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Toxic Effect of Peganum Harmala L. on Some Physiological Parameters in the Livers of Rabbits

Hajir Mohammed Kadhim[1*]

[1] *College of Education for Pure Sciences, Dep. Of Biology, University of Kerbala, Iraq*

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A B S T R A C T

In this study, (20) adult male laboratory white rabbits (Lepus arcticus) were used, with an age ranging from eight months to one year. Their weights ranged between 1,500 to 1,600 kg .. It was divided into the first and second group . where the first group included the group injected subcutaneously with the "alcoholic extract" of Harmala plant seeds with a concentration 3 mg / kg , which included (10) rabbits. The second group was the control, which contained (10) rabbits. In this research, the levels of the AST and ALT enzymes were estimated, and the percentage of some blood proteins such as total protein, albumin, and globulin were measured. Through this study, it was found that:

1- There was a significant increase (P<0.05) in the levels of AST - ALT enzymes compared with the "control group. "

2- There was also a significant decrease (P<0.05) in the average total protein level and the average albumin level compared with the "control group. "

3- No significant differences (P>0.05) were observed in the average globulin level compared with the "control group" .

1. INTRODUCTION

Harmala is a prevalent medicinal herb in the realm of traditional medicine. The plant is herbaceous and is commonly utilized as a medicinal herb in several nations including Africa, Europe, Central Asia, East Asia, and North Asia. It is particularly prevalent in the Arabian Gulf region, such as the Kingdom of Saudi Arabia and some countries like Iran, Pakistan and India [1]. P. harmala belongs to the family of Zygophyllaceae [2]. This family includes approximately 25 genera and more than 250 species [3]. It is considered as one of the perennial plant groups in semi-arid regions around the world. [4] It is used as a treatment for rheumatism, epilepsy, jaundice, sciatica, headaches, forgetfulness and all types of pain.

The Greeks also used powdered caramel seeds to treat fever and worms, and in Yemen it was used as a drink to treat chronic malaria after mixing it with tamarind, and in Turkey it was used in the form of dry capsules to treat envy and to expel evil eye and witchcraft.[5] Harmala is also burned after mixing it with some substances for inhalation for the purpose of sneezing or to purify the air and numb the nerves [6]. About 56 medical cases were recorded in Tunisia on patients to determine the toxic effects of harmala from 1983 to 1998. Concerning the ratio of men to women aged 26 years, it was 1/2, in addition to the occurrence of neurological effects of harmala at a rate of 91%, and intestinal and gastrointestinal effects 73%.For the percentage of its effect on the heart and vessels, it was 18% [7]. Previous research has shown that environmental factors may affect the chemical composition of this plant [8] because it contains alkaloids of the type beta-carboline (B-Carboline), which are the active medicinal substances in the plant [9]. The percentage of these compounds ranges between (2-7) % in dry seeds [10]. The biological and medicinal effectiveness of the Harmala plant is due to its alkaloids, especially Harmala Indolic Alkaloids, especially Harmaline, Harmine, Harmlol, and peganine [11]. Indole compounds contain more than (1400) different compounds that have efficient functions and are used in medical fields. Harmala was mentioned in ancient Arab medicine recipes to treat joint pain, colon, and diseases of asthma, chest pain, epilepsy, headaches,

*Corresponding Author Institutional Email: hajer.m@uokerbala.edu.iq (Hajir Mohammed Kadhim)

and other diseases [12]. The powder extracted from the seeds of the caramel plant is exploited in Greece as an anti-tapeworm and fever-reducing agent [13]. However, excessive consumption of this herb can also cause toxic complications for the consumer [14]. The species P Harmala (multi sectum) which belongs to the genus Haramel, has the ability to hinder the germination and growth of soil-borne fungus and disease-causing pesticides. It is also effective against two types of aphids (Schizaphis graminum and M. persicae).[15]

