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Kidney protective and burn healing effect *Plantago lanceolata* aqueous extract on albino male mice

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Abstract

This study aimed to in vivo evaluate the effect of *Plantago lanceolata* aqueous extract on kidney function test (total serum protein, urea and creatinine) and anti-inflammatory effect through determined the day require to heal burn in mice skin.. *Plantago lanceolata* is a species of flowering plant in the Plantaginaceae (plantain) family, *Plantago lanceolata* seems to be a very versatile in addition to the medicine cabinet being an antifungal, antioxidant, analgesic and many other medicinal application. Two design of mice groups used: one for kidney protective and other for anti-inflammatory activity. In kidney protective activity eight mice groups were assessed: group I: mice treated with first plant dose (500 mg\kg) , group II: mice treated with second plant dose (250 mg\kg), Group III :negative control; mice administrated with distill water, Group IV: mice were administrated with CCL_4, Group V: An interaction between CCL_4 and first dose of plant extract (0.02 mg\kg CCL_4+ 500 mg\kg plant dose), Group VI: An interaction between CCL_4 and second dose of plant extract (0.02 mg\kg CCL_4+ 250 mg\kg plant dose), Group VII:: An interaction between first dose of plant extract and CCL_4 (500 mg\kg plant dose +0.02 mg\kg CCL_4), Group VIII: An interaction between second dose of plant extract and CCL_4 (250 mg\kg plant dose +0.02 mg\kg CCL_4). While for anti-inflammatory activity, three mice groups were assessed: Group I: Mice without any treatment, Group II: Mice were administrated with *Plantago lanceolata* and Group III: Mice were administrated with silverin cream of burns. The results showed that CCL_4 drug produced an experiential decrease in protein concentration (3.76±0.15 g\dL) in contrast with negative control (5.40±0.26 g\dL), while direction of plant and plant with CCL_4 at all doses tested instigated an increase in total protein concentration (7.46±0.40, 6.43±0.41, 6.43±0.41 and 5.60±0.73 g\dl) for groups (V, VI, VII and VIII respectively).Creatinin also match other results of kidney function by increasing their concentrations in mice treated with CCL_4 (0.80±0.10 mg\dL) as compared to negative control (0.3±0.1 mg\dl) while plant extract showed the ability to cause reduction in a concentration in a dose dependent manner and according to deferent treatments (0.60±0.10, 0.62±0.15, 0.36±0.05 and 0.50±0.10 mg\dl) for (group V,VI,VII and VIII respectively). The results of anti-inflammatory activity declared the ability of plant to heal burn in 14 days as compared to negative and positive controls which require 28 and 18 days) respectively. All obtained results may be attributed to active secondary metabolite presented in plant like tannin, mucilage and silicic acid etc.

Keywords: *Planntago lanceolata*; Anti-inflammantory; Ura; creatinine; Total serum protein

1. Introduction

Medicinal plants are secondhand wide-reaching to cure various infections. They are abundant suppliers of phytochemicals with prospective curative influence in medications using immediate application of natural material. Moreover, they also play a role for the advancement of new therapeutic drugs (1).

Plantago lanceolata is one of the greatest abundant and extensively spread medicinal plant in the world. It is a persistent plant species that goes to the genus Plantago and the family Plantaginaceae. *Plantago lanceolata* is an herbal plant that

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goes to the Plantagiaceae family with broad geographic distribution in the world. It certainly spreads in Asia and Europe. This plant has revealed to contain 5 groups of biochemically effective compounds (2).

Plantago lanceolata leaves are described to have antifungal, anticancer, anti-inflammatory, analgesic, antioxidant, and wound healing that useful for repair tissue, it is common in conventional medicine for wound healing as well as care for diseases associated to skin, infection, cancer, and pain (3). Also, the traditional requests, it has been medicinal properties such as antiulcerogenic, anti-inflammatory and antioxidant, and anticarcinogenic activities (4). *Plantago lanceolata* leaves possess numerous bioactive compounds such as flavonoids, terpenoids, pectin, iridoid glycosides, and tannins which express anti-inflammatory and antioxidant activities (5,6).

The objective of current study was to investigate the potential protective consequences of *Plantago lanceolata* on CCL_4-induced nephrotoxicity in the mice kidney and its effects as anti-inflammatory.

2. Materials and Methods

2.1. *Plantago lanceolata*

Plant aerial parts (leaves) were collected during the period of September 2021 from Erbil\city at Iraq north, and then recognized by Dr. Ibrahim S. Al-Jubouri, College of Pharmacy, Al-Mustansiriyah University, Iraq.

2.2. Preparation of Plant Extract

Plantago lanceolata aqueous extract was ready rendering to method described by (7), briefly about 50 g of plant were located in a clean flask and 250 ml of distilled water supplementary to it followed by putting it in the shaking incubator at a temperature (40 C°) for a period of 3hours, then filtered and distributed on plates. After drying in the oven at 37 C° to collect dry powder, each sample was placed in closed tubes, and putted in refrigerator at 4 C° until use in biological application.

