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Effect of Lion's Foot (*Alchemilla vulgaris*) on Liver and Renal Functions in Rats Induced by CCl_4

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Abstract

The present work aims to study the influence of antioxidants activity of lion's foot (*Alchemilla vulgaris*) leaves at different concentrations to give more protection against chronic liver disease. Results indicated that dried lion's foot leaves had rich in total polyphenolic and flavonoids content (395.65 and 183.10 mg/100g, respectively). These results were reflected to the antioxidant activity (DPPH); it's noticed that the antioxidant activity of dried lion's foot leaves was high (131.74%). The major polyphenolic components were benzoic acid (1084.63 ppm) followed by ellagic acid, catechol, and catechin (614.16, 580.54, and 566.53 ppm, respectively) then salicylic acid and protocatechuic acid (479.71 and 444.43 ppm, respectively). On the same trend, flavonoids fractions indicated the highest content in luteo-6-arabinase 8-glucose, apig. 6-rhamnase 8-glucose, acatein, narengin and luteolin (40.01; 15.04; 8.07; 6.64 and 6.42 ppm, respectively). Fifty-six male albino rats were used in biological experiments. Rats fed on basal diet for two weeks before the performance of the experiment. At the beginning, rats divided into eight main group were fed on diets for 45 days as follows: Negative control group (first group) was fed on basal diet. Forty nine rats were fed on basal diet and induced by CCl_4, in paraffin oil (50% v/v, 2 ml/Kg) twice weeks subcutaneous injection to induce chronic damage in the liver, then divided into 7 groups numbered from group 2 to group 8. Positive control group rats fed on basal diet till final experiment (second group). Group 3 and 4 rats treated with 50 and 100 ppm ethanolic leaves extracts, respectively. Also, group 5 and 6 treated with 50 and 100 ppm aqueous leaves extracts, respectively. All extracts were fed on orally every day. While, rats in group 7 treated with 1% and 2% dried lion's foot leaves. At the end of the experimental period, serums were collected to determine liver and

renal functions. The liver was removed surgically for histopathological observation. The results revealed that CCl_4 intoxication impaired liver function. Serum AST, ALT, ALP and total bilirubin levels were elevated by CCl_4 administration, while significant decreasing was noticed in serum albumin in CCl_4 group. Histopathologically, CCl_4 caused congestion of central vain, fatty change of hepatocytes, and focal inflammatory cells in filtration. Treatment with lion's foot with different forms and concentration attenuated these adverse effects and markedly ameliorated histopathological and biochemical alterations caused by CCl_4 especially with 2% powder and 100 ppm ethanol extract administration. Therefore, the results of this study concluded that lion's foot can be proposed to protect hepatotoxicity induced by CCl_4 in rats. The results also revealed that the hepatoprotection effect of lion's foot may be attributed to its antioxidant contents and free radical scavenger effect.

Keywords

Lion's Foot (*Alchemilla vulgaris*), Polyphenols, Flavonoids, Chronic Liver Disease, Liver and Renal Function, Histopathological Evaluation

1. Introduction

Alchemilla vulgaris (Lion's foot or Lady's mantle) is an uncommon herbaceous member of the rose family (Rosaceae). Lady's mantle is native to Europe and Asia and grows well in New Zealand. The medicinal part of the *Alchemilla vulgaris* is aerial part with flowers and stems. The recommended official dose of Lion's foot, the main medicinal plant weigh level is 5 - 10 g/day of the dried plant noticed by [1]. Toxicity, as defined by the LD_{50} in rats, was observed only at the high concentrations of 5 g/Kg. Concentrations as high as 360 mg/mL did not show any sign of cellular toxicity in the LDH test. Higher concentrations did not substantially add to such properties. These observations show a very high therapeutic index [2]. Also, [3] reported that LD_{50} for *A. vulgaris* leaves is 17.3 g/Kg.

The liver is a highly sensitive organ which plays a major role in maintenance and performance of the homeostasis in the body. It is the chief organ where important processes like metabolism and detoxification take place. The entry of these toxicants into the body is principally *via* the gastrointestinal tract and after absorption; they are transported through the hepatic vein to the liver revealed by [4] and [5].

Hepatic disorders are one of the most global health problems, considering drugs, chemicals and/or alcohol as main triggers of the disease [6]. Non-alcoholic fatty liver disease (NAFLD) is the main cause of hepatic diseases in the developed countries. As benign steatosis to steato hepatitis and dramatically cirrhosis, NAFLD indicates various hepatic disorders may due to mal-deposition of fats deposition in hepatic cells, which varies in terms of severity from simple

non-inflamatory hepatic steatosis, to steato hepatitis (NASH) that finally progress to liver cirrhosis. NAFLD is mainly found as "a metabolic syndrome" participant with central obesity, hyperlipidemia, impaired glucose tolerance and hypertension revealed by [7] and [8].

Alchemilla mollis (Buser) Rothm aerial part and root methanolic-water extracts were evaluated for their hepatoprotective activity on carbon tetrachloride induced hepatotoxicity and hypoglycemic activity on alloxan-induced diabetic mice. Hepatoprotective activity results have revealed that serum ALT levels were significantly lowered by both the aerial part and root extracts at doses of 100 mg/Kg and 200 mg/Kg. Histopathological examination showed that *A. mollis* aerial parts and roots induced significant recovery from cellular damage; when compared to the carbon tetrachloride group, the most significant activity was observed with *A. mollis* aerial part extracts at a dose of 200 mg/Kg [9]. There is evidence of a hepatoprotective activity of *A. mollis* on the polyphenolic contents of the plant, especially in the case of flavonoids, which have potent antioxidant properties.

