

Determination of total flavonoid, antioxidant, active constituents and liver function enzyme of *Plantago lanceolata* aqueous extract

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Abstract

Plantago lanceolata considered as an ancient herb promising plant. In this work, the target of the study was designed to estimate total flavonoids, anti-oxidant and chemical composition of *Plantago lanceolata* aqueous extract *in vitro*. Total flavonoids content was;evaluated in the extract, and it was $317.0000 \pm 9.64365\text{mg /ml}$. also, anti-oxidant activity of *Plantago lanceolata* aqueous extract *in vitro* was assessed *in vitro* via evaluation of reductive ability. At all concentrations of plant extract tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml) in reductive ability; the absorbance was increase significantly in all dependent concentration manner. also, the aqueous plant extract was increase significantly The results of chemical composition of plant showed contain Tannins, polysaccharide, Alkaloids,Flavonoids and Polyphenolic Compounds . Also plant extracts counteract the damage caused by methotrexate drug through the reduction in liver enzymes GOT, GPT and ALP in all groups of mice the results of interactions indicated the ability of plant to modulating harmful effects of the drug in liver and its enzymes when administration to albino mice male in 200mg/kg.

Keywords: *Plantago lanceolata*; Anti-Oxidant; Chemical Composition; Reductive Ability; Alkaloids; Methotrexate

1. Introduction

Among the medicinal herbs species of therapeutic interest is included *P. lanceolata*. which is a type of the genus in the family named Plantaginaceae originated from Europe, commonly spread throughout the world. *Plantago lanceolata*. is a common recurrent weed, It is inborn in herb places on basic or neutral soils [1].

Plantago lanceolata, was grows to a tall about 40–60 cm and yields a rosette of leaves, sometimes up to 50 cm, in tall [2]. The size of the plantago be determined by the growth habitats.

The complex chemical composition of *P. lanceolata* L. species is represented by a series of categories of substances, among which the main components are: iridoid glycosides, polysaccharides (plantaglucide, glucomannan), flavonoids and flavone glycosides, phenolic carboxylic acids, alkaloids (indicain, plantagonin), terpenoids (loliolide, ursolic acid, oleanolic acid. [3]. As a result of the presence of active metabolites, *Plantago major* L. is used in cosmetic and pharmaceutical industries. Treating dermal diseases, anti-diarrhea, anti-cancer and antibacterial activates have been described in different sources as some of the health benefits of this plant [4]. which concluded that plant had beneficial effect on health.

Several studies develop methods for investigating the antioxidant properties of different, such as: Ferric Reducing Antioxidant Power (FRAP) assay, Trolox Equivalent Antioxidant Capacity (TEAC), DPPH (diphenylp-picrylhydrazyl

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radical), oxygen radical absorbance capacity (ORAC), chemiluminescence (CL), to name a few [5]. These methods are being applied by the food, pharmaceutical and cosmetic industries.

The long-term problem of MTX stay the basic causes of worried for both patients and therapists. Then, new methods to improve patients' tolerance and decrease MTX side effects are necessary needed [6]

This study had been approved out to estimate the effects of Aqueous extract of *Plantago lanceolata* extract against MTX-induced hepatic damage in mice.

2. Material and methods

2.1. Plant collection and identification

The aerial part of the plantago was collected from the local markets during October (2020), which had been identified by National Herbarium of Iraq.

2.2. Plant extract Preparation

Aqueous extract of *Plantago lanceolata* was obtained according to. [7] Fifty grams of the plant aerial parts powdered and extracted with hot distilled water (250 ml) 65°C for 1 hour in soxhlet device The plant extract solution was dryness under reduction pressure to concentrated in a rotary evaporator to yield crude dried plant extract at 37°C until dryness, then frozen at -20°C until use the essential concentration.

2.3. Determination of Total Flavonoids

Total flavonoids contented was spectrophoto-chemically resolute in the *P. lanceolata* aqueous extract by way of rutin standard equal by $AlCl_3$ colorimetric method [8].

