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Hepatoprotective Activity of *Plantago Lanceolata* Aqueous Extract on Albino Female Mice

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Abstract

This study gives an overview of the widely distributed herb plantain *Plantago lanceolata* and aqueous extraction of the plant, by study the effect of *Plantago lanceolata* aqueous extract on the hepatoprotective activity in albino female mice through measuring the three main liver enzymes aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) with the histological effect of extract on liver. Mice were separated into eight groups treated intraperitonially for seven days: (1) control, (2) CCl4 (0.02%), (3) aqueous extract (250 mg/kg), (4) aqueous extract (500 mg/kg), (5) CCl4 (0.02%) plus aqueous extract (250 mg/kg), (6) CCl4 (0.02%) plus aqueous extract (500 mg/kg),(7) aqueous extract (250 mg/kg) plus CCl4 (0.02%), and (8) aqueous extract (500 mg/kg) plus CCl4 (0.02%). Mice were killed, then the liver were histopathological examined and the serum levels of (AST), (ALT) and (ALP) tested.

The results of histopathology indicated that CCl4 cause severe damage to the mouse liver tissues, however, the protective effects of aqueous extract on liver were observed when CCl4 combined with it. The analysis of the serum indicated a significant increase in the values of AST, ALT and ALP in mice treated with CCl4 and pre and post treatment of the animals with the CCl4.

Keywords; *Plantago lanceolata,* AST, ALT, ALP.

Introduction:

Plants were widely used as feed, food and experimental medicinal reasons associated the development of human civilization [1]. Develop of recent medicine led to the loss a lot of ethnomedicinal information, coupled with the dissimulation or the minimization of the growing region for useful plant in medicine [2]. Among these medicinal plants is the plant of a research subject, *Plantago lanceolata* from the plantaginaceae family, which is an herb originally grown in pastures to feed animals, or in good fields for cultivation to manufacture the drugs, medicinal uses to make a cough-reducing syrup or make herbal sweets. [3]. This plant is original to Eurasia; but it has also naturalized in Australia, North America, New Zealand, South America, Hawaii, Southeast Asia, and eastern Africa [4]. It is deserves noting that *Plantago lanceolata* is currently used for medical uses [5], with different applications in many aspects, nowadays about 45 patents are registered depended on the activity of plantain leaves, but still, a few of them are in use [6]. The most important plant parts in medicine are shredded or husks, or completely dried of leaves [7]. The plant extract obtained from narrow leaf, the herb is a source of many active components, and the most important active components of them are flavonoids, phytosterols, glycosides, phenylethanoid, and iridoids, in addition to tannins, organic acids, mucilaginous substances, pectins, and mineral salts [8]. Flavonoid is the most important secondary metabolites having potent antioxidant effects [9,10]. The *Plantago* spp are rich in many form of iridoids, such as catalpol, asperuloside

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,aucubin , and flavonoids like scutellarein and apigenin-7- O-glucoside [11]. The plant extract prevented the increasing of plasma and evidence of lipid peroxidation, in addition to the levels of liver enzyme function of aspartate transaminase (AST) and alamine transaminase (ALT). Such findings improved the plant extract constituents that may have an active hepatoprotective activity [12].

Materials and Methods

Plantago lanceolata gathering and Identification

Plantago lanceolata classified by Dr. Ibrahim S. Al-Jubouri, College of Pharmacy, Al-Mustansiriyah University, which was collected in August 2021.

Preparation of plant aqueous extract

The leaves of the plants were minced after drying, and 150 grams of them were extracted for four hours in 250 ml of distilled water by the apparatus of Soxhlet. A water bath (45°C) was the source of heating. Then the obtained leaf extract solution put in plates and incubated in the oven at 37°C to prepare plant extract powder. This powder was dissolved in distilled water to prepare the required concentration for the laboratory mice [13].

Dose of plant aqueous extract

In albino female mice, two doses were used depending on plant LD50, the first dose of plant extract (250 mg/kg), while the second increased to (500 mg\kg).

Dose of drug carbon tetrachloride (CCL4)

In albino female mice the only used dose of the drug was (0.02%) .

Animal acclimatization

Swiss female white mice were supplied from the animal house of the University of AL-Nahrain, Research center of Biotechnology. The ages ranged from (8-12) weeks, weight ranged from 23-27g. Mice divided into 8 groups in a plastic cage (6mice/cage), with water and standard pellets.