The aim of the present work was to high light the reverse effect of dried Lion's foot*;* its aqueous and ethanol extracts at various ratios against the toxicity of CCl_4, also to evaluate the various ratios on body weight, feed intake, liver and renal functions and histopathological changes that may occur on rats suffering from chronic liver disease.

2. Materials and Methods

2.1. Materials

Lion's foot dried leaves was obtained from Horticultural Research Institute, Agriculture Research Center Giza, Egypt. Carbon tetrachloride (CCl_4), casein, vitamins, minerals, cellulose and choline chloride will be purchased from El-Gomhoreya Company, Cairo Egypt. Animals: Fifty six male albino rats will be obtained from Helwan breeding farm, Cairo-Egypt. Weighing an average will be between (200 ± 10 g). Casein, cellulose, all vitamins and minerals were obtained from El-Gomhoria Pharmaceutical Company, Cairo, Egypt. Corn oil and starch were obtained from the local market. Kits to determine serum aspartate amine transaminase (AST), alanine amine transaminases (ALT), and alkaline phosphates (ALP), total bilirubin, albumin, glutathione reductase and malonaldehyde were purchased from Biodiagnostic Company in Egypt.

2.2. Methods

2.2.1. Chemical Analysis of Raw Materials

Total polyphenolic compounds were determined by the Folin-Ciocalteau method [10]. The absorbance was measure at 760 nm. Results were express as gallic acid equivalents (GAE) per 100 g sample. Also, the content of flavonoids was determined by Chen and Li [11]. The absorbance of the solution at a wavelength of 510 nm was determined. Total flavonoid content in each spice extract was then calculated using a standard curve prepared as rutin per 100 g sample.

Soluble tannins were evaluated using the Folin Denis method as described by Taira [12]. Absorbance was measured with a spectrophotometer at 725 nm. While, saponins in Lion's foot leaves was determined by gravimetrically methods as described by Anhawange *et al.* [13].

Polyphenols and flavonoids compounds determined by HPLC according to method Goupy *et al.* [14] and Mattila *et al.* [15], respectively as follow: 5 g of sample were mixed with methanol and centrifuged at 1000 rpm for 10 min and the supernatant was filtrated through 0.2 μm Millipore membrane filter, then 1 - 3 ml was collected in avail for injection in HPLC Hewlett-packard (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set as 289 nm and 330 nm and quarter HP pump (series 1050). The column temperature was maintained at 35˚C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate 1ml/min.

DPPH scavenging activity tests were carried out according to the method of Brand-Williams *et al.* [16]. 0.01 g of sample was dissolved in 10 ml DMSO and seven different concentrations (1 mg/ml to 0.015 mg/ml) were prepared with ½ dilutions. 2.9 ml DPPH solution (10^{-4} M in ethanol) was added into 0.1 ml of sample solutions. The mixture was shaken vigorously and incubated 30 minute in 30°C water bath. Absorbance of the resulting solution was measured at 517 nm UV/visible spectrophotometer (Shimadzu). Percentage of inhibition (DPPH scavenging activity) determined as follows:

$$\%\ \text{DPPH radical-scavenging} = \left[\left(\text{Absorbance of DPPH} - \text{Absorbance of sample}\right)/\text{Absorbance of DPPH}\right] \times 100$$

2.2.2. Preparation of Extracts

Lion's foot (*Alchemilla vulgaris*) leaves were washed in aqueous 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 w/v) or in distilled water (1:100 w/v) in dark bottle for 48h in refrigerator at 4˚C temperature. To obtain extracts, then mixtures were filtered by filter paper (Whatman1). Aqueous and ethanolic extracts were evaporated in rotary evaporator at 50˚C.

2.2.3. Diet Composition and Animal Groups

1) Diet composition*:* Basal diet was prepared according to Reeves *et al.* [17]. The vitamin and mineral mixture had the prepared according to Campbell [18].

2) Experimental design: 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 aqueous 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 CCl_4, in paraffin oil (50% v/v 2 ml/Kg) twice weeks subcutaneous injection to induce chronic damage in the liver [19], 7 groups numbered from 2 to group 8. Group 2: Positive control group had fed on basal

diet till final experiment. Group 3: treated as group 2 with 50 ppm Lion's foot leaves ethanol extract daily orally. Group 4: treated as group 2 with 100 ppm Lion's foot leaves ethanol extract daily orally. Group 5: treated as group 2 with 50 ppm Lion's foot leaves aqueous extract daily orally. Group 6: treated as group 2 with 100 ppm Lion's foot leaves aqueous extract daily orally. Group 7: treated as group 2 with 1% Lion's foot leaves powder. Group 8: treated as group 2 with 2% Lion's foot leaves powder. During the experiment period, the quantities of diet, which were consumed and/or waste, were recorded every day. In addition, rat's weight was recorded weekly, to determine feed intake, Body weight gain%, and feed efficiency ratio according to Chapman *et al.* [20].

3) Blood 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.

2.2.4. Biochemical Analyses of Serum

1) Liver functions: Aspartate amine transaminase (AST), Alanine amine transaminases (ALT), and Alkaline phosphates (ALP) were measured according to the method described by Tietz *et al.* [21]. Albumin was measured according to the method described by Burtis *et al.* [22]. Bilirubin was determined according to the method described by Tietz and Saunders [23].

2) Kidney Functions: Uric acid was determined in the serum according to the method described by Fossati *et al.* [24]. Urea nitrogen and Creatinine were determined according to Young and Friedman [25].

