Archives of Razi Institute, Vol. 77, No. 5 (2022) 1845-1851

DOI: 10.22092/ARI.2022.357982.2128

Original Article

Effects of *Avena sativa* and *Glycyrrhiza glabra* Leaves Extracts on Immune Responses in Serum Cytokine and Liver Enzyme Levels in NIH Mice

Nori Mater, H[1], Manaf Abd Al-Rhman, R[1]*, Ibraheem, S. R[1], Shaker Mahmood, M[1]

1. Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq

Received 28 February 2022; Accepted 22 May 2022
Corresponding Author: quiet_randuna@yahoo.com

Abstract

In addition to their high quantities of active chemicals, medicinal plants are well-known for their pharmacological qualities, which include immunological modulation. T Consequently, this study aimed to examine the effects of *Avena sativa* and *Glycyrrhiza glabra* leaf extracts on immunological responses as measured by blood cytokine and liver enzyme levels. The phytochemical analysis of *Avena sativa* crude leaf extracts revealed the presence of alkaloids,flavonoids, tannins, phenolic compounds, and saponins but the absence of resins and violet oils. On the other hand, violet oils, flavonoids, tannins, saponins, and glycosides were detected in significant concentration in *Glycyrrhiza glabra* ethanolic extract, although resins and phenolic compounds were not present. Fifty male NIH mice were randomly divided into five groups: Except for the control group, all animals were given subcutaneously and orally with extracts (50 mg/kg) for 14 days prior to LPS-induced (1 mg/kg body weight) liver injury. LPS-induced liver damage was induced on day 15, and mice were starved.Group 1 was injected subcutaneously with normal saline as a control. Group 2 received an injection of 100 l of crude oat extract subcutaneously. Group 3 was administered 100 l (50 mg/kg) of crude Oat extract orally. Group 4: administered 100 l (50 mg/kg) of crude Licorice extract subcutaneously. Group 5 ingested 100 l (50 mg/kg) of crude Licorice extract orally. IL-4 levels were significantly elevated (P 0.05) in the subcutaneously and orally treated groups compared to the control group (12.3 0.23 pg/ml). IL-6 was significantly elevated (P<0.05) in mice given subcutaneously or orally with *Avena sativa* or *Glycyrrhiza glabra* extracts compared to mice treated subcutaneously or orally with a control substance (44 0.57 pg/ml). The concentration of TNF- was significantly elevated (P<0.05) in subcutaneous and oral treated groups (283.6 1.7 and 280.6 12.2; 233.9 0.6 and 241.2 2.8) compared with the control group (130 0.42) pg/ml. When mice were exposed to LPS-containing extracts, both GOT, and GPT levels fell relative to the control group.

Keywords: *Avena sativa*, *Glycyrrhiza glabra*, Liver features (GOT and GPT), Interleukins, Mice

1. Introduction

According to the World Health Organization (WHO), over 80% of the world's population uses herbal remedies for their medical requirements. With biotechnological advancements, the propagation, cultivation, and conservation of medicinal plants have increased in many countries in Sub-Saharan Africa. A growing body of evidence suggests that medicinal plants effectively treat a wide range of noncommunicable diseases, including cancer, diabetes, and various infectious diseases (1). The Oat (*Avena sativa*), so often known as the common Oat, is a species of cereal grain grown for its seed. While oats are suitable for human consumption as oatmeal, one of the most common uses is livestock feed. Oats are nutrient-rich meals related to lower blood low-density