Scheme of Experiment

Mice were divided into eight groups. The first one was negative group that treated with distal water, Group 2 taken a only dose of the drug CCl4 intraperitonially (i. p). for a 7days. Group number 3 and number 4 were taken 250 mg/kg and 500 mg/kg of plant extract i.p. for a week, respectively. Group number5 and number 6 on the first day, drug CCl4 was taken, posttreatment with 250 mg/kg and 500 mg/kg of plant extract i.p. for the remaining six days. Group 7 and group 8 treated with 250 mg/kg and 500 mg/kg of plant extract i. p. for six days then treated with CCl4 (i. p). for the last day. The groups were treated i. p. with (0.1 ml) of the dose. Before sacrificing, blood was collected by heart to obtain the serum for enzyme estimation. After that, the mice were killed to assessment liver histological segments.

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Hepatoprotective Effects Assessment

The effects evaluated after stimulating the damage with the drug CCL4. Assessment parameters were liver enzymes function in serum, in addition to histopathological examination of liver tissue to indicate the damage.

Evaluation of Alanine Aminotransferase(ALT), Aspartate Amino-Transferase(AST) and Alkaline Phosphatase (ALP).

Liver enzymes activities, AST, ALT and ALP were detected in serum of the mice by kit [14]. (Randox Company).

Assessment of Liver Histopathological

The liver fixation was in (10%) of formalin for two days. The process started with sample washing using ethanol 70% overnight and then dehydration with ethanol at various of concentrations. liver sample put in paraffin-xylene for half hours at 57-58ºC, followed by using the wax of paraffin alone for 2 hours at temperature 60-70ºC. Embedding the liver sample in pure of wax of paraffin which melting at temperature: 60-70ºC and leave to be solid at temperature of room. The paraffin block was sectioned using microtome with 5 microns thickness, then transferred the sections to a slide covered with Mayer's albumin. Slide was put in a water bath at temperature (35-40ºC). Followed by staining and cleared with xylene for ten minutes, then the slide was mounted using a Canada balsam and covered by cover slip to be prepared for microscopical examination [15].

Statistical analysis:

One mode examination of variance ANOVA (Duncan) was made to test whether group alteration was important or not, statistical significance was defined as ($p \leq 0.05$). Data were expressed as mean± standard error and statistical significances were carried out using Graph Pad Prism version 6 (Graph Pad Software Inc., La Jolla).

Results

Enzyme Estimation of **Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP)**

The level of AST in negative control of mice was (18.21±3.0) Unit/L, while the value of ALT was (30.04±2.4) Unit/L, and for ALP was (35.00±2.08) Unit/L. After, *Plantago lanceolata* aqueous extract treatment (250 mg/kg), the level of AST was (19.8±2.6) Unit/L, ALT was (31±2.6) Unit/L, and ALP was (35.6±3.05) Unit/L. These values increased to (20.4±4.1) for AST, (32.6±2.0) for ALT and (36.6±3.4) for ALP after treatment with aqueous extract (500 mg/kg). positive treatment which represented by ccl4 increased all these values to (57.1±1.3) for AST, (97.2±2.4) for ALT and (93.11±1.3) for ALP. In interaction groups between aqueous extract (250 mg/kg) with ccl4 and aqueous extract (500 mg/kg) with ccl4, the level of AST was (35±5.03) Unit/L (33±2.5) Unit/L respectively, while the activity of ALT was (80.3±4.0) Unit/L (70.3±2.6) Unit/L, and the level of ALP was (88.3±3.7) Unit/L

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(85±2.6) Unit/L. In interaction groups between ccl4 with plant extract (250 mg/kg) and ccl4 with plant extract (500 mg/kg), the level of AST was (30.5±3.5) Unit/L (28.3±4.5) Unit/L respectively, while the level of ALT was (73±3.6) Unit/L (66±3.6) Unit/L, and the level of ALP was (66±3.6) Unit/L (66±3.6) Unit/L

(Table 1).

Table 1. Effects of *Plantago lanceolata* aqueous extract and carbon tetrachloride (CCL4) on Aspartate Aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in mice serum.