2.2.5. Histopathological Studies

Liver and kidney tissues stained by Hematoxylin and Eosin according to Bancroft *et al.* [26] Hisropathological examination was performed at the Histology laboratory, Faculty of Veterinary Medicine, Cairo University.

2.3. Statistical Analysis

Results were expressed as the mean standard deviation ± SD. Data were statistically analyzed for variance "ANOVA" test at $P \leq (0.05)$ using SPSS statistical software, "version 20" will be used for these calculations [27].

3. Results and Discussion

3.1. Antioxidant Contents

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 of soluble tannins and saponins (150.64 and 296.32 mg/100g) in dried leaves. These

Table 1. Antioxidant contents in dried lion's foot leaves (mg/100g).

Compounds	mg/100g
Total polyphenols	395.65 ± 21.43*
Total Flavonoids	183.10 ± 8.70
Soluble Tannins	150.64 ± 10.22
Saponins	296.32 ± 14.51
Antioxidant Activity% (DPPH)	131.74 ± 2.05

*Values are means of triplicates ± Standard deviation.

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 [28].

Table 2 and Table 3 indicated high contents of polyphenolic and flavonoids compounds in dried lion's foot leaves (ppm on dry weight basis). The major polyphenolic components were benzoic acid (1084.63 ppm) followed by ellagic acid, catechol, and catechin (614.16, 580.54, and 566.53 ppm, respectively) then salicylic acid and protocatechuic acid (479.71 and 444.43 ppm, respectively). Also, flavonoids fractions indicated the highest content in luteo-6-arabinase 8-glucose, apig. 6-rhamnase 8-glucose, acatein, narengin and luteolin (40.01; 15.04; 8.07; 6.64; 6.42 mg/100g, respectively). These data was adapted by [29] they reported that, 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.

3.2. Biological Parameters

Data in Table 4 showed that, control positive group was significant decrease in body weight gain%, feed intake and feed efficiency ratio compared with control negative group, while all treatment groups with lion's foot at different concentration whether aqueous extract, ethanol extract or powder showed significant increase in these parameters compared with positive control group. These data was adapted by [30] they reported that *A. vulgaris* L. are mainly the flavonoids reported to increase the metabolic rate in cold environments, regulate digestive enzymes, and metabolic stimulation.

As seen in Table 5, AST, ALT, ALP and total bilirubin in serum were elevated significantly by CCl_4 administration while, there was significant decrease in serum albumin compared to negative control. Significant reduction was observed in AST, ALT, ALP and bilirubin with *A. vulgaris* treatment in all different groups except for 50 ppm aqueous extract group. The best result was in group treated with 100 ppm ethanol extract and 2% powder. In contrast rats fed on lion's foot leaves dried and aqueous or ethanol extract were significant increase in serum albumin compared with positive control. The enhancement of our results on liver function may be due to that *A. vulgaris* contains antioxidant,

Table 2. Polyhenolic compounds profile in dried lion's foot leaves (ppm).

Phenolic compounds	ppm
Gallic acid	85.65
4-Amino-benzoic acid	33.19
Protocatchuic acid	444.43
Catechin	566.53
Chlorogenic acid	70.10
Catechol	580.54
Epicatachin	264.32
Caffeine	59.32
P-OH-benzoic acid	182.68
Caffeic acid	50.46
Vanillic acid	285.94
P–coumaric acid	153.95
Iso-ferulic acid	69.95
Reversetrol	11.97
Ellagic acid	614.16
Alpha-coumaric acid	66.86
Benzoic acid	1084.63
3,4,5-methoxy-cinnamic acid	30.16
Coumarin	27.67
Salycilic acid	479.71
Cinnamic acid	21.32

Table 3. Flavonoids compounds profiles in dried lion's foot leaves (ppm).

Flavonoids Compounds	ppm
Luteo.6-arbinose 8-glucose	40.01
Luteo.6-glucose 8-arabinose	3.19
Apig. 6-arabinose 8-galactose	1.31
Apig. 6-rhamnose 8-glucose	15.04
Luteolin	6.42
Narengin	6.64
Rutin	0.44
Rosmarinic	0.30
Apig.7-O-neohespiroside	1.07
Kamp.3,7-dirhamoside	1.93
Apig.7-glucose	0.69
Quercetrin	0.22

Continued

Quercetin	0.75
Kaemp.3-(2-p-comaroyl)glucose	6.00
Naringenin	0.24
Hespirtin	0.63
Kampferol	0.33
Rhamnetin	0.56
Apegnin	0.14
Acacetin	8.07

Table 4. Body weight gain, feed intake and feed efficiency ratio in rats fed on dried lion's foot leaves and its extracts.

Parameters / Groups	Body Weight Gain (%)	Feed Intake (g/d)	Feed efficiency ratio (FER)
Normal group	$20.22^{a} \pm 2.84$	$18.98^{a} \pm 1.38$	$0.59^{a} \pm 0.016$
Control positive group (PG)	$11.03^{c} \pm 1.94$	$13.50^{b} \pm 1.65$	$0.22^{b} \pm 0.008$
Ethanol extract 50 ppm	$15.00^{b} \pm 1.52$	$18.89^{a} \pm 1.19$	$0.53^{a} \pm 0.011$
Ethanol extract 100 ppm	$16.00^{b} \pm 1.62$	$18.66^{a} \pm 1.92$	$0.56^{a} \pm 0.017$
Aqueous extract 50 ppm	$15.00^{b} \pm 1.99$	$19.30^{a} \pm 1.50$	$0.58^{a} \pm 0.009$
Aqueousextract 100 ppm	$16.13^{b} \pm 1.15$	$19.05^{a} \pm 1.30$	$0.59^{a} \pm 0.013$
Dried lion's foot 1%	$16.00^{b} \pm 1.70$	$18.50^{a} \pm 1.4$	$0.55^{a} \pm 0.017$
Dried lion's foot 2%	$17.18^{b} \pm 2.39$	$19.00^{a} \pm 1.19$	$0.58^{a} \pm 0.019$

Data are presented as mean (n = 7 rats) ± standard deviation, values with different superscripts within the column are significantly difference at $P < 0.05$, while those with have similar or partially are not significant.

