Effect of Pomegrante Extract (*Punica Granatum*) on Interleukin Levels and Liver Enzyme in Mice

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Abstract

Pomegranate (*Punica granatum*) crude plant extract has been prepared by way of using ethanol 80% solvent. Dried peeling and pulp in room temperature, 100 gm weight of powder plant and was solubilized in 500 ml of 80% ethanol alcohol. The consequences of phytochemical screening of crude extracts found that: alkaloids, flavonoids, tannins, phenolic and glycosides had been present in ethanolic extract while resins, saponines and violet oils had been absent. *In vivo* observe discovered that, the effect immunized mice with a 100 µl (50 mg/kg) of ethanol crude extracts, were given subcutaneously and orally for 14 days on extraordinary parameters. The first its effect on immune markers in mice (Interleukins 4, 6 and TNF). The second its effect on biochemical exams for liver enzymes (GOT and GPT) as compared to control mice were intraperitoneal injected with LPS alone (1 mg/kg body weight), damage of liver by LPS was induced. Than pre-treated mice with (50 mg/kg) extracts subcutaneously and orally for 14 days injected with the LPS on day 15 (after 14 days administration of extracts). The results showed that there was an decrease in the (IL-4), (IL-6) and (TNF) compared to the control mice group, cause decreases the immune system when use pomegranate crude extract contains bioactive compounds present a wide range of flavonoids, polyphenolic, and other anti-inflammatory phytochemicals compared with control. When mice were exposed to extracts with LPS, GOT and GPT levels decrease in all groups compared to injected control LPS group. The protective effect of extracts on hepatic damage, induced by LPS, was investigated.

Keywords: *Punica granatum*, liver enzyme (GOT and GPT), interleukins, mice.

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Introduction

Pomegranate (*Punica granatum*) contains many phytochemicals that have many therapeutic properties, as the chemical composition of pomegranate juice includes fructose, sucrose, glucose and some organic acids such as ascorbic acid (vitamin C) and citric acid, fumaric and malic, in addition to a little quantity of all amino acids, especially methionine, proline and acids, which is a rich source of polyphenols [1]. Especially tannins and flavonoids, which gives it its therapeutic properties, while it contains oil-rich seeds rich in bans kids and some phytoestrogen compounds that are similar in most cases, steroids in the human body [2]. Pomegranate interacts with drugs altered by the liver via cytochrome P450 2D6 (CYP2D6 substrates), such as amitriptyline, codeine, desipramine, flecainide, fluoxetine, ondansetron, tramadol, etc[3]. Where it interacts with pressure medications due to its hypotensive effect, which can cause a significant decrease In

blood pressure, which also interacts with Rosuvastatin, reduces the ability of the liver to eliminate it, which increases the risk of its side effects. Natural plants are an important source in treating many diseases, as they contain a large number of biologically active compounds. As for the outer shell, the pomegranate contains tannic acid, an astringent powder used as an excellent antidote to treat diarrhea, and boiled chips act as an anthelmintic agent, especially the worm. It is the only one that contains many alkaloids, including bileterin [4]. This substance was found with other alkaloids are ethyl methylizoplilirin-N, isobiliferinpseudobilticrine, ethyl pelletcrine, in the roots and stems of the pomegranate tree. It has been observed in many studies that pomegranate has a lethal inhibitor and growth inhibitor of negative cationic microbes and a number of fungi such as tunzuran and ribrom[5]. Given the types of chemical compounds that contain pomegranate fruits and their many benefits in treating many diseases [6]. Pomegranate contains a lot of antioxidants, as well as pomegranate peel occupies weight of the fruit about 26% to 30%, is characterized by the presence of great amounts of antioxidants, such as phenolic compounds such as flavonoids (anthocyanins) and catechins, other flavonoids, as well as tannins (buncaline), pedunculase, buncalagin, gallic acid and ellagic acid. These substances are determined in the pomegranate juice and peel and they represent 92% of the antioxidants present in this fruit [7]. Where the pomegranate peel contains T most people get rid of it with much more antioxidants than the pulp, so the supplemented supplements can be a summary pomegranate peel, which will be more beneficial than the extract of pomegranate pulp itself [8]. The current research aims to evaluate the effect of pomegranate extract (*Punica granatum*) on the levels of interleukins and liver enzyme in mice.