Groups	Dose (mg/kg)	AST (mean ±S.E) (Unit/L)	ALT (mean ±S.E) (Unit/L)	ALP (mean ±S.E) (Unit/L)
Negative Control		18.21 ±3.0d	30.04 ±2.4e	35.00±2.08d
Drug	0.02%	57.1±1.3a	97.2±2.4a	93.11±1.3a
Plant extract	250 mg/kg	19.8±2.6d	31±2.6e	35.6±3.05d
Plant extract	500 mg/kg	20.4±4.1d	32.6±2.08e	36.6±3.4d
Drug +Plant	250+0.02%	30.5±3.5bc	73±3.6c	83.3±3.5b
Drug +Plant	500+0.02%	28.3±4.5c	66±3.6d	78.3±4.04c
Plant+ Drug	0.02%+250	35±5.03b	80.3±4.0b	88.3±3.7b
Plant+ Drug	0.02%+500	33±2.5b	70.3±2.6c	85±2.6b

Different letters: Significant difference ($P \leq 0.05$) between means

Histopathological Changes in Liver

- **Negative Control**

Normal structure appearance of hepatic tissue which consist of central vein with thread like arrangement of hepatocyte cells.

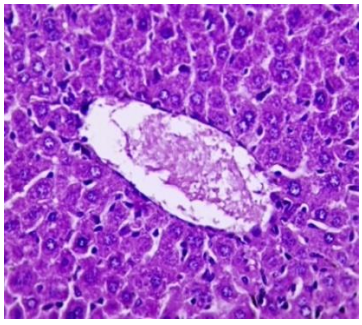

Figure 1. Section showing negative control with normal liver area section H& E, (40X)

- **carbon tetrachloride (CCL4)**

Section showing focal area of necrosis, inflammatory cells infiltration with necrotic hepatic cells.

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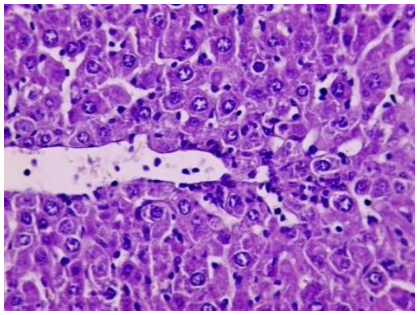

Figure 2. Histopathological section showing liver from treated mice by CCL4 showed focal area of necrosis, inflammatory cells infiltration with necrotic hepatic cells (X40)

(H & E).

- **After treated with *Plantago lanceolata* aqueous extract (250 mg/kg)**
  No changes in histological were seen in the liver of *Plantago lanceolata* extract treated group, just a few cells with mild depletion of glycoprotein.

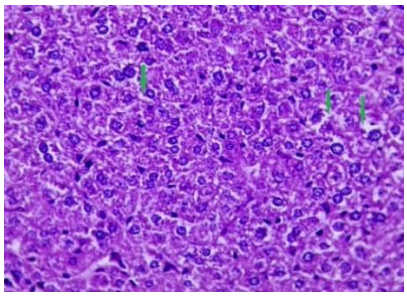

Figure 3. The liver tissues of treated mice with *Plantago lanceolata* aqueous extract showed a few cells with mild depletion of glycoprotein (X40) (H & E).

- **After treated with *Plantago lanceolata* aqueous extract (500 mg/kg)**
  structure appearance looks like structure of hepatic tissue of the liver

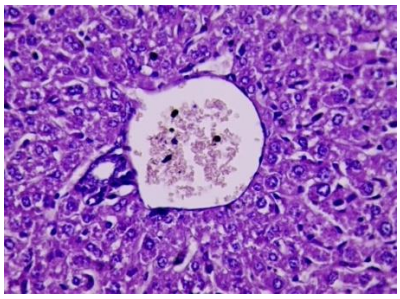

Figure 4.The liver tissues of treated mice with *Plantago lanceolata* aqueous extract showing look like appearance structure of hepatic tissue (X40) (H & E).

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- **After treated with CCl4 1 day + (250 mg/kg) of extract for 6 days**
  Liver showed mild depletion of glycoprotein, and a few cells with fatty changes

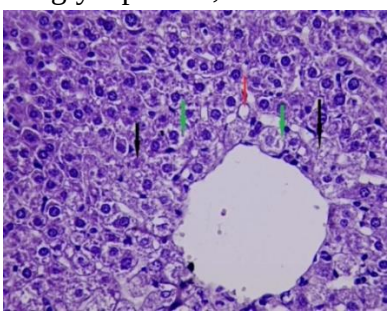

Figure 5. Section showing mild depletion of glycoprotein, few cells with fatty changes (X40) (H & E).