Table 5. Effect of dried lion's foot leaves and its aqueous and ethanolic extracts on liver function of rats.

Parameters / Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Albumin (g/dl)	Total Bilirubin (mg/dl)
Normal group	$53.42^{c} \pm 5.88$	$24.50^{c} \pm 4.00$	$160.57^{c} \pm 17.83$	$3.85^{a} \pm 0.11$	$1.70^{b} \pm 0.09$
Control positive group (PG)	$66.57^{a} \pm 9.24$	$37.50^{a} \pm 6.69$	$219.42^{a} \pm 29.37$	$2.05^{b} \pm 0.48$	$2.18^{a} \pm 0.18$
Ethanol extract 50 ppm	$58.28^{b} \pm 9.64$	$29.85^{b} \pm 3.09$	$183.28^{b} \pm 10.50$	$3.83^{a} \pm 0.20$	$1.75^{b} \pm 0.17$
Ethanol extract 100 ppm	$56.57^{bc} \pm 8.36$	$24.14^{c} \pm 5.10$	$175.14^{bc} \pm 15.09$	$3.45^{a} \pm 0.08$	$1.75^{b} \pm 0.14$
Aqueous extract 50 ppm	$60.00^{ab} \pm 13.85$	$34.42^{ab} \pm 6.27$	$195.42^{ab} \pm 16.51$	$3.75^{a} \pm 0.26$	$2.07^{a} \pm 0.19$
Aqueousextract 100 ppm	$58.28^{b} \pm 9.15$	$30.85^{b} \pm 5.79$	$186.00^{b} \pm 11.92$	$3.39^{a} \pm 0.20$	$1.90^{b} \pm 0.15$
Dried lion's foot 1%	$55.00^{bc} \pm 5.90$	$29.71^{b} \pm 4.13$	$187.14^{b} \pm 15.92$	$3.71^{a} \pm 0.35$	$1.82^{b} \pm 0.10$
Dried lion's foot 2%	52.70c ± 6.49	$24.28^{c} \pm 4.06$	$180.00^{b} \pm 10.69$	$3.82^{a} \pm 0.44$	$1.78^{b} \pm 0.14$

Data are presented as mean (n = 7 rats) ± standard deviation, values with different superscripts within the column are significantly difference at $P < 0.05$, while those with have similar or partially are not significant.

Table 6. Effect of dried lion's foot leaves and its water and ethanolic extracts on renal function of rats.

Parameters	mg/dl		
Groups	Creatinine	Urea	Uric Acid
Normal group	$0.65^{d} \pm 0.10$	$35.00^{d} \pm 2.44$	$1.80^{d} \pm 0.25$
Control positive group (PG)	$1.30^{a} \pm 0.02$	$68.20^{a} \pm 7.08$	$3.98^{a} \pm 0.42$
PG treated with ethanol extract 50 ppm	$0.78^{c} \pm 0.08$	$43.43^{c} \pm 4.99$	$2.73^{bc} \pm 0.11$
PG treated with ethanol extract 100 ppm	$0.66^{d} \pm 0.04$	$39.52^{cd} \pm 3.12$	$2.25^{c} \pm 0.25$
PG treated with aqueous extract 50 ppm	$0.93^{b} \pm 0.07$	$62.30^{ab} \pm 5.12$	$3.10^{ab} \pm 0.19$
PG treated with aqueous extract 100 ppm	$0.85^{bc} \pm 0.10$	$58.60^{b} \pm 4.83$	$2.82^{bc} \pm 0.94$
PG fed on Lion's foot dried 1%	$0.69^{d} \pm 0.08$	$40.55^{cd} \pm 6.45$	$2.90^{c} \pm 0.92$
PG fed on Lion's foot dried 2%	$0.61^{d} \pm 0.08$	$38.52^{cd} \pm 7.47$	$2.29^{c} \pm 0.80$

All results are expressed as mean ± SD. Values in each column which have different letters are significantly different ($p < 0.05$).

polyphenols, tannins composed of some gallic and mostly ellagic acid, flavonoids such as orientin, quercetin, quercitrin, isoquercetin, vitexin, rutin, hyperoside, with others, including luteolin and pro-anthocyanidins to which the main pharmacological activities of the plant [31] Also, [32] reported that *A. vulgaris* contain flavonoids which important for the treatment of inflammation and wound healing. Our results are agree with [33] which reported that medicinal plant *A. vulgaris* serve as a vital source of potentially useful new compounds for the development of effective therapy to combat liver problems.

Results in Table 6 showed a significant decrease in creatinine and urea in the groups fed on 1% and 2% dried lion's foot leaves compared with other groups which rats fed on 50 and 100 ppm ethanol extracts orally, then in rats fed on water extracts compared with positive group. Also, uric acid was decreased significant in rats fed on 1% and 2% dried lions foot (2.90 and 2.29 mg/dl, respectively), then rats groups fed on ethanol extracts (2.73 and 2.25 mg/dl) followed by rats fed on water extracts (3.10 and 2.82 mg/dl) 50 ppm and 100 ppm, respectively compared with positive group (3.98 mg/dl). It appears from results that lion's foot was safe on renal functions. These data were adapted by [34] they found that reduction in serum uric acid level may be attributed to the increased utilization of uric acid against increased production of the free radical since it has a capable especially of reacting with free radicals.

