis (%)* |
|---|---|
| 0.24 | 32.8 |
| 0.5 | 34.2 |
| 0.75 | 47.1 |
| 1 | 48.09 |

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