The aqueous plant extract in weight (3.2 mg) was once as soon as dissolved among five ml in regard to fifty % methanol, accompanied with the aid of addition concerning one ml concerning a five percentage (w/v) ($NaNO_3$) solution. After six minutes, one ml involving a ten percentage (w/v) aluminium chloride answer was once brought yet the combination used to be allowed in accordance with remain because of a in addition five minutes before ten ml in regard to a ten % (w/v) NaOH reply used to be added. The mixture used to be committed up in imitation of fifty ml along DW but mixed well. Then the absorbance used to be modest at 450 nm with a spectrometer below fifteen min. A similar process used to be applied in accordance with vii after concentrations (two and half, five, ten, twenty, forty since eighty μg) concerning rutin as like standard, yet ancient in imitation of put together standard curve.

2.4. Determination the active chemical constituent in plant [9]

2.4.1. Detection of Tannins tests

The one % Lead acetate solution have been added to the aqueous plant extract. A gelatinous or white precipitate have been made that the presence of tannins.

2.4.2. Detection of polysaccharides

One ml of the aqueous plant extract was mixed with two ml of the Benedict reagent, then put the mixture in a boiling water bath for five minutes and left to cool. The red deposit indicated that a presence of polysaccharides.

2.4.3. Detection of alkaloids (Dragangroff test)

A solution of 60 mg of Bismuth sub-nitrate have been dissolved in 0.2 ml HCl (solution A) and Solution B contains 600 mg potassium iodide in one ml D.W The solution A + B were mixed and additional to the plant aqueous extract, an orange to brown color will detected expression the founded of alkaloids.

2.4.4. Detection of the Saponins

The detection process was happened by mixing the solution of the plant aqueous extract well. Formation of foam at the top of the extract showed of saponins.

2.4.5. Detection of Flavonoids

This test was prepared by using Sodium hydroxide solution had been mixed with restricted amount of plant extract solutions and left, a bright yellow color was showed that appeared of flavonoids.

2.4.6. Detection of Polyphenolic Compounds

Three % ferric chloride (FeCl_3) solution have been added to the plant aqueous extract solution a brown deposition will be show.

2.5. Determination of free radical scavenging assay method

This method was complete by estimate the reductive ability by using [10], used to be adopted in conformity with consider the reductive ability, inside namely 1 ml regarding every attention of the drive into put off [0.02, 0.04, 0.08, 0.16, 0.32 yet 0.64 mg/ml] used to be combined collectively along one ml as regards 0.2M phosphate clink (pH 6.6) after one and half ml concerning one % potassium ferricyanide, afterward then incubated at 50°C due to the fact on 20 minutes. Then, 1ml related to ten % trichloroacetic sour style was once introduced below the combination after quit the reaction. The mixture was once centrifuged due to the fact x minutes at 3000 rpm, but 2.5 ml above the supernatant used to be mixed including two ml of distilled water or 0.5 ml over anew.

2.6. Determination the Liver protection ability of plant

For enzymatic examined test, blood had been collected by heart puncture, which had been transported to small tubes (Eppendorf) and a permitted to clot at room temperature 37 °C for fifteen minutes, after that serum was parted by centrifugation at 3,000 rpm for about 10 minutes. The collected serum had been used for the determined of liver enzyme parameters [11], commercial kits (Randox Company) were used then liver was taken for histopathological examination.

2.6.1. Animal's used in study

Six to eight weeks aged Albino male mice 23-25 g, and were taken from the animale house of Biotechnology Research Center, Al-Nahrain University, animals were kept in cages (4 mice in each cage) were separated into three groups as indicated in Table 1.

Table 1 Groups of animals investigated in the Laboratory tests

Type of interaction	Dose mg/kg	number of animals
Distilled water	-----	4
Methotrexate	40	4
<i>Plantago lanceolata</i>	200	4
Methotrexate + plantago plant	40+200	4
Total number of animals		16

2.7. Experimental design

Animals were kept (four mice in one cage) with add libitum contact to water and food pellets. mice have been divided into four groups: The first group was treated with 200 mg/kg of plant extract, the second group was treated with 40 mg/kg of methotrexated drug which was the product of Pharmacia Company (Belgium), the third group was treated with an interaction of 40 mg/kg methotrexate and 200 mg/kg plant extract, and the last group represent the control group (normal mice without any treatment). Each investigated group was injected intraperitoneally (i.p.) with a single dose per day (0.1 mL) of the tested material in 7 days. On day eight of the experiment the animals have been dissected to complete research calculations [12]

3. Results and discussion

3.1. Total flavonoid

Total flavonoids content were spectrophoto-chemically determined in aqueous extract of *P. lanceolata* as rutin equivalent. The aqueous extract was present to contain $317.0000 \pm 9.64365 \mu\text{g/ml}$ flavonoids Such finding is agreement with a study carried out by [13].