Haram is an abortifacient and it is antibacterial, which is used to treat psychological diseases. It also has therapeutic anti-inflammatory effects [16] and is a pain reliever for eczema and skin diseases such as visceral leishmaniasis [17]. Experiments and scientific research have proven that the alkaloid of the Harmala plant possesses biological activity that kills microorganisms, antibacterial activity that acts on the cell wall, kills fungi and bacteria, and is an antitussive [18,19]. The harmala plant thrives in steppe regions, semi-arid environments and sandy soils [20,21] showed that harmala has great anti-allergy and therapeutic anti-tumor effectiveness. The seeds also have a superior ability to activate phagocytic cells [22]. Harmala has been used for a long time against diabetes [23]. It is used to lower blood pressure and as an emetic agent, in addition to using the smoke of caramel seeds as incense to eliminate epidemics and as a sedative agent [24]. A study [25] showed the effect of poisonous harmala on rabbits, small camels, monkeys, sheep, and horses when the animal consumes lethal doses and its effect on the digestive system and the nervous system. Poisoning rabbits with poisonous harmala causes congestion in the heart, lungs, kidneys, stomach, intestines, and bleeding in the liver, as shown. [26]. Today, harmala is considered one of the most promising plants and the most attractive to the attention of scientists in the field of pharmaceutical manufacturing, especially after more in-depth studies have been carried out to determine the pharmacological and toxicological effects of many of the extracts and components of this plant. The need has arisen to conduct a number of studies and research on this subject, and therefore the current study aims to know the functional effects of the alcoholic extract of the seeds of the harmala plant on some enzymes and proteins of the blood serum **.**

2. MATERIALS AND METHODS

The experiment in this study was designed to determine the effect of harem seeds on some functional blood parameters in white male rabbits. The experiment began from February 2024 to May 2025, and the study was conducted in several places (College of Education for Pure Sciences/ Department of Biology / University of Kerbala- and private laboratories to conduct tests). The study was carried out on (20) rabbits, divided into two groups. The first group N= (10) male rabbit were given 3 mg/kg of alcoholic extract of the seeds of the Haramel plant (patient group) , and the second group N= (10) male rabbit were given distilled water (control group). The animals were injected with the concentration mentioned below. Skin using sterile medical syringes with a capacity of 5 milliliters every day for 30 days were used. The animals were anaesthetized using chloroform. The procedure involved accessing the abdominal cavity and extracting blood directly from the heart by puncturing it, in order to acquire a substantial quantity of blood. The blood samples were deposited in sterile test tubes without anticoagulant, each with a volume of 10 ml, and were allowed to sit for a duration of 20-25 minutes. At laboratory temperature, then the tubes were transferred to the centrifuge at a speed of (3000) rpm for 15 minutes in order to obtain the serum, which was kept in the refrigerator at a low temperature (-4) degrees Celsius, [27] until some analyzes and functional standards are conducted, such as (ALT-AST Globulin-Albumin-total protein).

2.1 .Functional Laboratory Tests for Blood

1-The estimation of the enzyme level of Glutamic Oxaloacetic Transaminase (AST) & Glutamic Pyruvate Transaminase (ALT). The enzyme level (AST-ALT) in the serum was estimated by colorimetric method according to method [28].

2 -The level of total protein in blood serum was estimated calorimetrically according to the Biuret method [29].

3 -The estimation of the level of albumin. The level of albumin in the blood serum was estimated by the colorimetric method and based on the ability of the albumin to bind with the dye (BCG) Bromocresol Green, as the color changes from yellow-green to blue-green according to method [30].

4 -The estimation of the level of globulin in the blood serum. The level of globulin was measured indirectly, after measuring the level of albumin. After that, the result was subtracted from the result of the total protein measurement according to the following equation: Globulin Conc. (g/dl) = Total protein Conc.– Albumin Conc [31].

2.2 .Statistical Analysis

The primary data of the research results were analyzed statistically using the T-test at the level of significance ($P < 0.05$) [32].

3. RESULTS AND DISCUSSIONS

The results of the functional study shown in Table 1 revealed a concentration of 3 mg/kg for the group of alcoholic extract of the seeds of the caramel plant in the serum of the rabbit. There was a significant increase ($P < 0.05$) in the enzymatic rate (AST-ALT) , which reached , respectively , (26.21±1.23) U/L

(±24.31±1.90) compared with the control group (15.49±1.59) , (13.18±3.21) U/L individually. This result matches the result of [33] reached in his study through the effect of injection into the peritoneal cavity and administration of orally administered for six consecutive weeks of caramel seed extract at a dose of 200 gr / kg in albino mice. It caused a significant increase in the level of liver enzymes (AST-ALT) as well as severe tissue damage to the liver represented by swelling of hepatic cells as a result of the accumulation of fats inside them. This indicates that it leads to fatty degeneration of the cells and damage and necrosis in the liver lobules. These results can be explained as an outcome of treatment with harmala due to the toxicity of harmala seeds. This is especially the case with the toxic alkaloids, harmine and harmaline, which led to an imbalance in the physiological function of the liver. The reason may be due to an increase in the AST-ALT enzyme as a result of the kidney injury. Due to decreased cardiac output resulting from kidney injury, as blood flow to the kidney is reduced, leading to its inability [34].