3.3. Histopathological Examination of Liver

Liver of rat from group 1 revealed the normal histological structure of hepatic lobule (Figure 1). Meanwhile, liver of rats from group 2 revealed congestion of central vein (Figure 2). Examined sections from group 3 revealed congestion of central vein and hepatic sinusoids as well as vacuolar degeneration of hepatocytes (Figure 3) and Kupffer cells activation. Moreover, liver of rats from group

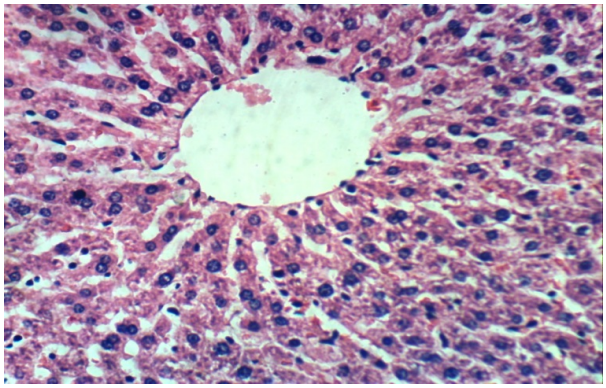

Figure 1. Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E × 400).

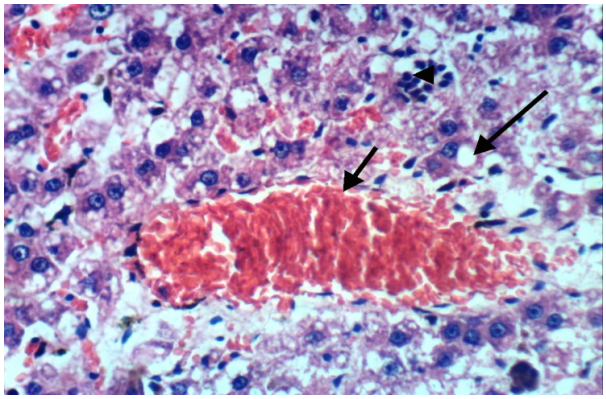

Figure 2. Liver of rat from group 2 showing congestion of central vein, fatty change of hepatocytes and focal inflammatory cells infiltration (H & E × 400).

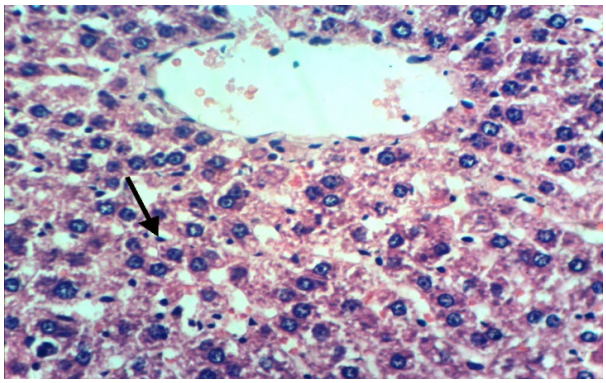

Figure 3. Liver of rat from group 3 showing Kupffer cells activation (H & E × 400).

4 showed slight vacuolar degeneration of hepatocytes (**Figure 4**). However, liver from group 5 showed Kupffer cells activation and strands of fibroblasts proliferation in the portal triad (**Figure 5**). Liver of rats from group 6 revealed sinusoidal leukocytosis (**Figure 6**). Slight Kupffer cell activation was the only histopathological change observed in liver of rats from group 7 (**Figure 7**). Moreover, liver of rats from group 8 revealed few leucocytes in the hepatic sinusoids (**Figure 8**).

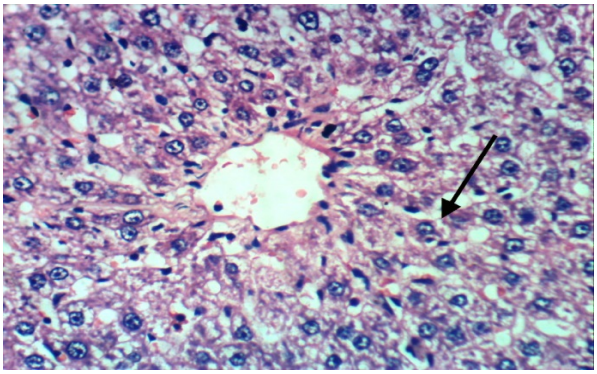

Figure 4. Liver of rat from group 4 showing slight vacuolar degeneration of hepatocytes (H & E × 400).

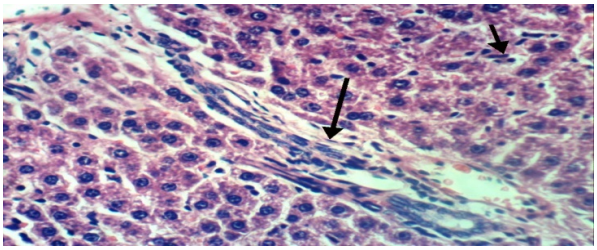

Figure 5. Liver of rat from group 5 showing Kupffer cells activation and strands of fibroblasts proliferation in the portal triad (H & E × 400).