Table 2 Total Flavonoid of *Plantago lanceolata* aqueous extract

Total Flavonoid	Mean $\pm$ Std
<i>Plantago lanceolata</i>	317.0000 ± 9.64365

3.2. Active chemical constituent in plant

Determination active constituents of *Plantago lanceolata* aqueous extract showed that chemical composition of plant contain (Tannin, polysaccharide, alkaloid, flavonoid, polyphenol compound) as in table 3.

Table 3 Investigation of Active chemical compounds of *Plantago lanceolata* aqueous extract

Sample	Test name	Reagent	Result
1g. residue in 100ml Ethanol50%	Tannins	Lead Acetate 1% solution	++ve
1g. residue in 100ml Ethanol50%	Polysaccharides	Benedict reagent	++ve
1g. residue in 100ml Ethanol50%	Alkaloids	Dragangroff reagent	++ve
1g. residue in 100ml Ethanol50%	Saponins	Foam formation	-ve
1g. residue in 100ml Ethanol50%	Flavonoids	Alkaline reagent (NaOH)	+ve
1g. residue in 100ml Ethanol50%	Polyphenolic Compounds	ferric chloride 3% solution	++ve

3.3. Reductive ability

At six concentration have been tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml), of *P. lanceolata* aqueous plant extract then absorbance was measured that presented significantly increase in 0.64 mg/ml (0.272467 ± 0.005033) while 0.124167 ± 0.016073 in 0.02mg/ml and such finding showed that the plant aqueous extract is more effective than trolox in the scavenger of free radicals.

Table 4 Reductive ability of *Plantago lanceolata* aqueous extract and trolox (vitamin E)-

Con. (mg/ml)	Abso. (Mean $\pm$ SD)	
	<i>Plantago lanceolata</i>	Trolox (Vitamin E)
0.64	0.272467 ± 0.005033^A	0.211 ± 0.015^A
0.32	0.188167 ± 0.007638^B	0.132 ± 0.007^B
0.16	0.173100 ± 0.008718^C	0.114 ± 0.004^C
0.08	0.158600 ± 0.009539^D	0.108 ± 0.001^{CD}
0.04	0.143980 ± 0.004703^E	0.101 ± 0.001^{CD}
0.02	0.124167 ± 0.016073^F	0.100 ± 0.001^D

4. Hepatoprotective activity

4.1. Histopathological of liver

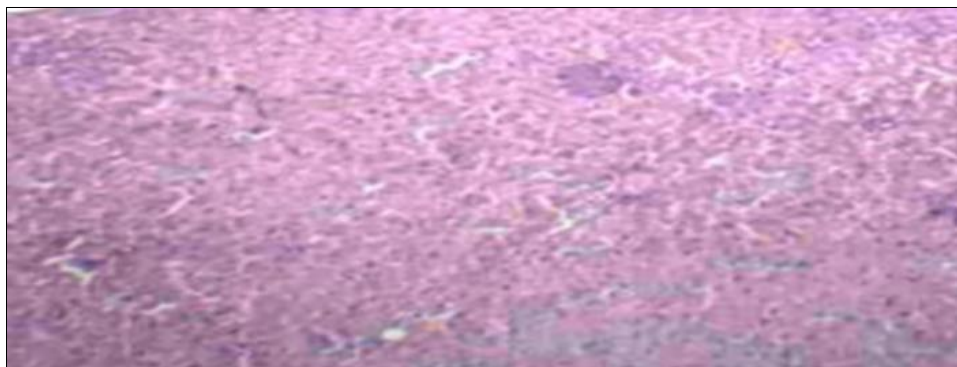


Figure 1 Section showing normal appearance of liver (H & E)(X40)

