Protective Effect on Lipids Profile of Lion’s Foot (Alchemilla Vulgaris) Leaves against CCl₄ Toxicity and its Fortified to Guava and Mango Pulp

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Abstract

Lion’s foot (Alchemilla vulgaris L.) which is widely known as lady’s mantle, bear’s foot or lion’s foot is traditionally used due to their tannin contents for the treatment of inflammations, diarrhea, wound healing and inhibit cardiovascular diseases and cystic fibrosis. The present work aims to highlight the reverse effect of dried lion’s foot (Alchemilla vulgaris) leaves and its water and ethanol extracts at various ratios against the toxicity of CCl₄ on serum lipids profile that may occur on albino rats. Also, preparation of guava and mango pulp that fortified with lion’s foot powder and its water and ethanol extracts as an available product rich in bioactive component to help hyperlipidemic patients. Fifty-six male albino rats were used in this experiment. These rats were divided into eight main groups (seven rats of each) and were fed on diets for 45 days as follows: Group 1: Negative control group was fed on basal diet. Forty nine rats were fed on basal diet and treated with CCl₄ subcutaneous injection to induce toxicity, then divided into 7 groups form group 2 to group 8. Group 2: Positive control group will be fed on basal diet till final experiment. Groups 3:8 as the same of the second group with (50 and 100 ppm ethanol extract, 50 and 100 ppm water extract, 1% and 2% dry leaves powder respectively) daily orally. Moreover, guava and mango pulps were classified to 7 groups as follows: control sample, and the samples (2 to 7) were fortified with lion’s foot as follows ethanol extract 50ppm and 100ppm, water extract 50ppm and 100ppm , water extract 50ppm and 100ppm, 1% and 2% dried leaves for sensory evaluation. All these samples were organoleptically evaluated for general appearance, color, flavor, taste, consistency and overall acceptability.

Results: The results revealed that treatment with lion’s foot leaves and its ethanol and water extracts caused significant decreased levels of serum lipid profile of total cholesterol, triglycerides, total lipids, LDL-C and VLDL-C induced by CCl₄ and enhancement HDL-C in all groups. The best result of lipid profile was in groups treatment with the high level 2% dried lion foot followed 1% dried lion’s foot and 100ppm ethanol extract. These results due to the antioxidants content in Lion’s foot as polyphenols, flavonoids, tannins and saponins (359.65, 183.10, 150.64 and 296.32, respectively). The best sample of fortified guava pulp was 1% dried leaves. While, the best result of mango pulp was in the samples fortified with 50ppm ethanol extract, water extracts (50ppm and 100ppm) and 1% and 2% dried leaves.

Conclusion: This study showed that the fortification of Alchemilla vulgaris L. can be proposed to protect the toxicity induced by CCl₄ in rats, also to help inhibit the improvement cardiovascular diseases and cystic fibrosis.

Keywords: Lion’s Foot (Alchemilla Vulgaris); Dried Leaves; Water and Ethanol Extract; CCl₄; Cytotoxicity; Lipids Profile; Hyperlipidemic; Mango and Guava Pulp.

Abbreviations: GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents; ANOVA: Analysis of One Way Variance; MDA: Malondialdehyde.

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Introduction

Lion's foot (*Alchemilla vulgaris L.*) which is widely known as lady's mantle, bear's foot or lion's foot is traditionally used due to their tannin contents for the treatment of inflammation of the upper digestive tract, diarrhea internally and as wound healing and astringent externally [1] and [2]. Another use of *Alchemilla* Species is for the adaptation to the hormonal levels of the body in case of menopause [3]. Reference [4] revealed that antioxidants content in dried lion's foot leaves (mg/100g) was total polyphenols 395.65 ± 21.43, total flavonoids 183.10 ± 8.70, antioxidant activity% (DPPH) 131.74 ± 2.05, soluble tannins 150.64 ± 10.22 and Saponins 296.32 ± 14.51. Also, benzoic acid, ellagic acid, catechol, catechin, ellagic acid, salicylic acid and vanillic acid recorded the highest contents which known with their antioxidant and anti-inflammatory properties [5]. Lady's Mantle has been described as an important herb for the cleansing and healing of topical and internal wounds (ulcers, festering wounds and shot wounds), to diffuse internal blood clots, for ulcers and inflammation in the mouth and throat. The pulped leaves, he says, can be applied directly or via a linen cloth soaked in the juice of the leaves [6] and [7]. Reference [8] reported that carbon tetrachloride (CCl₄) is assumed to initiate the biochemical processes leading to oxidative stress, which is the direct cause of many pathological changes in liver, kidney, testes, lungs, nervous system and blood tissues by producing free radicals. These free radicals frequently damage different cell organelles through lipid peroxidation.