Material and method

Preparation of crude ethanol extract of *Punica granatum* plant [9]

Crude plant extract was prepared by using ethanol 80%solvent. *Punica granatum* has been assemble from the gardens in Baghdad. Dried peel and pulp of *Punica granatum* in room temperature and through means of the blender floor into a powder. after that, weight (100gm) of powder plant and soluble 500 ml ethanol alcohol 80% then soak it 3day. Than using a filter paper (Whatman No.1) for filtered extracted sample and the filtrate was put in petri dish to dry and obtain crude extract. Finally, at 4°C in a refrigerator in a glass container kept the extract.

Tests of phytochemical compounds

1-Alkaloids test:

The method was achieved according to[10].A few drops of modified Dragendorff's reagent were added to 5ml of plant extract after filtration. A positive result is indicating appearance of orange –red color.

2-Flavones test:

The test was done according to [11] by adding 10 ml of ethyl alcohol(50%) to 10 ml of KOH solution (50%) and then mixed with 5 ml of plant extract after filtration. The appearance of yellow color indicated the presence of flavones in plant.

3-Volatile Oils test:

The method was conducted according to [9] by adding few drops of plant extract, after filtration, in the middle of filter paper, until saturation by capillary tube. Then the paper was examined under UV Light. The appearance of pinkish color on filter paper indicates the presence of volatile oils.

4-Tannins test:

The procedure was depend on the method described by[11]. 1% of lead acetate was added to plant extract sample. The appearance of gel precipitant indicated positive result.

5-Saponines test:

This test was done based on the method described by[10]. 1ml of 1% mercuric chloride was added to2ml of plant extract after filtration, the observation of white precipitant indicated a positive result.

6-Glycosides test:

The procedure was carried out based method described by[12]. Kidde's reagent (Few drops) was added to 3ml of plant extract after filtration. A positive result was indicated by appearance of blue –purple color ring.

7-Resins test:

This test was achieved according to [13]. 10ml of 4% HCL solution was added to 5ml of plant extract after filtration, the presence of resins was indicated in the plant by appearance of turbidity.

8-Phenol compound test:

This test was performed according to [14]. 2 ml of plant extract after filtration was added to 1% of ferric chloride, dark blue color was appearance indicated the presence of phenol compound.

Mice animal and immunization

Healthy white mice were used in this experiment, weighing (1) kg, were separated into four groups every group contains sex mice.

Group 1: injected control group with normal saline

Group 2: subcutaneous immunization with a 100µl of crude extract.

Group 3: oral immunization with a 100µl of crude extract.

All three group immunization was apply according to the method by [15].Mice were injected with a (50mg/kg) 100µl of crude extract, was given subcutaneously and oral for 14 days for interleukins test.

Group 4: control for enzyme liver test mice were intraperitoneal injected with commercial Lipopolysachride (LPS) (1 mg/kg body weight).

All three group mice were pre-treated with (50 mg/kg) extracts subcutaneously and oral for 14 days before the LPS-induced damage liver. On day 15 (after 14 days extracts administration), damage of liver by LPS was induced and the mice then fasted, killed by breaking of the neck after anaesthetized with ether [16].

Collection of blood

Collection blood from mice by cardiac puncture before injected with LPS and after, than using centrifugation at 5000 rpm for 10 min to separated serum from blood samples with and saved at -20°C for serological exam (before injected with LPS) measure interleukins using ELISA test and after injection LPS for liver enzymes test.

Estimation of interleukins 4,6,TNF levels by ELISA

This test was used for detection of interleukins which produced against pomegrante extract (*Punica granatum*),was performed using producer's protocol ELISA Kits (EIA-ab/china) of a sandwich enzyme immunoassay for *in vitro* quantitative measurement of (IL-4), (IL-6) and (TNF) levels in mice serum.

Biochemical tests enzymes liver (GOT and GPT)

Liver function (Aspartate Aminotransferase AST (GOT) and Alanine Aminotransferase ALT (GPT) were examined:

A) Aspartate Aminotransferase AST (GOT) - Activated Pyridoxal Phosphate:

Principle of testing: pyridoxal-5'-phosphate.3,4 AST in the sample stimulates the transport of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. the oxaloacetate formed is reduced to malate by malate dehydrogenase (MDH) with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). Pyridoxal phosphate AST acts as an enzyme aid in the amino transport reaction and is assayed on an automated biochemical analyser COBAS INTEGRA a bichromatic in absorbance at 340nm in hematology laboratory of in Gastroenterology and Hepatology Hospital by using kit (Siemens - USA). [17].

