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EFFECT OF GUNDELIA TOURNEFORTII L. EXTRACT ON LIPID PROFILE AND FLORA BALANCE IN RATS EXPOSED TO EXPERIMENTAL PSEUDOMONAS AERUGINOSA INFECTION

I. Gh. Sarhan[1] Y. I. Al-Hadidy[1] Sh. F. Weshah*[2]

[1]Tikrit University - College of Agricultural
[2]College of Health and Medica Techniques - Al-Dour

***Correspondence to:** Shaimaa Fadhil Weshah, College of Health and Medica Techniques - Al-Dour, Northern Technical University, Tikrit, Iraq.
Email: Shaimaa.fadhil@ntu.edu.iq

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Abstract

The study aimed to investigate the effect of alcoholic *Gundelia tourneofortti L.* extract on lipid profile and the balance of microbial flora in the intestines of laboratory rats when infected with *Pseudomonas aeroginosa.* The study was used 20 laboratory rats (Albino Sprague - Dawley) at the age of 7-8 weeks, with an average weights 143-148 gm, divided into four groups, with five replications, and the inhibition activity *Pseudomonas aeruginosa* was studied by the concentrations of 200, 400 and 600 µg/ml of the extract using the well diffusion assay method. The results found an increase in the alcoholic extraction effectiveness by increasing its concentration and that the alcoholic extraction was the most effective compared with aqueous extract, with the highest inhibition diameter of 30 mm at a concentration of 600 µg/ml. The results also show an increase ($P<0.05$) in the level of total cholesterol, triglycerides and LDL of groups of rats infected with *Pseudo. aeruginosa* was 70.5, 73.0, and 14.2 mg/dl, respectively and HDL levels decreased at 27.0 mg/dl, compared with the control group. As for the effect of infection with *Pseudo. aeruginosa* on the balance of normal flora in rats, it caused a significant decrease at ($P<0.05$) in the total number of intestinal bacteria, while the total number of LAB bacteria decreased compared to the control group rats.

The oral administration of the alcoholic extract at a concentration of 230 and 430 µg/kg with infection has a significant effect in reducing the negative effect of groups infected with Pseudo. aeruginosa on all the above-measured parameter.

Keywords: Alcoholic extract, Gundelia tourneofortti L., Pseudomonas aeroginosa, Lipid profile, Micro flora.

تأثير مستخلص نبات الكعوب *Gundelia tournefortii L.* في مرتسم الدهن وتوازن النبيت الطبيعي في الجرذان المعرضة للإصابة التجريبية ببكتريا *Pseudomonas aeroginosa*

ايناس غازي سرحان[1] ياسمين اسماعيل الحديدي[1] شيماء فاضل وشاح*[2]