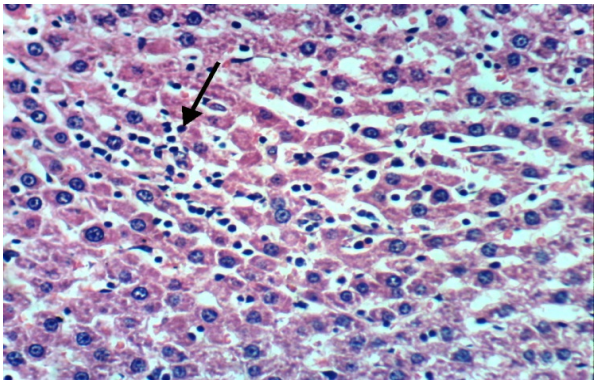

Figure 6. Liver of rat from group 6 showing sinusoidal leucocytosis (H & E × 400).

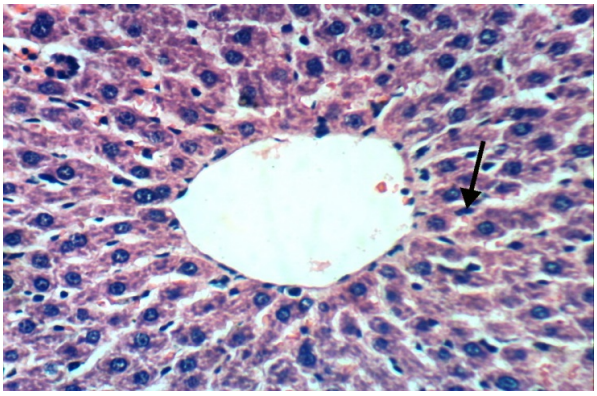

Figure 7. Liver of rat from group 7 showing slight Kupffer cell activation (H & E × 400).

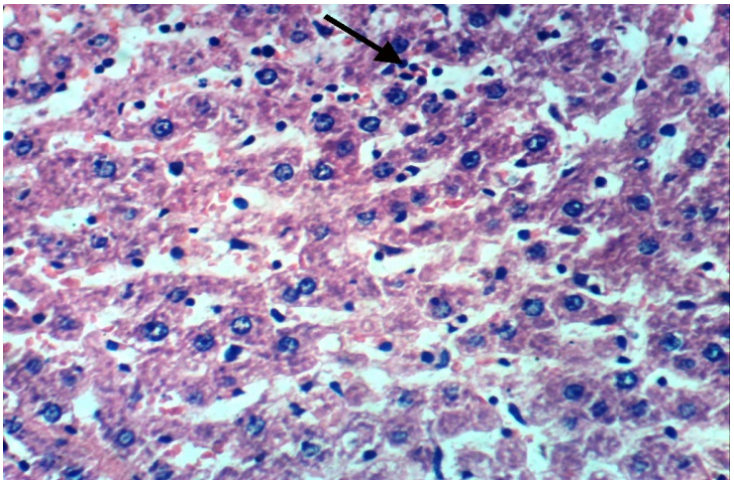

Figure 8. Liver of rat from group 8 showing few leucocytes in the hepatic sinusoids (H & E × 400).

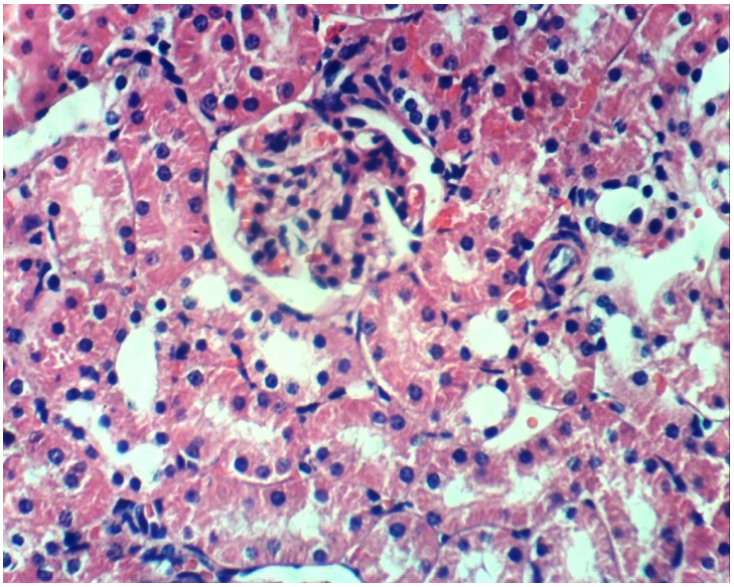

Figure 9. Kidney of rat from group 1 showing the normal histological structure of renal tubules (H & E × 400).

3.4. Histopathological Examination of Kidneys

Microscopically, kidneys of rat from group 1 showed the normal histological structure of renal tubules (**Figure 9**). Meanwhile, kidneys of rat from group 2 revealed vacuolation of epithelial lining renal tubules as well as congestion of glomerular tuft and renal blood vessels (**Figure 10**). Meanwhile, kidneys of rats from groups 3 and 4 revealed no hiatopathological changes (**Figure 11** and **Figure 12**, respectively). On the other hand, kidneys of rats from group 5 showed vacuolation of renal tubular epithelium (**Figure 13**). Some sections from group 6 revealed no histopathological changes (**Figure 14**). However, kidneys of rats from groups 7 and some sections from group 8 revealed no histopathological changes (**Figure 15** and **Figure 16**, respectively).