lipoprotein (LDL) cholesterol while consumed regularly. Soluble fiber influences cholesterol levels by decreasing cholesterol absorption into the bloodstream inside the intestines (2-4). Healthy bacteria inside the small gut digest soluble fiber; insoluble fiber does not digest; however, it facilitates ordinary bowel moves. For the past decades, there has been a growing interest in investigating different extracts received from plants for nutritional and therapeutic purposes. Soluble fiber influences cholesterol levels by lowering the absorption of LDL into the bloodstream in the intestines (5). *Avena sativa* is a rich supply of protein, nutrients, lipids, mineral materials, and high amounts of precious nutrients, which include soluble fibers, b- glucan, carboxy acid, and other phytochemicals, saponins, and flavonoids. It was discovered that taking licorice root extract reduced the tiers of liver enzymes, whose high ranges illustrate non-alcoholic fatty liver ailment. It becomes used for diarrhea, dysentery, and colitis. The mature seeds of *Avena sativa* are used as food (6). Licorice (*Glycyrrhiza glabra)* is a perennial herbaceous plant. The licorice plant has been used medicinally for more than three thousand years due to its medicinal and therapeutic advantages of licorice. The historical Egyptian papyri mentioned it as a medicinal plant, as licorice was used inside and beyond to treat kidney ache, cough, liver and spleen ache, heartburn, and constipation (7). Licorice is excessive in flavonoids which are the number one source of licorice medicinal blessings because of its antioxidant and anti-inflammatory homes, and it also boosts the health of the immune machine. The benefits of licorice for the prevention of liver disorders have been approved. It is well documented that the licorice liver protection functions are mediated by its chemicals, such as glycyrrhizin, which restores the stability of liver enzymes (8).

Therefore, this study aimed to investigate the effects of *Avena sativa* and *Glycyrrhiza glabra* leaves extracts on immune responses in serum cytokine and liver enzyme degrees.

2. Materials and Methods

2.1. Preparation of Crude Ethanol Extract of Oat (*Avena sativa*) and Licorice (*Glycyrrhiza glabra*)

Crude plant extracts were prepared using ethanol 80٪ solvent. Oat and Licorice leaves had been assembled from the gardens in Baghdad. The leaves of *Avena sativa* and *Glycyrrhiza glabra* are derided at room temperature and made into a powder of plants by means of the blender. Then, 100 g of plant powder and resolve it in 500 ml ethanol 80% for 3days.The extracted samples were filtered through a filter paper (Whatman No.1), and the filtrate was turned into Petri Dish to dry and achieve crude extract. The extract is placed in a container at 4°C in a refrigerator until use (9, 10).

2.2. Tests of Phytochemical Compounds

2.2.1. Alkaloids Test

As previously described by Leli, Mencacci (11), a few drops of Dragendorff's reagent were added to 5ml of plant extract after filtration. If the appearance of the solution changed to orange-red color, the sample was considered positive for the presence of alkaloids.

2.2.2. Flavones Assessment

As previously described by Suh, Park (12), for the assessment of flavones presence, 10 ml of ethyl alcohol (50%) was added to 10ml of KOH (50%), then 5 ml of filtrated plant extract was mixed with them to evaluate the mixture appearance. The appearance of yellow color indicated the presence of flavones in the plant extract.

2.2.3. Volatile Oils Assessment

The technique was carried out in keeping with Indian herbal pharmacopeia (1998) by adding a few drops of plant extract, after filtration, within the middle of filter paper until saturation by way of the capillary tube. Then the paper becomes examined below UV Light. The appearance of pinkish coloration on filter paper suggests the presence of volatile oils.

2.2.4. Tannins Test

The technique depended on the approach defined by Suh, Park (12). After filtration, 2 ml of plant extract

pattern was brought to 1% of lead acetate. The look of the gel precipitant indicated the result. The positive result was the appearance of gel precipitant.

2.2.5. Saponines Assessment

This assessment was achieved based on the technique defined by Leli, Mencacci (11). 1ml of 1% mercuric chloride was delivered to2ml of plant extract after filtration; the positive result indicated white precipitant.