[1]جامعة تكريت – كلية الزراعة

[2]الجامعة التقنية الشمالية / كلية التقنيات الصحية والطبية / الدور

***المراسلة الى:** شيماء فاضل وشاح، كلية التقنيات الصحية والطبية /الدور، الجامعة التقنية الشمالية، تكريت، العراق.

البريد الالكتروني: Shaimaa.fadhil@ntu.edu.iq

الخلاصة

هدفت الدراسة إلى معرفة تأثير مستخلص نبات الكعوب Gundelia tourneofortti L. الكحولي على مرتسم الدهون وتوازن الفلورا الميكروبية في أمعاء الجرذان عند إصابتها تجريبيا ببكتيريا Pseudomonas aeroginosa، واستخدمت في الدراسة 20 جرذًا ذكر (Albino Sprague – Dawley) بعمر 7–8 اسابيع بمتوسط اوزان 143–148 جرام مقسمة الى أربع مجموعات بخمس مكررات وتم دراسة القدرة التثبيطية للمستخلص بتراكيز 200 و400 و600 ميكروغرام/ مل تجاه بكتيريا Pseudomonas aeruginosa باستخدام طريقة الانتشار بالحفر. ووجدت النتائج زيادة في فاعلية المستخلص الكحولي للنبات بزيادة تركيزه وأن المستخلص الكحولي كان الأكثر فاعلية مقارنة بالمستخلص المائي، حيث كان قطر التثبيط الأعلى 30 مم بتركيز 600 ميكروغرام/ مل. كما أظهرت النتائج ارتفاع عند الاحتمالية ($P<0.05$) لمؤشرات الكوليسترول الكلي والدهون الثلاثية وLDL لمجموعات الفئران المصابة ببكتيريا Pseudo. aeruginosa كانت 70.5، 73.0، 14.2 ملجم/ ديسيلتر على التوالي وانخفضت قيمة HDL عند 27.0 مجم/ ديسيلتر، مقارنة مع مجموعة السيطرة. أما عن تأثير الإصابة على توازن النبيت الطبيعي في الجرذان، فقد تسبب في ارتفاع معنوي عند ($P<0.05$) في العدد الكلي للبكتيريا المعوية، بينما انخفض العدد الإجمالي لبكتيريا LAB مقارنة بمجموعة السيطرة.

كلمات مفتاحية: المستخلص الكحولي، Gundelia tournefortii L، Pseudomonas aeroginosa، مرتسم الدهن، توازن الفلورا.

Introduction

The majority of research and studies have tended to use natural sources, such as plants and their extracts with medicinal properties, which are used in the treatment of many diseases due to their biological effectiveness and due to their content of phytochemical compounds , Phytochemicals, which are biologically active chemical compounds with multiple preventive and therapeutic properties, as they protect the plant itself from microbial infections and parasites, which may be phenolic compounds, phenols, flavonoids, Essential Oil, and others (4, 19, 22 and 32) , which have proven effective in treating various pathological conditions and also work to strengthen and boosting the immune system (32).

High cholesterol and atherosclerosis are among the most common diseases at the present time that threaten human health, and food is one of the main causes of high cholesterol in the blood, which in turn is the main factor in injuries to blood vessels, arteries and liver, and thus human life has become a threat at the medium and long term (14)

Most of those who have hypercholesterolemia develop many diseases over time, most notably coronary heart diseases (CHD), atherosclerosis, heart attack, and stroke, in addition to disabilities, which amount to about 4% (28 and 44), and that the methods used to lower cholesterol with cholesterol-lowering drugs, the most important of which are statins, following a healthy diet and exercising regularly, but the use of this drug is accompanied by many health problems, especially in the elderly (37) Therefore, most research centers are moving towards the use of natural alternatives and as far as possible from chemicals Food additives, as natural resources contain many effective compounds with therapeutic properties, whether plant or microbial, as safe and effective alternatives against many different pathological conditions (32).

The medicinal plant, the subject of our current study, is *Gundelia tournefortii L.* A natural, perennial herbaceous plant. This plant belongs to the Asteraceae family, that grows in semi-desert or sandy plains. Its original habitat is Middle Eastern countries in Palestine, Iraq, Syria, Jordan, and other countries in Iran, Azerbaijan, Anatolia, and temperate regions such as Armenia, Turkey, and other regions. Other (13).

This plant is distinguished by its therapeutic properties as well as its nutritional benefits, as it is considered a good source of protein, mineral salts, vit. A, B, and C, and essential fatty acids such as linoleic acid, oleic and palmitic 7, and fiber, which works to reduce constipation and also works to reduce cholesterol and it is used in the manufacture of salads, pickles, appetizers and soups (15).

As for its pharmacological properties, it is used in the treatment of many pathological conditions, as it works to reduce fat and blood sugar levels and treat intestinal and stomach infections (7, 24, 25 and 34) It is also used to treat atherosclerosis (heart disease, atherosclerosis, stroke, chest infections, stomach pain, and a diuretic (20, 29, 36 and 38) and as an antimicrobial (20 and 33), Antioxidant (6), and its seeds for the treatment of vitiligo (7).

