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Estimation of total flavonoid, antioxidant and lung protective activity of *Plantago ethanolic* extract on albino male mice

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Abstract

The impartial of this research was to determine whole flavonoid content (TFC) based on flavonoid-aluminum chloride ($AlCl_3$) and antioxidant action by spectrophotometric method and resolve of lung protective ability of *Plantago* plant. One of these plant *Plantago*. Results indicated that *Plantago* possesses great flavonoid contents which was (281± 0.210 µg/ml) in addition to incredible antioxidant potential in a concentration dependent manner (ranged from 0.150 to 0.538 for 0.02 and 0.64 mg/ml respectively for reductive ability and possessed lung protective ability by counteract damage induced by CCL_4 treated albino male mice.

Keywords: *Plantago*; Antioxidant; Flavonoid; Ethanolic extract; Albino male mice; Health care; Medicinal plant; Free radical; Secondary metabolite.

1. Introduction

Traditionally, consuming the medicinal plants for therapeutic of respiratory diseases for many years ago (1). Investigators have been informed that ginger plants could be active in treating asthma. Plantain distributed all completed the planet and is one of the most plentiful accessible medicinal herbs (2). Plantago lanceolata L. (Plantaginaceae) is a recurrent plant species with a worldwide distribution. It has many bioactive composites containing allantoin, aucubin, ursolic acid, flavonoids, and asperuloside.

Many technical studies also report that the plantain extract has a broad variety of biologic effects including "damage therapy action, anti-inflammatory, analgesic, antioxidant, weak antibiotic, immunomodulating, and anti-ulcerogenic activity" (3). Iridoid Glycosides are a class of monoterpene-developed combinations that have been documented in over 50 plant families (4). The major Iridoid Glycosides found in P. lanceolata are catalpol and its precursor aucubin (5).

The leaf was conventionally used to cure injuries, including respiratory infections combined with fever. This potted plant contains of phenol mixtures and has antioxidant estate (6). The valuable within cure of the soreness of the higher site of respiratory system because of containing Tanen and Mucilage. (7). Plantago major,) along with certain polysaccharides enhances damage. Study gets indicated that can be helpful in the medication chronic bronchitis (8). Numerous healing belongings involving beneficial impacts on gastrointestinal, bloodline and respiratory (asthma and dyspnea) ailments consume been described for the *Plantago lanceolata* in (9). So it is used within to overpower coughs related to bronchitis and upper respiratory inflammation, to decrease skin inflammation, medication of injuries and equally a laxative (10).

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2. Material and methods

2.1. Plant collection, identification and extraction

Plants aerial parts (leaves) were supplied from the local markets of Baghdad during Sep. / 2021 and recognized by Dr. Ibrahim S. Al-Jubouri, College of Pharmacy, and Al-Mustansiriyah University, Iraq. Briefly, after plant parts cleaned and dried at shade for a week. Then, via electrical grinder the plant grinding and stored in air tight container and maintained at room temperature until used. The method of (11) were adopted in which about 50 grams of the plant leaf were extracted with 80% methanol (vol: m250 ml) at 65°C for 3 hours using the soxhlet apparatus then dryness in a rotary evaporator to yield dried crude extract.

2.2. Dose of *Plantago* in mice

In albino male mice. A dose of 400 mg/kg was tested depending on LD50 of *Plantago lanceolata* to 2,940 mg/kg.

2.3. Research laboratory Animals

Biotechnology Research Centre (Al-Nahrain University) supplied the animals (Albino male mice).

2.4. Experimental Design

Experimental designs were divided into two groups, as explained below, (4 animals per group)

- **Group I:** Mice were managed with *Plantago* ethanolic extract at dose of (400mg/kg).
- **Group II:** Mice were administrated with the CCL_4 (7th days) at dose of (40mg/kg).
- **Group III:** Mice were administrated with CCL_4 (1st and 2ed days) + *Plantago* ethanolic extract (from 3rd to 7th day).

The mice were injected intraperitoneally (IP) as a single dose (0.1ml) per a day for 7 days using plant extract and sacrificed in day 8.

2.5. Determination of Total Flavonoids

Total flavonoids substance was determined using spectrophotometer in methanolic extract of plantago as rutin (as standard) followed method of (12). In this assay methanolic extract at weight (3.2 mg) was dissolved in 5 ml of 50% methanol, then 1 ml of a 5% (w/v) sodium nitrite and 1 ml of a 10% (w/v) aluminum chloride solution was added after 5 and 6 minutes respectively. Finally 10 ml of a 10% (w/v) NaOH solution was added. The mixture was completed to 50 ml with distilled water. The absorbance was measured at 450 nm with a spectrometer after 15 min. same procedure followed to create equation of the standard curve.

2.6. Assessment of Anti-oxidant Activity *in vitro* (reductive ability)

The method described by (11) was adopted, briefly 1 ml of different plant concentrations (from 0.02 to 0.64 mg/ml) mixed with 1ml of 0.2M phosphate buffer (pH 6.6) and 1.5 ml of 1% potassium ferricyanide, and then after incubation for 20 min at 50°C. 1ml of 10% TCA was added to the mixture and centrifuged for 10 minutes and 2.5 ml of the supernatant was mixed with 2 ml of distilled water and 0.5 ml of freshly prepared 1% Ferric chloride. After that, the absorbance was measured at 700nm. Trolox used as control by following same method to estimate the reductive ability.

2.7. Lung Tissue Preparation for Histology Preparation

Lung of mouse are set for histopathologically learning by way of termed (10). Sections remained stable in (10%) formalin about 24h, afterward dehydration through measured series of alcoholic liquid ranged from (30-100%) for (5) min apiece. Formerly the tasters cleared in xylene and paraffin wax. Cross sections of (5)µm fatness are ready and stained with hematoxylin (Harison) and eosin rendering to typical technique. (Ibrahim *et al*., 2017).