2.2.6. Glycosides Assessment

The method was achieved based on the method defined by Gil, Tomás-Barberán (13). After filtration, a few drops of Kidde's reagent were added to 3ml of plant extract. The appearance of blue–red color ring indicated a positive result**.**

2.2.7. Resins Assessment

The technique was achieved based on the method defined by Vinothapooshan and Sundar (14); 10ml of 4% HCL was added to 5ml of plant extract after filtration, and the observation of turbidity indicated the presence of resins in the plant.

2.2.8. Phenol Compound Test

This test was performed by Liao, Shen (15). After filtration, 2 ml of plant extract was added to one% of ferric chloride, and the darkish blue color indicated the presence of phenol compound.

2.3. Animals and Immunization

Fifty healthy male NIH mice were randomly divided into 5 groups: Except for the control group, all the animals were pre-treated with extracts (50 mg/kg) subcutaneously and orally for 14 days before the LPS-induced (1 mg/kg body weight) damage liver. On day 15 (14 days after extracts administration), liver damage was induced by LPS, and the mice then fasted (9). Group 1: control group was injected subcutaneously with normal saline. Group 2: injected subcutaneously with 100µl of crude Oat extract. Group 3: orally received 100µl (50 mg/kg) of crude Oat extract. Group 4: injected subcutaneously with 100µl (50 mg/kg) of crude Licorice extract. Group 5: orally received 100µl (50 mg/kg) of crude Licorice extract.

2.4. Blood Sampling

Blood was collected from mice by cardiac puncture before being injected with LPS, and after, serum became separated from blood samples with the aid of centrifugation at 5000 rpm for 10 min and saved at -20°C for the serological exam (before injected with LPS) measure interleukins using ELISA test and after injection LPS for liver enzymes test.

2.5. Detection of Interleukin 4, 6 and TNF

Inflammatory cytokines were detected, which were produced against crude extracts; when immunization subcutaneously and orally in mice, it becomes done the use of ELISA kits of a sandwich enzyme immunoassay for *in vitro* quantitative dimensions of (IL-4), (IL-6), and (TNF) in mice serum. The assays were accomplished in accordance with the producer's protocol (EIA-ab/china).

2.6. Biochemical Assay for Liver Enzymes

Liver enzymes (Aspartate Aminotransferase AST (GOT) and Alanine Aminotransferase ALT (GPT) have been tested as follows:

AST (aspartate aminotransferase; GOT, glutamate oxalacetate transaminase) and ALT (alanine aminotransferase; GPT, glutamate pyruvate transaminase) are sensitive indicators to monitor liver function under drugs treatment or with acute viral hepatitis. The blood sample's elevated AST and ALT values indicate liver damage or injury (16).

AST and ALT were measured using the following kits according to the manufacturer's instructions. AST (GOT) detection kit (Roche, catalog number: 11876848216) ALT (GPT) detection kit (Roche, catalog number: 11876805216).

2.7. Statistical Analysis

USING AN ANOVA TABLE, IBM SPSS computer program version 25.0 was used to calculate the median, standard error (SE), and probability (two-tailed).

3. Results and Discussion

The results of phytochemical screening of crude

leaves extracts from Oat discovered that: alkaloids, flavonoids, saponins, glycosides, tannins, and phenol compounds were found in ethanolic extracts, while volatile oils and resins were absent (Table 1). Al-Snafi (8) noted that Oat leaves include these compounds in various proportions. Phytochemical analysis showed that crude extracts of Licorice leaves incorporate numerous compounds, together with volatile oils, saponins, flavonoids, tannins, alkaloids, and glycosides at the same time as phenol compounds and resins have been absent (Table 1). Chopra, Saraf (17) referred to that Flavonoids, saponins were found in the methanol extract, and Alkaloids, proteins, and tannins were not detected. Those differences inside the existences of energetic secondary metabolites within the leaves crude extracts of the plants beneath study should be because of the degree of the polarity among solvents (18).