As mentioned by some researchers, this plant contains compounds that prevent the division of cancer cells and shorten the life for these cells (8 and 23) and through the aforementioned, the current study aimed to study the effectiveness of alcoholic extract of tournefort's gundelia on experimental infection with *Pseudomonas aeruginosa* in terms of their effects on Lipid profile and the balance of the normal intestinal flora of laboratory rats ,therefore; The purpose of this study to investigate the effect of alcoholic *Gundelia tourneofortti L.* extract on lipid profile and the balance of microbial flora in the intestines of rats when infected with *Pseudo. aeroginosa*.

Materials and Methods

Sample collection**:** *Gundelia tourneofortti L.* plant sample was collected from the northern regions of Iraq and the diagnosis was made based on the Iraqi flora taxonomic keys and to confirm the diagnosis, and the herbarium at the University of Tikrit was used.

Preparation of Plant Extracts**:** The plant was collected, cleaned, washed, dried and ground to a fine powder using an electric grinder, and then the method was described (21) to prepare the extracts as follows **(**The aqueous extract was prepared by mixing 50 grams of dry powder with 500 milliliters of distilled water, then the mixture was left at room temperature for 24 hours, then it was filtered by several layers of medical gauze to get rid of plankton, then it was centrifuged at a speed of 3000) cycles. One minute for 15 minutes, filter using filter papers No o.1, what man. To obtain a clear solution, the resulting extract was poured into glass dishes and placed in an electric oven at 40 ° C, then the dry extract was kept in sealed and opaque glass bottles and refrigerated until it was needed.

The alcoholic extract was prepared in the same method as the aqueous extract, taking into account the replacement of water with ethanol alcohol 80%. The filtrate was concentrated in the Rotary vacuumed evaporator of indiamart type (India).

Bacteria used in the study**:** *Pseudomonas aeruginosa* was obtained from the laboratories of the Tikrit, the diagnosis was made to the Spices level using Vitek 2 compact system.

Inhibitory Activity of the extracts of *Gundelia tourneofortti L.*

Antibacterial activity of the aqueous and alcoholic extracts of *Gundelia tourneofortti L.* against. *Pseudo. aeruginosa* was estimated by the Well diffusion assay method, spread 0.1 µl of the bacterial suspension on the Muller-Hinton agar plates, then drills are made with a diameter of 7 mm using a Cork borer, then the specified concentrations are added, which are 200, 400, 600 µg / mm for each extract and bacteria of the enhancer, with a volume of 0.1 µl. It is left for 2 hour in the refrigerator to spread, then the occlusion is incubated at 37 °C / 24 hours. The effectiveness of the extract was determined by measuring the Inhibition zone around the hole and measured in millimeters (10 and 43).

Vital Experience :Twenty male rats, ages ranged from 7 to 9 weeks and weights 142-149 g, were divided into four groups and five replicates per group in stainless steel cages and raised under the appropriate conditions of temperature 25 ° C and humidity at a rate of 45-70%, lighting, and ventilation and the appropriate diet and drinking water were provided, in addition to paying attention to the cleanliness of the cages and changing the sawdust periodically and regularly throughout the experiment period, as stated in (30). The experiment was designed by dividing the group of laboratory rats randomly into four groups with five replicates:

G1: rats of the control group.
G2: group of rats infected with *Pseu.aeroginosa* and left untreated
G3: group of rats infected with *Pseu.aeroginosa* and treated with alcoholic *Gundelia tourneofortti L*.extract at 230 µg/kg.
G4: group of rats infected with *Pseu.aeroginosa* and treated with alcoholic *Gundelia tourneofortti L*.extract at 430 µg/kg.