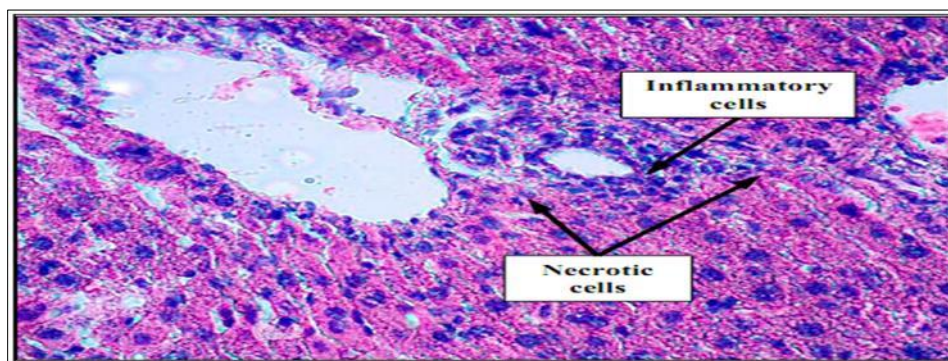


Figure 2 Necrosis is present together with mild inflammatory cell infiltrate (mononuclear cells) and fatty changes in liver tissue of mice treated with methotrexate drug (H & E)(X40)

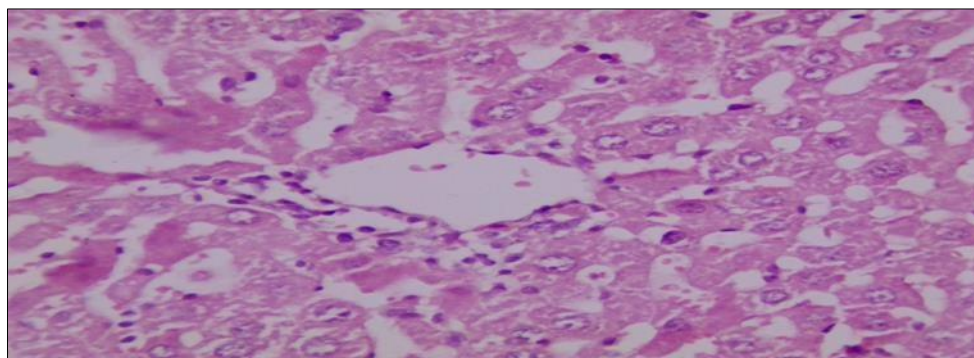


Figure 3 Degenerative changes, dispersed necrosis of hepatocyte, mild inflammatory cells infiltration, sinusoidal dilation and fatty changes in liver tissue of mice treated with plant extract. (H & E)(X40)

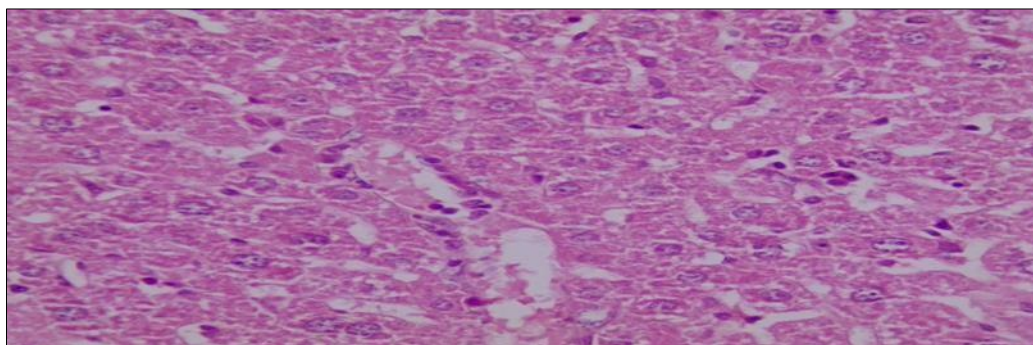


Figure 4 Look like near normal histological structure appearance but still there was dilatation of sinusoid in liver tissue of mice treated with plant extract and MTX. (H & E)(X40)

4.2. Liver enzyme

Table 4 Liver enzymes of mice groups treated with *Plantago lanceolata* aqueous extract and interaction

Groups	GOT	GPT	ALP
Distilled water	396	146	179
Methotrexate	214	77	92
<i>Plantago lanceolata</i>	149	42	118
Methotrexate + plantago plant	305	39	91

Results for liver function enzymes showed the ability of plant to reduce the level of GOT, GPT and ALP. (149, 42 and 118 U/L), when it compare with control (396, 146 and 179 U/L) respectively.