Antioxidants and anti-inflammatory agents play a critical role against CCl₄ intoxication by scavenging active oxygen and free radicals and neutralizing lipid peroxides [9] and [10]. Catechin reduced the total cholesterol, and LDL cholesterol [11]. Reference [12] revealed that the phytochemicals ellagic acid showed hepatoprotective activities in CCl₄-induced hepatotoxicity in mice. The protective action was improved further by doubling the dose of the phytochemicals. Apart from the anti-lipid peroxidative and antioxidant actions, these active phytochemicals might have played a role in restoring the cytochrome P450 enzyme system or promoted the liver regenerative activity.

The aim of the present work was to high light the reverse effect of dried lion foot and its water and ethanol extracts at various ratios against the toxicity of CCl₄ on serum lipids profile that may occur on albino rat. Also, to determine sensory evaluation of guava and mango pulp that fortified with lion's footwater, ethanol extracts and dry powder.

Materials and Methods

**Materials**

Lion’s foot (*Alchemilla vulgaris*) leaves was obtained from Horticulture Research Institute, Agriculture Research Center Giza, Egypt. While, Guava (*Psidium guajava L.*) and Mango (*Mangifera indica L.*) fruits were obtained from private farm. Carbon tetrachloride (CCl₄), casein, vitamins, minerals, cellulose and choline chloride will be purchased from El-Gomhoreya Company, Cairo Egypt. Corn oil and starch were obtained from the local market. Fifty six male albino rats (Sprague Dawley strain) weighing an average (200 ± 10g) were obtained from animal house in Food Technology Research Institute, Agriculture Research center, Giza - Egypt. Serum cholesterol, triglycerides, HDL, LDL, and VLDL kits were purchased from Biodiagnostic Company in Egypt. Guava and mango were obtained from local markets.

**Methods**

**Preparation of extract:** Lion’s foot were washed in several times to remove any adhering flesh, dried in oven under vacuum, then ground well. Ground lion’s foot was dipping in ethanol 80% (1:100 v/v) in dark bottle for 48h at ambient temperature (25°C) to obtain extracts, and then mixtures were filtered by filter paper (Whatman1). Water solution was evaporated at 55°C in rotary evaporator, while ethanolic extract was evaporated 40°C till completely evaporation.

**Determination of Antioxidants content:** Total phenolic content was determined by the Folin-Gioacalte METHOD [13]. The extracted samples were mixed with Folin-Gioacalte reagent for 6 min, then 7% sodium carbonate (NaCO₃) was added. The absorbance was measured at 760 nm after 90 min of incubation at room temperature. Results were expressed as mg gallic acid equivalents (GAE) per 100 g sample. While, total flavonoids were determined using the method of [14]. Briefly, 0.5 ml of sample, 0.5 ml of 2% AlCl₃, and methanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm. Total flavonoids content was calculated as quercetin equivalent from a calibration curve and expressed as quercetin equivalents (QE) per 100g. Total tannins content (mg/100g) were evaluated according to the method of [15]. Saponins were determined by the method of [16]. The antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay described by [17].

**Diet composition and animal groups:** Diet composition: Basal diet was prepared according to [18]. The vitamin and mineral mixture had the prepared according to [19].

**Experimental design:** In animal house in Food Technology Research Institute, Agriculture Research Center; albino rats were adapted for one week prior to commencement of the experiment, housed in well aerated cages under hygienic condition and water was introduced *ad-libitum*. After this week, rats were divided into 8 main groups (seven rats of each) and fed on diets for four weeks as follows: Group 1: Negative control group fed on basal diet. Forty nine rats fed on basal diet and treated with CCl₄, in paraffin oil (50% v/v 2 ml/kg) twice weeks subcutaneous injection to induce chronic damage in the liver [20], 7 groups numbered from 2 to group 8 were classified as follows: Group 2: Positive control group had fed on basal diet till final experiment. Group 3: treated as group 2 with 50 ppm ethanol extract daily orally. Group 4: treated as group 2 with 100 ppm ethanol extract daily orally. Group 5: treated as group 2 with 50 ppm water extract daily orally. Group 6: treated as group 2 with 100 ppm water extract daily orally. Group 7: treated as group 2 with 1% leaves powder in diet. Group 8: treated as group 2 with 2% leaves powder in diet.