B) Alanine aminotransferase ALT (GPT) - pyridoxal phosphate activator:

Principle of testing: pyridoxal 5'-phosphate. 3,4 ALT stimulates the transamination of L-alanine and 2-oxoglutarate forming L-glutamate and pyruvate. The pyruvate formed is reduced to form Lactate and NAD + by a lactate dehydrogenase-stimulated (LDH). Pyridoxal phosphate acts as an enzyme aid in the amino transport reaction and the rate of NADH oxidation is directly proportional to the alanine aminotransferase activity and is assayed on an automated biochemical

analyser COBAS INTEGRA a bichromatic in absorbance at 340nm in hematology laboratory of in Gastroenterology and Hepatology Hospital by using kit (Siemens -USA) [17].

Statistical analysis

IBM SPSS computer program version 25.0 was used to calculate the median, standard error (SE), probability (two tailed) by using ANOVA table, Duncan test and independent T-test.

Result and discussion

Detection of active compounds:

After extraction methods, then the chemical analysis was carried out to detect the active constituents of each extracts (Table 1). The results of phytochemical detections for pomegrante extract *(Punica granatum*) were positive for (Alkaloids, Flavones, Tannins, Glycosides and Phenol compound). It was negative for (Volatile Oils, Saponines and Resins). [18] who noted that pomegranate (*Punica granatum*), as byproducts, possess strong antioxidant activities and are rich sources of phenolic compounds. [19] referred to the antioxidant activities of natural plant extract and the phenolic compounds have reduced the risk of disease and the ability to maintained human health. The phytochemical properties depend on the types of solvent and depend to polarity and nonpolarity. The results showed extraction of major amounts of flavonoids and phenolics and other phytochemical when using of 80% ethanol polar solvent.

Table 1:Chemical analysis of *Punica granatum* crude extract

Chemical compound	Ethanolic extract of *Punica granatum*
Alkaloids	(+)
Flavones	(+)
Volatile Oils	(-)
Tannins	(+)
Saponines	(-)
Glycosides	(+)
Resins	(-)
Phenol compound	(+)

Estimation of cytokines levels:

The serum levels of cytokines, counting IL-4, IL-6 and TNF-α have been assessed in diverse groups and in contrast with the control group using immuno-linked enzyme screening technology (ELISA):

The results showed that there was an decrease in the values of (IL-4), (IL-6) and (TNF), compared to the control mice group. The IL-4for subcutaneously and oral groups (39.6 ± 5.1, 12.3 ± 0.23) pg/ml respectively, we found that there was decrease compared to the control (42.5 ± 1.2pg/ml) , IL-6 for subcutaneously and oral groups (103.8 ± 1.7 , 34 ± 0.57) pg/ml

respectively, we found that there was decrease compared to the control (112.3 ± 1.0pg/ml), and TNF for subcutaneous and oral groups (193.4 ± 0.7, 120 ± 0.42) pg/ml respectively, also we found that there was decrease compared to the control (217.5 ± 2.1pg/ml). All of these cytokines revealed a very significant difference (P≤0.05) between groups compared to the control group table (3), cause decreases the immune system when use crude extract compared with control. As proven in figure 1, a higher mean was observed in TNF-α comparison to others. Pomegranate crude extract contains bioactive compounds present a broad range of flavonoids , polyphenolic and other anti-inflammatory phytochemicals, all of which may aid in efforts toward prostate and breast cancer and as an adjunct nutritional therapy [20]. When administration of pomegranate extracts reduce inflammation in a respiratory inflamed also the symptoms of IBD and all inflammation recovered in model of mice [21]. The pomegranate has been used medicinally as antibiotic for thousands of years [22].