3. Results

3.1. Total flavonoid of *Plantago*

Total flavonoids content in methanolic extract of plant was (281± 0.210 µg/ml) flavonoids.

3.2. Reductive ability

In all concentration tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml), plant possessed more reductive ability than trolox as shown in table 1 below (ranged from 0.150 to 0.538 for 0.02 and 0.64 mg/ml respectively).

Table 1 Reductive ability of *Plantago* ethanolic extract and Trolox (vitamin E)

Concentration (mg/ml)	**Reductive Ability Absorbance (Mean ± SD)**	
	***H. Plantago ethanolic* Extract**	**Trolox (Vitamin E)**
0.02	0.150 ± 0.010	0.100 ± 0.001
0.04	0.292 ± 0.007	0.101 ± 0.001
0.08	0.351 ± 0.007	0.108 ± 0.001
0.16	0.460 ± 0.013	0.114 ± 0.004
0.32	0.488 ± 0.016	0.132 ± 0.007
0.64	0.538 ± 0.013	0.211 ± 0.015

3.3. Lung histological section result

In mice treated with **plant extract**, Section showing widening of white pulp and reduction of red pulp (H & E) (x40).figure 1

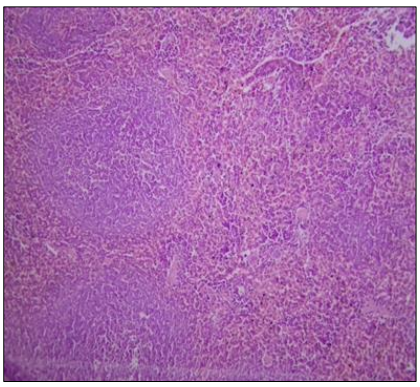

Figure 1 Widening of white pulp and reduction of red pulp

The results of mice treated with CCL4 indicated the ability to cause damage to organ tested. Histological examination of the CCL4 group showing interstitial lymphocytic inflammation and interstitial fibrosis and hyperplasia was also detected in the tissue (x200).figure 2

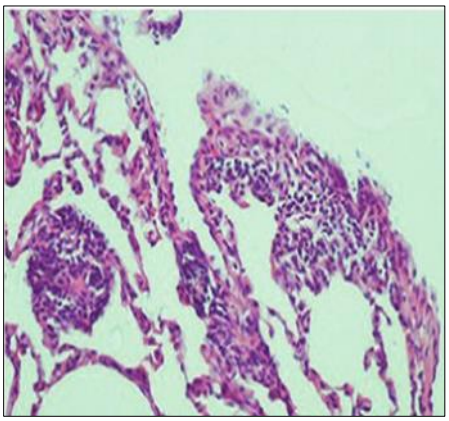

Figure 2 Interstitial lymphocytic inflammation and interstitial fibrosis and hyperplasia.

The results of interaction group Section of the lung showing thickening of alveolar septa with congestion, and mild inflammatory cells infiltration and destruction of alveolar septa forming the beginning of emphysema structure (H & E)(X40).figure 3

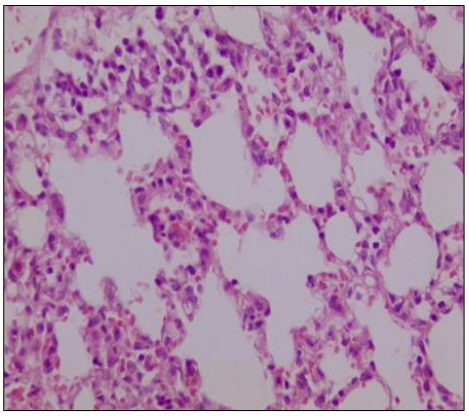

Figure 3 Formation of emphysema structure

4. Discussion

Alcoholic extract of Plantago major is anti-phlogistic in carrageenan and pge1- stimulated in Flammarion's in mice (13). Aceteoside, the most important phenyl ethanoid that prevents ara-chidonic acid stimulated mouse ear oedema (14). Therefor it can inhibit oedema in wound .Compounds such as the iiridoid glyciosides auccubin and catallpol, the aglycone aucubiigenin, and caffeiic acid products containing plant amajoside and acetonidethat have been remote from Plantago spp. and have established antimicrobial activity thus Plantago plant extracts can decline germ capability in wound site which is a suitable propensity to increase speed damage treatment.

P. major is one of the greatest plentiful and broadly distributed medicinal crops in the world. The dynamic chemical ingredients are aucubin (an anti-microbial agent), allantoin which stimulates cellular growth and tissue regeneration, and mucilage which reduces pain and discomfort (15). Composed, these components are supposed to provide plantain mild anti-inflammatory, antimicrobial, antihemorrhagic, and expectorant actions (16). In this research, in the group treated with the extract of Plantago due to allantoin constituents, goblet cells in bronchioles were improved and regenerated. Additionally, due to mucilage compounds in Plantago, the mice of lymphoid cells and nodules were small and decreased. Moreover, the thickness of epithelium was reduced and enhanced (17).

5. Conclusion

The present study explained the ability of plant Plantago to scavenge potassium fericynide free radical in a concentration dependent manner. In addition to ability of plant to counteract the damage to lung caused by toxic compound CCL4 and all these biological activities attributed to plant flavonoid content and many other secondary metabolites presented in plant.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There was no conflict of interest.

Statement of ethical approval

This work done after taking a permission from the head of animal laboratory house\Biotechnology Research Center\Al-Nahrain University \Baghdad\Iraq.

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