Table 1. Chemical analysis of*Avena sativa* and*Glycyrrhiza glabra* crude leaves extracts

	Avena sativa	*Glycyrrhiza glabra*
Chemical compound	**Ethanolic extract**	
Alkaloids	**(+)**	**(+)**
Flavones	**(+)**	**(+)**
Volatile Oils	**(-)**	**(+)**
Tannins	**(+)**	**(+)**
Saponins	**(+)**	**(+)**
Glycosides	**(+)**	**(+)**
Resins	**(-)**	**(-)**
Phenol compound	**(+)**	**(-)**

As shown in table 2, serum levels of cytokines, counting IL-4, IL-6, and TNF-α, significantly increased in groups 2-5 compared to the control group. The level of IL-4 showed significant elevation ($P \leq 0.05$) in subcutaneous and orally treated groups (75.6±2.8 and 83.3±1.7; 61.0±0.4 and 55.8±2.8) pg/ml for *Avena sativa* and *Glycyrrhiza glabra* extracted respectively as compared with control (12.3±0.23) pg/ml. The amount of IL-6 showed significant elevation ($P \leq 0.05$) in subcutaneous and orally treated mice (128.5±4.6 and 135.6±2.7; 119.6±5.1 and 115.2±1.9) pg/ml for *Avena sativa* and *Glycyrrhiza glabra* extracted respectively compared with control (44±0.57) pg/ml. Finally, the concentration of TNF- α found significant elevation ($P \leq 0.05$) in subcutaneous and oral treated groups (283.6±1.7 and 280.6±12.2; 233.9±0.6 and 241.2±2.8) pg/ml for *Avena sativa* and *Glycyrrhiza glabra* extracted respectively compared with control (130±0.42) pg/ml (Table 2). Figure 1 shows a higher mean was observed in TNF-α compared to other groups. This result agreed with Hong, Wu (19), and Baba, Acar (20) that higher concentrations of TNF-α may assist the defense mechanism towards plant extracts. Phytochemical *Avena sativa* plant extracts induce secretion of tumor necrosis component-α (TNF-α) and IL-6 from macrophages through Toll-like receptor four (TLR4) (21, 22). Our study observed that the percentages of CD3+ and CD4+ T cells were extensively significantly increased *Avena sativa* extracts treatment, indicating development in T cell quantity and proportion, particularly in T cells, that may secrete cytokines and mediate cell immune responses with antigen offering cells (APCs), in accordance with our adaptive immunity assessment consequences. To be precise, CD4+ T cells consist of T helper type 1 (Th1) cells, which stimulate cellular-mediated immune responses through producing inflammatory cytokines and TNF-α (23). This interleukin is secreted by lymphocytes, mast cells, eosinophils, natural killers, and endothelial cells, and its function is the proliferation and differentiation of hematopoietic precursors and macrophages; therefore, the increased level of leukocytes explained, and this agreed with previously published works Banchereau and Steinman (24) Saikia and Das (25). This difference in results may be attributable to the variation in phytochemical substance and immunomodulating properties based on chemical compounds' structure, molecular weight, and compositional function, which led to cytokine production (21, 26, 27).

In white mice, the effect of crude *Avena sativa* and *Glycyrrhiza glabra* extract on liver function, particularly Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase ALT (GPT), the most prevalent diagnostic markers of liver damage, as compared to control values (28, 29). The two enzymes

were significantly less (P<0.05) in mice treated subcutaneously and orally with 100 µl (50mg/kg) of extracts with LPS compared to the control LPS animals, as shown in table 2. The subcutaneously and the orally treated animals with Oat extract showed that GOT levels were 116.0 3.5, 117.0 1.2 IU/L, respectively, and there was a decrease compared to the control LPS values (195.031.63) IU/L. We found a decrease in the GPT levels in animals treated subcutaneously and orally with Oat extract with LPS (10.5±1.2, 12.8±1.7) IU/L, respectively, and there was a decrease compared with the control LPS values (70.2±0.99) IU/L. The GOT levels in animals treated subcutaneously and orally with Licorice extract were 90.0±1.7 and 84.0±1.7 IU/L, respectively; we found a decrease compared with the control values (195.03±1.63) IU/L. The subcutaneously and oral GPT levels of Licorice extract with LPS (11.2±0.1, 12.1±0.1) IU/L, respectively. The results decreased compared to the control LPS values (70.2±0.99) IU/L. The increase in liver enzymes strongly depends on the serum and the degree of damage and change in the tissues of the organs induced by LPS (30, 31). The extracts treatment significantly decreased the activities of GOT and GPT compared to those observed in mice with LPS-induced liver damage (P≤0.05). This test was conducted to determine extracts' liver protective effect (Table 3).