2.3. Dose of plant

In this study two designed protocol were adopted and according to that different plant doses were used (for kidney protective activity 250 and 500 mg\kg were used) while for anti-inflammatory activity in albino male mice, A dose of 400 mg/kg was tested depending on LD50 of *Plantago lanceolata* to 2,940 mg/kg .

2.4. Laboratory Animals of Research

Albino male mice (*Mus muscles)* were the laboratory animals which supplied from the Biotechnology Research Centre (Al-Nahrain University). Their ages at the first of research were eight-ten weeks, and their weight was twenty three – twenty seven grams.

2.5. Experimental Design

2.5.1. Kidney protective activity

In this experiment, mice groups were divided into eight groups, as shown below, in which each group contain 4 animals (total no.32 mice)

- Group I: Mice were injected with *Plantago lanceolata* aqueous extract at dose of (500mg/kg).
- Group II: Mice were injected with *Plantago lanceolata* aqueous extract at dose of (250mg/kg).
- Group III :Mice were administrated with distill water
- Group IV: mice were administrated with CCL_4.
- Group V: An interaction between CCL_4 and first dose of plant extract (0.02 mg\kg CCL_4+ 500 mg\kg plant dose)
- Group VI: An interaction between CCL_4 and second dose of plant extract (0.02 mg\kg CCL_4+ 250 mg\kg plant dose)
- Group VII:: An interaction between first dose of plant extract and CCL_4 (500 mg\kg plant dose +0.02 mg\kg CCL_4)
- Group VIII: An interaction between second dose of plant extract and CCL_4 (250 mg\kg plant dose +0.02 mg\kg CCL_4)

The mice were injected intraperitoneally (IP) as a single dose of plant extract (0.1ml) per a day for 7 days. In day 8, the mice sacrificed. Groups (V and VI) mice injected with CCL_4 at first day and from (2-7 day) mice injected with plant extract at dose (500 and 250 mg\kg respectively) while mice groups (VII and VIII) mice injected with plant extract at dose (500 and 250 mg\kg) at first day then CCL_4 from (2-7 day respectively). The parameters of assessment were kidney function test (urea, creatinin and total serum protein). Beforehand losing the mouse, blood was saved by applying heart puncture, also transferred to Eppendorf tube and permitted to clot at room temperature for 15 minutes, and then serum was separated by centrifugation at 3000 rpm for 10 minutes. To carry out these assays in mouse serum, methods described by Ref. [8] was followed and a commercial kits (Randox Company) ready to use were adopted.

2.5.2. Anti-inflammatory activity

In this experiment, the ability of plant extract at dose 400 mg\kg to heal burn was assessed. The animals were divided into three groups:

- Group I: Mice without any treatment (negative controls)
- Group II: Mice were administrated with *Plantago lanceolata* .
- Group III: Mice were administrated with commercially cream of burns (silverin Switzerland), which supplied form pharmacy of Baghdad.

Mice hair were removed hair and flame was used to induced burns of skin, then calculated the recovery days by determining number of days required to heal the wound (Tian et al., 2007). In this method mice monitor until full recovery is gained. The total number of mice in this stage was 9 animals.

2.6. Statistical Analysis

Data in this study were presented as mean±SD. The statistical programme GraphPad Prism version 5.01 (GrapgPad software, Inc., La Jolla, CA, USA), also statistical analysis system—SPSS version 14 was used.

3. Results

Concerning whole protein, CCL_4 drug produced an observed reduction in protein concentration (3.76±0.15 g\dL) in contrast by negative control (5.40±0.26 g\dL), while direction of plant and plant with CCL_4 at all doses tested produced an increasing in whole protein concentration (7.46±0.40, 6.43±0.41, 6.43±0.41 and 5.60±0.73 g\dl) for groups (V, VI, VII and VIII respectively).Creatinin likewise match other results of kidney function by increasing their concentrations in mice treated with CCL_4 (0.80±0.10 mg\dL) as compared to negative control (0.3±0.1 mg\dl) while plant extract showed the ability to cause reduction in a concentration in a dose reliant on way and according to deferential actions as shown in table (1) .