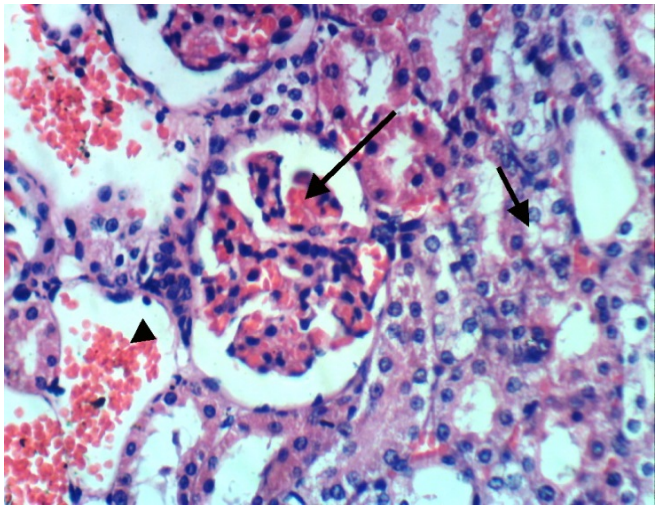

Figure 10. Kidney of rat from group 2 showing vacuolation of epithelial lining renal tubules, congestion of glomerular tuft and renal blood vessels (H & E × 400).

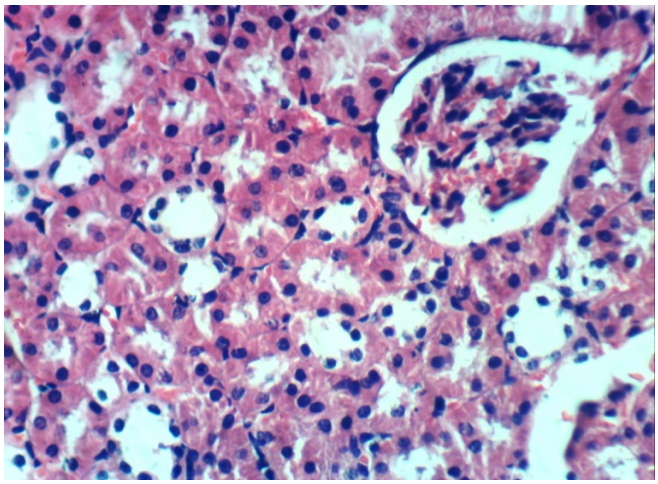

Figure 11. Kidney of rat from group 3 showing no histopathological changes (H & E × 400).

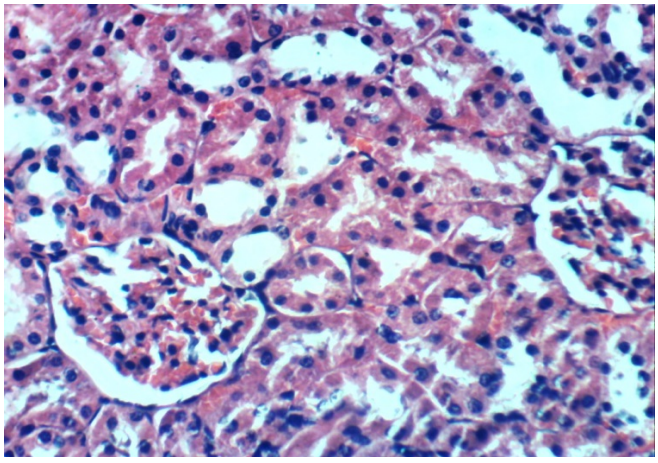

Figure 12. Kidney of rat from group 4 showing no histopathological changes (H & E × 400).

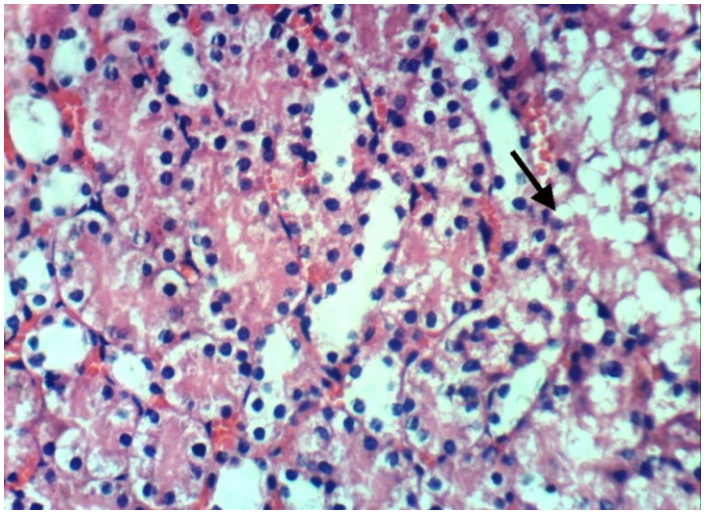

Figure 13. Kidney of rat from group 5 showing vacuolation of renal tubular epithelium (H & E × 400).

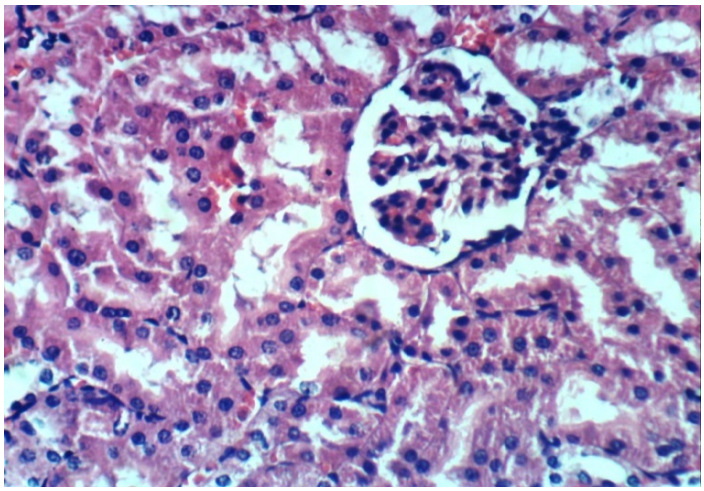

Figure 14. Kidney of rat from group 6 showing no histopathological changes.