This does not match the result reached by [35] in this study when giving the methyl alcoholic extract at concentrations of 200 and 300 mg/L to chicken broilers for 38 days with drinking water. While study [36] indicated a decrease in these two enzymes when giving the extract of harmala to male rats as a result of sulfur poisoning. This study indicated the effect of harmala in protection. The results of the functional study shown in (Table 2) also showed a concentration of 3 mg/kg for the alcoholic extract group of harmala seeds. In the rabbit's blood serum, there was a significant decrease in the average total protein level and the average albumin level compared to the control group. It reached (2.41±0.71) and (4.31±0.38 g/dl) compared to the control group, which is (4.91±0.63), (6.21±0.09) g/dl micrometer, respectively. The results of this study agree with the study [37] which was conducted on chickens when giving ram seeds extract for six weeks.

It was noticed that an increase in the weight of the liver took place, as well as a decrease in the level of proteins and albumin in the blood serum. The reason for the decrease may be due to a case of nephritis or inflammation of the glomerulus and nephrons, as the amount of protein in the urine increases or as a result of a condition. Lack of albumin concentration in the blood serum [38]. Protein deficiency often results in liver disease due to lack of protein intake in food or lack of absorption. Also, the level of albumin decreases in the blood serum due to liver damage and severe diseases [39]. [40] have stated that the presence of small amounts of urinary albumin is an indicator of the risk of developing diabetic nephropathy or large vessel disease. This condition is called micro albuminuria. The results of the current study also indicated that there are no clear significant differences (P > 0.05) in the rate of Globulin level compared with the control group reached (3.41±0.63) and (3.23±0.56) g/dl, separately.

TABLE 1. The effect of the alcoholic extract of the seeds of the harmala plant on the average level of the AST-ALT enzyme in white male rabbits after they were injected subcutaneously with the alcoholic extract of the seeds of the harmala plant at a concentration of 3 mg/kg for (30) days.

Average enzyme level of ALT U/L	enzyme level rate of AST U/L	Studied standards mean±S.D
Harmala	Harmala	Abstract Concentration
26.21±1-23 B	24.31±1.90 B	mg/kg3
13.18±3.21 A	15.49±1.59 A	Control distilled water

Values represent mean ± standard error, different capital letters indicate significance P<0.05

TABLE 2. The effect of the alcoholic extract of the seeds of the harmala plant on the average level of some proteins in the blood serum of white male rabbits after they were injected subcutaneously with the alcoholic extract of the seeds of the harmala plant at a concentration of 3 mg/kg for (30) days.

Average globulin level g/dl	Average albumin level g/dl	Average total protein level g/dl	Studied standards mean±S.D
Harmala	Harmala	Harmala	Abstract Concentration
3.23±0.56 A	2.41±0.71 B	4.31±0.38 B	mg/kg3
4.41±0.63 A	4.91±0.63 A	6.21±0.09 A	Control distilled water

Values represent mean ± standard error, different capital letters indicate significance P<0.05

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Arabic Abstract

استخدمت في هذة الدراسة (20) ارنبا من ذكور الارانب البيض المختبرية البالغة بعمر يتراوح ما بين ثمان اشهر الى سنة , وتراوحت أوزانهم بين 1500 إلى 1600 كجم . قد قسمت إلى مجموعتين الأولى والثانية حيث ضمت المجموعة الأولى المجموعة المحقونة تحت الجلد بـ "المستخلص الكحولي" لبذور الحرمل بتركيز (3ملغم /كغم) والتي ضمت على (10) ارانب أما المجموعة الثانية فكانت مجموعة السيطرة والتي ضمت (10) أرانب في البحث تم تقدير مستوى انزيمي AST,ALT وقياس نسبة بعض البروتينات الدموية كالبروتين الكلي والألبومين والكلوبيولين . وجد من خلال هذة الدراسة :-

1- هناك زيادة معنوية ($P<0.05$) في مستويات انزيمي ALT - AST مقارنة بمجموعة السيطرة .

2- كما حدث انخفاض معنوي ($P<0.05$) في متوسط مستوى البروتين الكلي ومتوسط مستوى الالبيومين مقارنة بمجموعة السيطرة .

3- لم يلاحظ أي فروق معنوية ($P>0.05$) في متوسط مستوى الجلوبيولين مقارنة بمجموعة السيطرة .