The treatment was done with *Gundelia tourneofortti L*. alcoholic extract by Oral administration to the animals at a dose of 2 ml 1.5 x 10^{8} CFU /day/ Rat, and the experiment continued for 28 days. Immediately after the end of the experiment, the rats were starved for a period of 20 hours, after which they were anesthetized with chloroform, and explained and blood was drawn for the tests. Libid profile criteria, total cholesterol, triglycerides, LDL, HDL were estimated as in (42), and the total number *of E.coli*, probiotic and *Salmonella* and *Shigella* was estimated according to (31).

The treatment was done with *Gundelia tourneofortti L*. alcoholic extract by oral dose to rats at a volume of 2 ml 1.5 x 10^{8} cells / ml of the extract, Libid profil criteria, total cholesterol, triglycerides, LDL, HDL, VLDL were estimated as in (42), and the total number *of E.coli*, and probiotic and *Salmonella* and *Shigella* bacteria was estimated according to the method (31).

Statistical Analysis: The results of the experiments were analyzed using the Linear Model General within the ready-made statistical program (39) to study the effect of factors on according to the complete random design (CRD) as well as the Duncan test (16) to determine the significance of the differences between the averages of the factors affecting the studied traits at the level 0.05.

Results and Discussion

Inhibitory Activity of aqueous and alcoholic extracts of *Gundelia tourneofortti L*.: The inhibitory activity of the aqueous and alcoholic extracts of *Gundelia tourneofortti L*. against *Pseudo. aeruginosa* by Well Diffusion Method is shown in Table 1, where all concentrations used for the extract showed inhibitory effectiveness, which varied according to the concentration of the extract, as the diameter of the inhibition increases with the increase in the concentration of the extract, and the effective biological effect was for the alcoholic extract, and the diameters of the inhibition zones were recorded 30, 19, 26 mm, while its aqueous extract recorded 21, 23, and 27 for concentrations of 200, 400 and 600 µg/ml

respectively, and the highest inhibition diameter of the alcoholic extract was 30 mm at 600 µg/ml.

Table 1 Inhibitory effect of alcoholic extract of *Gundelia tourneofortti L.* on the growth of *Pseudo. aeroginosa.*

Inhibition Zone (mm)					
The aqueous extract of of alcoholic extract of *Gundelia tourneofortti L.*			**The alcoholic extracts of alcoholic extract of *Gundelia tourneofortti L.***		
200	400	600	200	400	600
21	23	27	19	26	30

The reason for the alcoholic extract has a higher antimicrobial activity due to the polarity of alcohol in the precipitation and extraction of the active compounds such as flavonoids, phenols, alkaloids ,glycosides and Saponins, especially the flavonoids and the polyphenolic compound, quercetin, gallic acid and tocopherol, and that their antifungal action increases with the increase of the free hydroxyl groups and the formation of hydrogen bonds between these groups and sulfur groups in the protein of the microbial cell, which results in a change in the nature of the protein of the microbial cell, leading to its loss of function (5, 7, 12 and 24). And many studies have shown that plant contains multiple substances that are resistant to *Pseudo. aeruginosa* compared to antibiotics erythromycin and penicillin (20).

Effect of alcoholic extract of Gundelia tourneofortti L. on the lipid profile: Table 2 shows the effect of alcoholic extract of Gundelia tourneofortti L. on the lipid profile of rats induced by Pseudo. aeroginosa, according to the results, there were increase ($P<0.05$) in the values of total cholesterol, triiglycerides and LDL, and a significant decrease in the level of HDL of groups of rats infected with Pseudo. aeruginosa was 70.5, 73.0, and 14.2 mg/dl, respectively, compared with the control 52.0, 43, and 13.0 mg/dl, and HDL levels decreased at 27.0 mg/dl, compared with the group of control 39 mg/dl , and treatment with alcoholic extract at 230 and 430 mg to rats infected with this type of bacteria led to a significant decrease in the values of cholesterol, triglycerides and LDL, and a significant increase in the value of HDL for all treatments.

Table 2 Effect of alcoholic extract of *Gundelia tourneofortti L.* on the lipid profile of rats induced by *Pseudo. Aeruginosa*.