Table 3: Interleukins 4, 6 and TNF levels

		IL-4 level	IL-6	TNF
		Median ± SE	Median ± SE	Median ± SE
	Control	42.5 ± 1.2	112.3 ± 1.0	217.5 ± 2.1
Pomegranate extract	Subcutaneous	39.6 ± 5.1	103.8 ± 1.7	193.4 ± 0.7
	Oral dosage	12.3 ± 0.23	34 ± 0.57	120 ± 0.42
	Probability	0.474	0.084	**0.007**

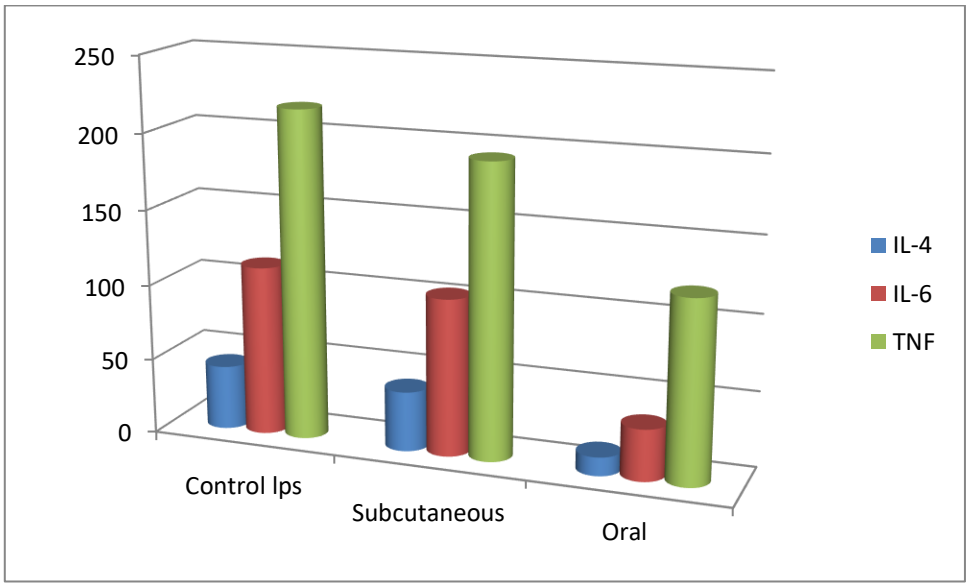

Figure 1 : Interleukins 4, 6 and TNF level

Liver functions

The effect of *Punica granatum* crude extract on liver function including Aspartate Aminotransferase (AST /GOT) and Alanine Aminotransferase (ALT /GPT) the most common diagnostic markers of liver damage, has been studied in white mice as compared with control [23]. Results showed that the two enzymes were significantly less (P≤0.05) as in table (2) in mice treated subcutaneously and oral with (50mg/kg) 100µl of *Punica granatum* extract with LPS compared with the control LPS animals, the subcutaneously and the oral GOT levels (74.0± 2.3,70.0 ± 1.2,0.19) IU/L respectively, we found that there was a decrease compared with the control (195.03±1.63) IU/L. The subcutaneously and oral GPT levels (13.0 ± 1.7,12.0 ± 4.6 ,0.752) IU/L respectively, we found that there was a decrease compared with the control LPS (70.2±0.99) IU/L. The increase and decrease in liver enzymes strongly depends on the serum and the degree of damage and change in the tissues of the organs induced by LPS [24]. The extracts treatment significantly decreased the activities of GOT and GPT compared to LPS-induced liver damage in mice with (P≤0.05). This test was conducted as follows to determine the liver protective effect of extracts [25].

Table 2: liver enzymes level

Plant extracts		GOT level	GPT
		Median ± SE	Median ± SE
	Control lps	195.03±1.63	70.2±0.99
Pomegranate extract	Subcutaneous	74.0 ±2.3	13.0 ± 1.7
	Oral dosage	70.0 ± 1.2	12.0 ± 4.6
	Probability	0.19	0.752

Conclusion

We concluded from chemical analysise of pomegrante extract (*Punica granatum*) crude extract contain (Alkaloids, Flavones, Tannins, Glycosides and Phenol compound) and not contain (Volatile Oils , Saponines and Resins) and we concluded from the study that Consumption of pomegranate crude extract (50 g/day) and phytochemical compound appears to have favorable effects on some markers of subclinical inflammation, The level of interleukins (IL-4, IL-6, and TNF) decrease and GOT and GPT levels decrease. The protective effect of extracts on hepatic damage, induced by LPS, was investigated.

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