Table 2. Interleukins 4, 6 and TNF level

Plant extracts		IL-4 level	IL-6	TNF
		Median±SE	Median±SE	Median±SE
Group 1 (Control)		12.3±0.23	44±0.57	130±0.42
Oat (*Avena sativa*) extract	Subcutaneous	75.6±2.8	128.5±4.6	283.6±1.7
	Oral dosage	83.3±1.7	135.6±2.7	280.6±12.2
	Probability	0.074	0.142	0.151
Licorice (*Glycyrrhiza glabra*) extract	Subcutaneous	61.0±0.4	119.6±5.1	233.9±0.6
	Oral dosage	55.8±2.8	115.2±1.9	241.2±2.8
	Probability	0.187	0.349	0.046

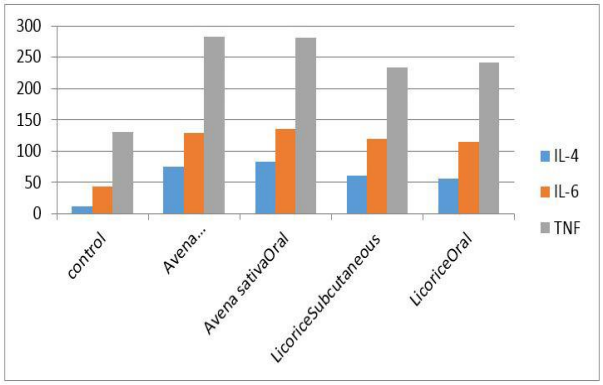

Figure 1. Interleukins 4, 6 and TNF level

Table 3. Detect enzyme liver (GOT and GPT) liver enzymes level

Plant extracts		GOT level	GPT
		Median±SE	Median±SE
Control lps		195.03±1.63	70.2±0.99
Oat extract	Subcutaneous	116.0±3.5	10.5±1.2
	Oral dosage	117.0±1.2	12.8±1.7
	Probability	0.74	0.471
Licorice extract	Subcutaneous	90.0±1.7	11.2±0.1
	Oral dosage	84.0±1.7	12.1±0.1
	Probability	0.064	0.776

According to the findings of the experiments, *Avena sativa* crude extract includes (Alkaloids, Flavones, Tannins, Saponins, Glycosides, and Phenol compound), whereas *Glycyrrhiza glabra* crude extract contains (Alkaloids, Flavones, Tannins, Saponins, Glycosides, and Phenol substance). The level of interleukins (IL-4, IL-6, and TNF) rose in mice given the *Avena sativa* extract rather than the *Glycyrrhiza glabra* extract that strong immune system. When mice were exposed to extract with LPS, GOT and GPT levels decreased in all groups compared to the control LPS group. The protective effect of extracts on hepatic damage induced by LPS was investigated.

Authors' Contribution

Study concept and design: H. N. M.
Acquisition of data: H. N. M.
Analysis and interpretation of data: R. M. A. A.
Drafting of the manuscript: S. R. I.
Critical revision of the manuscript for important intellectual content: R. M. A. A.
Statistical analysis: M. S. M.
Administrative, technical, and material support: H. N. M.

Ethics

All research was approved by our Institutional Ethics Board at the University of Baghdad, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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