Table 1 Effect of *Plantago lanceolata aqueous* extract and CCL_4 on biochemical parameter function in albino male mice

Groups	Total serum protein(g\dl)	Urea (mg\dl)	Creatinine (mg\dl)
Plantago lanceolata (500 mg\kg)	5.70±0.20	45.33±4.50	0.3±0.1
Plantago lanceolata (250 mg\kg)	5.36±0.30	42.01±2.64	0.16±0.11
Negative control	5.40±0.26	36.46±3.21	0.3±0.20
CCL_4 (0.02 mg\kg)	3.76±0.15	66.56±2.08	0.80±0.10
CCL_4+ *Plantago lanceolata* (500 mg\kg)	7.46±0.40	57.01±2.22	0.60±0.10
CCL_4+ *Plantago lanceolata* (250 mg\kg)	5.43±0.41	44.61±4.61	0.62±0.15
Plantago lanceolata (500 mg\kg)+ CCL_4	6.43±0.41	36.32±3.05	0.36±0.05
Plantago lanceolata (500 mg\kg) + CCL_4	5.60±0.73	42.36±2.51	0.50±0.10

3.1. Anti-Inflammatory results (Burns Healing Effect of plant extract)

The ability of *Plantago lanceolata* , sliver sulfadiazine (positive control) and negative control to heal the burns estimated by determining days required for recovering. The results showed that aqueous extract of plant *had* the ability to heal

burns in 14 days as compared to sliver sulfadiazine (18 day) and negative control which require (24 days) to heal the burns (Table2) and (Fig 1).

Table 2 The recovery of burn healing in mice after different treatments

Groups	Treatment	Period of recovery
1	Mice without treatment	Days 25
2	aqueous extract of *Plantago lanceolata*	Days 14
3	Sliver sulfadiazine	Days 18

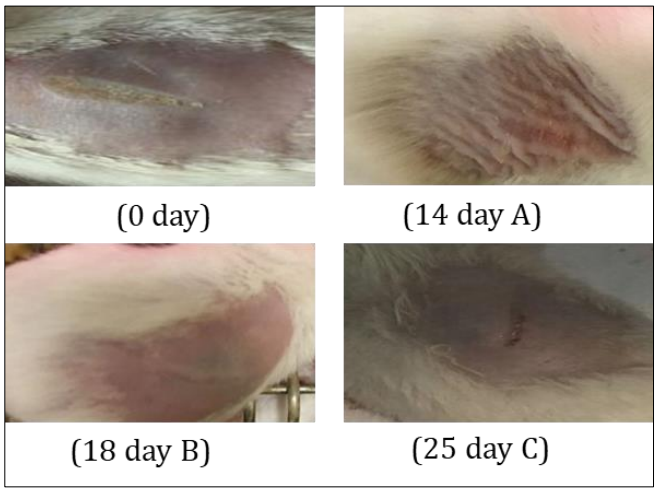

Figure 1 Mice healed from burns on (0-25) days after different treatments. A) Mice treatment with plant extract. B) Mice treatment with 1 % silver sulfadiazine. C) Mice without any treatment (negative control)

4. Discussion

Plantago lanceolata is an associated of the Plantaginaceae family, it is one of the excellent pharmaceutical and industrial significance. *Plantago lanceolata* has been used as an acerbic, anti-inflammatory, antitumor, analeptic, and anti-ulcer influence in traditional treatment (10). This herbaceous shrub includes saponin, terpenoids, flavonoids salicylic acid, pectin, and caffeic mucilage. In many studies, immunomodulatory, antimicrobial, antiulcer, anticancer, analgesic and antioxidant properties (11).

This study aimed to evaluate of plant extract of *Plantago lanceolata* aqueous extract effect on kidney function after treated with CCL_4.

The test Renal oxidative stress is prompted by a rising in the development of reactive molecular oxygen species, such as H2O2 and O2, because of the reduction of the action of antioxidant enzymes (12,13). Aggregate the molecular oxygen species production increases the production of greatly reactive free radicals for instance hydroxyl that reacts with the components of cell for example proteins, DNA as well as lipids and finally initiates failure of integrity and diminished function of cell (14). The oxidative stress induces nephropathy by damaging the glomerular membrane over destruction of endothelial cells, the pelvic cells of the glomerular membrane. In the present study, alcoholic plant extract of the kidney tissue in the CCL_4 group enhanced significantly compared to the control group (15).

And the extract of *Plantago lanceolata* with components for instance phenolic acid, flavonoids and coumarin can have a sweeping effect on reactive species of oxygen and nitrogen (16).

The in vivo anti-inflammatory activity of *Plantago lanceolata* extracts was determined effect on enzyme catalyses arachidonic acid to produce leukotrienes. Leukotrienes play role in most inflammatory infections. The *Plantago lanceolata* extracts or the phytochemicals which take inhibitory effect on this enzyme need the possibility to be used in inflammatory condition (17).

5. Conclusion

The present study showed that *Plantago lanceolata* extrac has a kidney protective activity as well as anti-inflammatory potentials after intraperetonially administration of the extract which may promote the immune-stimulatory activities. These effects attributed to chemical constituents of the plant like: flavonoid, alkaloid, terpenoids, caffeic acid derivatives, iridoid glycosides, fatty acids, polysaccharide, vitamins

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 has done after take permission from the head of animal laboratory house\Biotechnology Research Center\Al-Nahrain University \Baghdad\Iraq.

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