**Serum sampling:** At the end of the experiment period, the rats were fasted overnight then the rats were anaesthetized, sacrificed and blood samples were collected from the aorta. The blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was carefully separated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen till analysis at -20°C.
Biochemical analyses of serum: Serum lipid profiles: Serum samples were used for the determination of total cholesterol, triglycerides, Total lipids, and HDL-C, according to the methods described by [21]. Serum LDL-C, and VLDL-C were calculated according to the following equation:

1. VLDL-C Concentration (mg/dL) = TG (mg/dL)/5
2. LDL-C = Total Cholesterol – [(VLDL-C) + (HDL-C)]

Guava and mango pulp preparation: Guava and mango were washed, then mango fruits were peeled. The guava pulp was extracted by using pulper machine and strained through 1 mm stainless steel sieves to remove seeds. Guava or mango pulps were blended in the mixer without any additives such as (water and sugar). Guava and mango pulps were placed in transparent glass bottles and classified to 7 groups as follows: control sample, and the samples (2 to 7) were fortified with lion’s foot as follows: 50ppm and 100ppm ethanol extract, 50ppm and 100ppm water extract, and 1% and 2% dried leaves.

The sensory parameters: The sensory parameters of appearance, colour, flavour, taste, consistency and acceptability were evaluated by 10 trained panelist based on 10 point Hedonic rating scale with maximum score considered as the best [22].

Statistical analysis: Results were analyzed using SPSS 19.0 Program (2000). Means and standard deviations were determined using descriptive statistics. Comparison between means of samples was determined using analysis of one way variance (ANOVA) and multiple range tests. Statistical significance was defined at P ≤ 0.05.

Results
Tables and Figures given below.

Discussion
Total polyphenols, total flavonoids, soluble tannins, saponins and antioxidant activity of dried lion’s foot are recorded in Table 1. The data indicated that dried lion’s foot leaves had rich in total polyphenolic and flavonoids content (395.65 and 183.10 mg/100g, respectively). Also, it’s observed the highest content

| Constituents                      | mg/g                |
|----------------------------------|---------------------|
| Total polyphenols                | 395.65 ± 21.43      |
| Total flavonoids                 | 183.10 ± 8.70       |
| Antioxidant Activity% (DPPH)     | 131.74 ± 2.05       |
| Soluble Tannins                  | 150.64 ± 10.22      |
| Saponins                         | 296.32 ± 14.51      |

Values are means of triplicate ± Standard deviation.

Table 1. Antioxidant Contents In Dried Lion’s Foot Leaves (mg/100g).

Table 2. Effect Of Dried Lion’s Foot Leaves And Its Water And Ethanol Extracts On Lipid Profile Of Rats.

| Parameters                          | mg/dl |
|-------------------------------------|-------|
|                                     |       |
| Groups                              |       |
| Normal group                        |       |
| 120.76 ± 12.02                      |       |
| Control positive group (PG)         |       |
| 169.85 ± 16.93                      |       |
| PG treated with ethanol extract 50ppm|       |
| 140.12 ± 14.83                      |       |
| PG treated with ethanol extract 100ppm|       |
| 129.15 ± 11.67                      |       |
| PG treated with water extract 50ppm |       |
| 148.87 ± 12.12                      |       |
| PG treated with water extract 100ppm|       |
| 141.11 ± 12.21                      |       |
| PG fed on Lion’s foot dried 1%      |       |
| 131.31 ± 10.21                      |       |
| PG fed on Lion’s foot dried 2%      |       |
| 125.12 ± 11.15                      |       |

All results are expressed as mean ± SD.
A.V: Alchemilla vulgaris

Values in each column which have different letters are significantly different (p<0.05).
Figure 1. Effect of dried Lion's foot leaves and its water and ethanol extracts on lipid profile of rats.

![Graph showing lipid profile](image1)

Figure 2. Effect of dried Lion's foot leaves and its water and ethanol extracts on lipid profile of rats.

![Graph showing lipid profile](image2)

Table 3. Effect of dried *Alchemilla vulgaris* and its ethanolic and aqueous extracts on glutathione reductase and malonaldehyde of rats.

| Parameters                      | Glutathione Reductase (GSH–RX) ng/ml | Malondialdehyde (MDA) nmol/ml |
|---------------------------------|--------------------------------------|------------------------------|
| Groups                          |                                      |                              |
| Normal group                    | 25.72 ± 2.01                         | 8.51 ± 0.50                  |
| Positive group (PG)             | 10.69 ± 1.28                         | 17.95 ± 1.54                 |
| PG treated with ethanol extract 50 ppm | 16.07 ± 1.38                     | 10.52 ± 1.90                 |
| PG treated with ethanol extract 100 ppm | 22.84 ± 0.24                    | 9.63 ± 0.65                  |
| PG treated with water extract 50 ppm | 16.73 ± 1.28                      | 12.55 ± 1.06                 |
| PG treated with water extract 100 ppm | 18.87 ± 1.82                    | 11.60 ± 1.89                 |
| PG fed on A.V dried 1%          | 18.08 ± 1.24                        | 11.92 ± 1.60                 |
| PG fed on A.V dried 2%          | 23.51 ± 1.46                        | 9.80 ± 1.82                  |

All results are expressed as mean ± SD.