Treatment	CHOL	TG	LDL	HDL
	mg / dI			
G1	52b	43c	13a	39b
G2	70.5a	73a	14.2a	24c
G3	53b	66b	11.6b	55.5a
G4	49.5c	48c	5.4c	33.4bc

The different letters within the same columns are significant differences at ($P<0.05$). G1: rats of the group of control.

G2: group of rats infected with *Pseu.aeroginosa* and left untreated (positive control).

G3: group of rats infected with *Pseu.aeroginosa* and treated with extract of *Gundelia tourneofortti L.*at a concentration of 230 mg.

G4: group of rats infected with *Pseu.aeroginosa* bacteria and treated with extract of *Gundelia tourneofortti L.*at a concentration of 430 mg.

The results of this study agree with (3 and 41) which indicated a significant decrease in the concentration of total cholesterol and triglycerides after treatment with the extract. As indicated by (9, 35 and 40) lowering lipid indices in experimental animals exposed to oxidative stress after treatment with the extract and that the flavonoids and phenols, which act as antioxidants and saponins, as these compounds hydrolyze into sapogenin, and saponins work to reduce the absorption of cholesterol in the intestine.

As for the high concentration of HDL, it is because the extract contains many biologically active compounds such as flavonoids, phenols, and glycosides, which regulate the process of lipid metabolism. Also, the LDL concentration decreased as a result of the high level of HDL (32).

Effect of alcoholic extract of *Gundelia tourneofortti L*. on the balance of normal flora of rats induced by *Pseudo. Aeruginosa*: The effect of infection with *Pseudo. aeruginosa* on the gut microbial balance is shown in Table 3, it caused a significant increase in the number of intestinal bacteria, and it decreased the total count of LAB, it recorded 23, 5.0 CFU / ml, respectively, compared to the control group (G1). The results also showed that treatment of groups of infected rats orally of alcoholic extract at a concentration of 230 and 430 µg/kg, for treatments G2 and G3, respectively, led to an increase in the total count of Lactic acid bacteeria and a decrease in the total count of intestinal, compared to the group (G2), and there were no types of salmonella and Shigella in the different groups in the experiment.

Table 3 Effect of alcoholic extract of *Gundelia tourneofortti L*. on the balance of normal flora of rats induced by *Pseudo. aeroginosa.*

Type of treatment	Total count of LAB	Total count of *E. coli*	Total count of *Salmonella*& *Shigella*
		CFU / ml Log 10^6	
G1	61a	228b	**0.0a**
G2	5.0d	236a	0.0a
G3	53b	112.5c	**0.0a**
G4	16c	93.5d	**0.0a**

The different letters within the same columns are significant differences at (P<0.05).G1: rats of the group of control.

G2: group of rats infected with *Pseu.aeroginosa* and left untreated (positive control).

G3: group of rats infected with *Pseu.aeroginosa* and treated with extract of *Gundelia tourneofortti L*.at a concentration of 230 mg.

G4: group of rats infected with *Pseu.aeroginosa* bacteria and treated with extract of *Gundelia tourneofortti L*.at a concentration of 430 mg.

The treatment with alcoholic extract led to achieving a balance of the natural flora, increasing the types of probiotic, and reducing the number of pathogenic bacteria. The results of this study agreed with (1 and 2) they showed that the use of yogurt containing extracts of *Gundelia tourneofortti L*. alcoholic extract led to the improvement of the natural flora of the intestine by increasing the number of LAB. The addition of *Gundelia tourneofortti L*. extract results in modifying the intestinal environment, inhibiting the growth of pathological bacteria, and preventing their

attachment to the intestinal wall. It is also possible that these effective phytobiotic compounds increase the secretion of the digestive system and inhibit the growth of pathological bacteria.

These effects lead to improving growth performance when adding these vital plant extracts to the diet. Poultry (17 and 18).

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