Also, interactions between plant and MTX showed the ability of plant to modulating harmful effects of the drug (305, 39 and 91 U/L) for GOT, GPT and ALP respectively when it compare with control (396, 146 and 179 U/L) respectively.

5. Discussion

Medicinal plants are used for ages to maintain and enhance health of human body infections. This is for “their availability and arguably efficacious state, therefore expect an alternative remedy nowadays.

The flavonoid and phenolic compounds are the chief pharmaceutical components of pharmaceutical plants. These compounds are considered as effective anti-oxidant sources to scavenger of free radicals. Additional studies shown that normal products for example flavonoids and phenolic have showed to be effective free radical scavengers and lipid peroxidation inhibitors [14], and may be, the best designated and best beneficial assets of every group of flavonoids was their capability to work as antioxidants; protective the body of human against Reactive Oxygen Species (ROS) [15]. Therefore, there is increasing attention in the improving health benefits of dietary flavonoids, and the present study existing such interest in such medicinal plant such as *P. lanceolata* for examining their anti-oxidant and radical scavenging activity t, because of its richness in flavonoids and phenolic compounds.

P. lanceolata had in their compounds like apigenin, balcalein and luteolin in addition to their products with the main compounds apigenin 6.8-di-C-glucoside and luteolin-7-Oglucuronide, luteolin-7-O-glucoside and 7-Oglucuronide-3'-glucoside [16]. and among phenolic carboxylic acids could be mentioned: p-hydroxy benzoic acid, protocatechnic, gentisinic, chlorogenic and neochlorogenic acid and others. Inorganic ingredients that contain 1% salicylic acid and salts [17].

The free radicals could be cause diseases by lipid, protein peroxidation or by DNA damage inside the human body, many therapeutic plants extracts have antioxidant activities for scavenging these damaging compounds. It could be avoid free radicals from causes any disease in human. Phenols compounds that had been presented; in a high quantity in plants and showed antioxidant activity [18].

Phenolic compound such as phenolic acids, flavonoids, lignin and tannins that are general compounds found in plant parts such as leaves, flower tissues, had strong antioxidant activity found in these plant had their ability to scavenger free radicles through its reductive activity [19].

Hepatoprotective effects considered as the main source of human deaths in the world, aqueous plant extract of *P. lanceolata* seed, enhance the hepatoprotective effect had been done by the levels of liver enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) showed reduced [20].

A number of therapeutic plants have active elements and crude herbal extracts were investigated to test their possible protective probable activity against MTX (Methotrexate) induced renal and hepatic side effects disorders. Like (Rhein, chicoric acid, inulin, gallic acid, disomin, berberin) and other plant extracts were tested as an method preventing MTX toxicity by antioxidant and anti-inflammatory pathways [21]. The results of the present study showing that *Plantago lanceolata* aqueous extract which had the activity in antioxidant and anti-inflammatory could effectively reduce MTX-induced hepatic injuries in mice.

6. Conclusion

Plantago lanceolata considered as an herbal promising plant that have anti-oxidant ability for scavenging free radical in all concentrations tested, also the aqueous plant extract had different chemical composition of secondary metabolites that contain Tannins, polysaccharide, Alkaloids, Flavonoids and Polyphenolic Compounds. The plant extracts counteract the damage caused by methotrexate drug through the reduction in liver enzymes GOT, GPT and ALP in all groups of mice the results of interactions indicated the ability of plant to modulating harmful effects of the drug in liver and its enzymes,

Compliance with ethical standards

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Disclosure of conflict of interest

The author declare that they have no conflict of interest.

Statement of ethical approval

All procedures performed in studies involving animals participants were in accordance with the ethical standards of the institutional research and with the comparable ethical standards.

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