Values in each column which have different letters are significantly different (p<0.05).
of soluble tannins and saponins (150.64 and 296.32 mg/100g) in dried leaves. These results were reflected to the antioxidant activity, its noticed that the antioxidant activity of dried lion’s foot leaves was high (131.74%). European Pharmacopoeia described the aerial parts of lion’s foot and its chemical content as containing a minimum of 6% tannin, chlorogenic acid and caffeic acid [23]. The effects of lion foot leaves and its water and ethanol extracts on serum lipid profile of rats toxic with CCl₄.

In Parallel, rats fed on diets containing 2% and 1% dried leaves were highly significant decrease in serum total cholesterol (125.12 and 131.31mg/dL) respectively, followed by rats fed on ethanol extract 100 and 50 ppm orally (129.15 and 140.12 mg/dL, respectively), then rats fed on water extract 100 and 50 ppm orally (129.15 and 140.12 mg/dL, respectively), followed by rats fed on 100 and 50 ppm ethanol extract orally compared to rats fed on positive control diet (Table 2, Figure 2).

In contrast, rats fed on diets containing 2% and 1% dried lion’s foot were highly significant increase in serum HDL compared to rats fed on normal control diet (24.23 mg/dL). Also, Another rats groups either fed on ethanol or water extracts orally were significant increase in serum HDL compared to rats fed on positive control diet (Table 2, Figure 2).

Conclusively, serum total cholesterol, triglycerides, total lipids, LDL and VLDL were significant decrease in rats fed on diet containing dried lion foot’s foot and rats fed on either ethanol or water extracts, orally (Figure 2), while HDL was significant increase. Also, toxicity with CCl₄, caused significant rise in cholesterol, triglycerides, total lipids, LDL-C and VLDL-C while, there was significant decrease in HDL-C.

These results are may due to the content of phytocchemicals as antioxidant and its activity (DPPH) in lion’s foot which have a significant effect on lowering all lipids parameters except increase

| Table 4. Sensory evaluation of Guava pulp fortified with ethanolic, water extracts and dried lion’s foot. |
|-----------------------------------------------|
| **Pulp samples** | **General appearance** | **Color** | **Flavor** | **Taste** | **Consistency** | **Acceptability** |
| Control | 18.92 ± 1.21 | 18.42 ± 1.37 | 19.58 ± 1.58 | 18.33 ± 1.13 | 18.75 ± 1.15 | 94.04 ± 5.64 |
| 50ppm ethanol extract | 17.08 ± 1.17 | 18.21 ± 1.03 | 16.58 ± 1.48 | 15.46 ± 1.13 | 17.41 ± 1.32 | 84.74 ± 5.26 |
| 100ppm ethanol extract | 17.25 ± 1.37 | 17.38 ± 1.73 | 16.67 ± 1.15 | 15.58 ± 1.13 | 16.92 ± 1.15 | 83.83 ± 6.55 |
| 50ppm water extract | 17.33 ± 1.07 | 17.54 ± 1.61 | 16.71 ± 1.13 | 17.04 ± 1.13 | 16.79 ± 1.41 | 85.42 ± 6.50 |
| 100ppm water extract | 17.12 ± 1.27 | 17.62 ± 1.87 | 15.75 ± 1.58 | 17.29 ± 1.13 | 16.58 ± 1.88 | 84.39 ± 4.21 |
| Dry 1% | 18.17 ± 1.11 | 17.83 ± 1.01 | 19.33 ± 1.58 | 18.25 ± 1.13 | 18.35 ± 1.15 | 91.93 ± 3.24 |
| Dry 2% | 16.43 ± 1.07 | 16.83 ± 1.17 | 17.79 ± 1.18 | 15.25 ± 1.13 | 16.58 ± 1.15 | 82.87 ± 5.83 |