- **After treated with CCl4 1 day + (500 mg/kg) of extract for 6 days**
  Histopathological in the liver of treated mice look like normal histological structure appearance of hepatic tissue

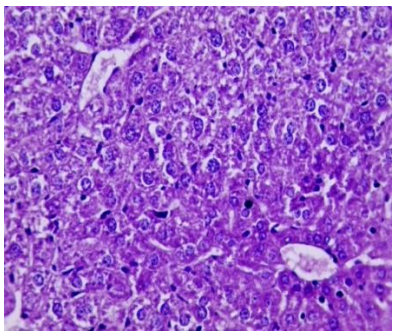

Figure 6. Section showing look like normal histological structure appearance of hepatic tissue (X40) (H & E).

- **(250 mg/kg) of extract for 6 days +CCl4 1 day**
  Liver showing few hepatic cells with mild depletion of glycoprotein

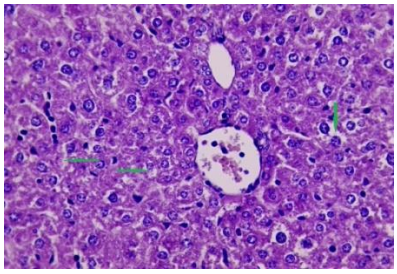

Figure 7. Section showing few hepatic cells showing mild depletion of glycoprotein. (X40) (H & E).

- **After treated with (500 mg/kg) of extract for 6 days +CCl4 1 day**
  In this treated group there is a wide area of hepatic cells showing depletion of glycoprotein

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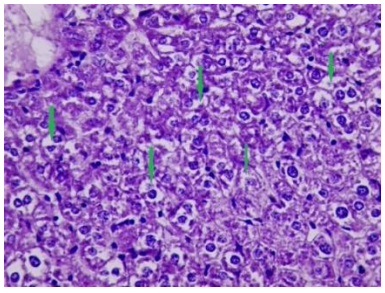

Figure 8. wide area of hepatic cells showing depletion of glycoprotein (X40)(H & E).

Discussion

In human body the principally place of detoxification and the major target of drug exposure is liver, drugs high levels led to different hepatic disorders by causing reactive oxygen species (ROS), that perfect to cause cellular injury by affecting different biomolecules of cells, such (proteins, lipids, and DNA) [16], also increased (ALP, ALT and AST) [17].Treated mice with carbon tetrachloride drug only showed cellular necrosis in the tissue section, and an increase in fat and infiltration of inflammatory cells appeared with necrotic hepatocytes, because this drug is one of the toxins that affect rapidly on the liver in humans. When the liver organ is exposed to the drug for 30 minutes, it leads to changes In fat metabolism. While the exposed for longer periods leads to tissue changes, decomposition of fats and necrosis, effects on the nervous system, and increase in liver enzymes levels[18],in addition to damage the double layer of phospholipids in the membranes and cirrhosis of the liver due to the drug carbon tetrachloride (CCL4), which causes an increase in the level of glucose in the blood, and the enzyme glucose-6-phosphate is reduced due to microsomes due to the toxicity of this drug and a decrease in the amount of total protein[19]. High dose of ccl4 drug appeared more damage, including increased liver weight, hepatic lipolysis, high cholesterol, growth retardation and weight loss [20]. The plant extract prevented the appearance of necrosis in the liver cells, as well as preventing the rise of malondehyde in the plasma and liver because malondehyde is evidence of lipid peroxidation as well as the levels of liver enzymes ALT, AST, and ALP were lower, and this confirms that the plant aqueous extract has an important role in protecting the liver and protecting it from toxins[21].The plant contains biologically active compounds such as polysaccharides, tannins, flavonoids, tannin, saponin, and glycosides and these compounds have been proven by studies, through the detection of chemical analysis of the plant [22], In addition to the presence of vitamins such as vitamin C [23]. a role in immune stimulation and an increase in the number of white blood cells and has a positive effect on lymphocytes, neutrophils, macrophages and numerically or functionally fatal [24].Such finding agree with the results of Shalash and Hussein (2020),who confirmed that the effect of CCL4 caused severe damage to tissue of the testes of mice[25]. This drug CCL4 causes diseases of the liver and other organs.

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