| Table 5. Sensory evaluation of Mango pulp fortified with ethanolic, water extracts and dried lion’s foot. |
|-----------------------------------------------|
| **Pulp Samples** | **General appearance** | **Color** | **Flavor** | **Taste** | **Consistency** | **Acceptability** |
| Control | 18.33 ± 1.76 | 18.37 ± 1.06 | 18.33 ± 1.17 | 18.29 ± 1.05 | 19.76 ± 1.22 | 93.09 ± 4.76 |
| 50ppm ethanol extract | 17.37 ± 1.61 | 18.08 ± 1.06 | 17.29 ± 1.71 | 16.95 ± 1.28 | 18.51 ± 1.24 | 88.42 ± 4.94 |
| 100ppm ethanol extract | 17.75 ± 1.21 | 18.09 ± 1.01 | 16.37 ± 1.57 | 16.41 ± 1.60 | 17.93 ± 1.37 | 86.52 ± 3.91 |
| 50ppm water extract | 17.58 ± 1.06 | 17.59 ± 1.72 | 17.87 ± 1.53 | 17.58 ± 1.83 | 17.81 ± 1.66 | 88.41 ± 4.91 |
| 100ppm water extract | 17.58 ± 1.67 | 17.37 ± 1.24 | 17.62 ± 1.58 | 17.54 ± 1.85 | 17.66 ± 1.54 | 87.71 ± 4.42 |
| Dry 1% | 16.63 ± 1.43 | 16.91 ± 1.16 | 18.41 ± 1.08 | 17.75 ± 1.95 | 19.54 ± 1.14 | 89.23 ± 3.47 |
| Dry 2% | 15.41 ± 1.51 | 14.25 ± 1.86 | 16.66 ± 1.87 | 15.25 ± 1.17 | 17.22 ± 1.21 | 78.76 ± 4.75 |
in HDL level. These data were in agreement of [4] and [11]. It could be noticed from the presented data in Table (3) that positive control (induced with CCL) showed a significant decrease of glutathione reductase activity (10.69ng/ml) compared to rats fed on basal diet (25.72ng/ml). It’s clear rats fed on diet containing 2% lion’s foot and 100ppm ethanol extract were highly significant increase in glutathione reductase (23.51 and 22.84 ng/ml, respectively) while, there was significant increase of other treated groups.

On the other hand, data showed marked increase in the serum Malondialdehyde (MDA) level of rats fed on the positive control group (17.95 nmol/ml) compared with normal group (8.5 nmol/ml). Rats in groups treated with dried lion’s foot and its ethanol and water extracts recorded a significant decrease in the serum MDA level. The best result was observed in the rats groups treated with 100ppm extract and 2% powder (9.80 and 9.63 nmol/ml, respectively). These data may due to polyphenols and flavonoids content in lion’s foot which effect working as a scavenging free radical to prevent liver cell damage [24]. Sensory evaluation scores of fortified Guava pulp with different levels of Alchemilla Vulgaris leaves powder (1% and 2%) and its extracts of water and ethanol (50ppm and 100ppm) are shown in Table (4). It was found that, there was no significant difference (P<0.05) of general appearance and color between control sample and all fortified samples except a sample fortified with 2% dry leaves powder.

On the other hand, table (4) showed a significant statistical difference between control sample and all fortified guava pulp samples in flavor evaluation except a sample fortified with 1% dry leaves powder which recorded (19.33 ± 1.58) compared with the control pulp sample (19.58 ± 1.58).

The best results of taste evaluation were in pulp samples fortified with 50ppm water extract, 100ppm water extract, and 1% dry leaves powder. The values were (17.04 ± 1.13, 17.29 ± 1.13, and 18.25 ± 1.30, respectively) compared with control sample (18.33 ± 1.3) which recorded no significant difference between them. While, there was significant statistical difference between control sample and the samples fortified with 100ppm water, and 50ppm ethanol extract, and 2% dry leaves powder of lion’s foot indicated that acceptable sweet taste which can be taken on its own or in a herbal combination [7].

Concerning the consistency of guava pulp, there were significant differences between all fortified groups and control sample except 1% dry lion foot leaves powder that showed no significant difference compared with control sample.

Fortification with dried lion's foot leaves and its extracts of water and ethanol on acceptability are shown also in table (5). It was found that, the best result was in the samples fortified with 50ppm ethanol extract, (50ppm and100ppm) water extract and 1% dry that showed no significant difference between them and control sample. While, there were statistical significant differences between control sample and 100ppm ethanol extract also, 2% dry. The sample fortifies with 2% dry recorded the lowest value in all fortified samples.

There were statistical significant differences between all fortified groups and control sample except the sample fortified with 1% dry lion foot leaves powder which recorded no significant difference compared with control sample.

The best results of taste were in pulp samples fortified with (50ppm and 100ppm) water extract, 1% dry followed (50ppm and 100ppm) ethanol extract, while there was significant difference between control sample and the samples fortified with 2% dry. That indicates its better sweet taste which can be taken on its own or in a herbal combination [7].

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