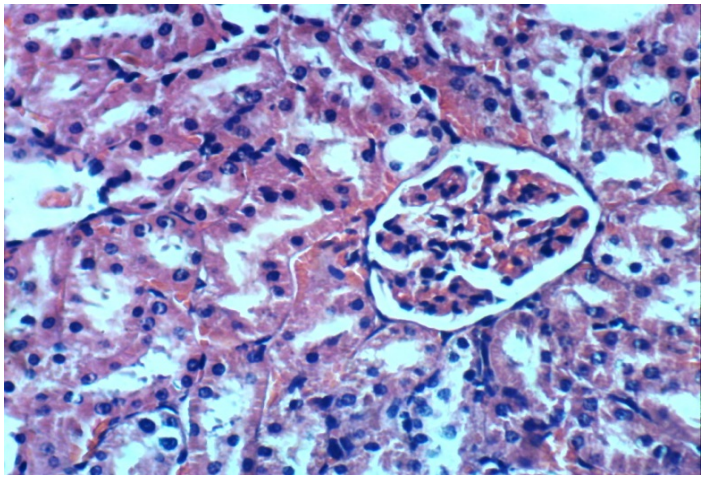

Figure 15. Kidney of rat from group 7 showing no histopathological changes (H & E × 400).

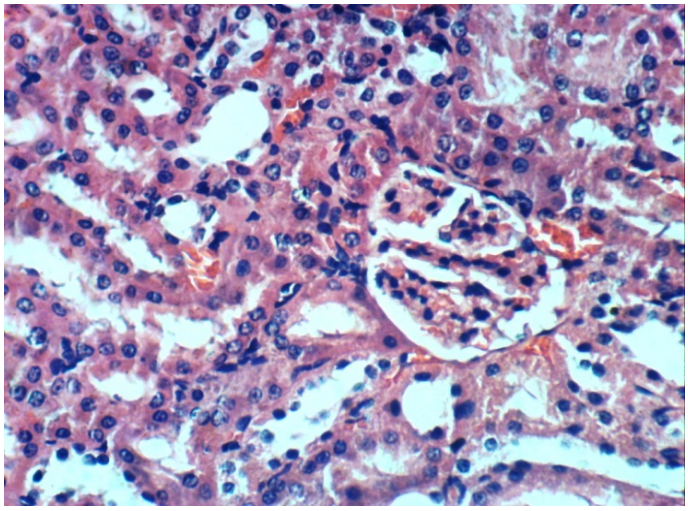

Figure 16. Kidney of rat from group 8 showing no histopathological changes (H & E × 400).

4. Conclusion

There is evidence of a hepatoprotective activity of *A. vulgaris* on the polyphenolic and flavonoids compounds of plant leaves, which have potent antioxidant properties.

References

[1] Blumenthal, M., Klein, J. and Rister, R. (1999) The Complete German Commission E monographs. Integrative Medical Communication, Newtown, Boston.

[2] Said, O., Saad, B., Fulder, S., Khalil, K. and Kassis, E. (2011) Weight Loss in Animals and Humans Treated with "Weigh Level". A Combination of Four Medicinal Plants Used in Traditional Arabic and Islamic Medicine. *Evidence-Based Complementary and Alternative Medicine*, Article ID: 874538.

[3] Saad, B., Azaizeh, H., Abu-Hijleh, G. and Said, O. (2006) Safety of Traditional Arab Herbal Medicine. *Evidenced Based Complementary and Alternative Medicine*, **3**, 433-439. https://doi.org/10.1093/ecam/nel058

[4] Olatosin, T., Akinduko, D. and Uche, C. (2013) Evaluation of the Hepatoprotective Efficacy of *Moringaoleifera* Seed Oil on CCl_4-Induced Liver Damage in Wistar Albino Rats. *The International Journal Engineering and Science*, **2**, 13-18.

[5] Devaraj, E. (2016) Hepatoprotective Properties of Dandelion. *Journal of Applied Pharmaceutical Science*, **6**, 202-205. https://doi.org/10.7324/JAPS.2016.60429

[6] Ung, C.Y., Lam, S.H., Zhang, X., Li, B. and Gongg, Z. (2011) Existence of Inverted Profile in Chemically Responsive Molecular Pathways in the Zebrafish Liver. *PLoS One*, 6, e27819. https://doi.org/10.1371/journal.pone.0027819

[7] Ezejindu, D.N., Udemezue, O.O. and Chinweife, K. (2014) Hepatoprtective Effects of *Moringa oleifera* Extract on Liver of Wistar Rats. *International Journal of Medical Research and Health Sciences*, **3**, 23-27.

[8] Halaby, M.S., El-Din, M.M. and Emara, N.E. (2015) Influence of *Moringa oleifera* on Non-Alcoholic Fatty Liver in Adult Albino Rats. *Middle East Journal of Applied Science*, **5**, 902-912.

[9] Ozbek, H., Acikara, O.B., Keskin, I., Kirmizi, N.I., Ozbilgin, S., Boz, B.E., Kurtul, E.,

Ozrenk, B.C., Tekin, M. and Saltan, G. (2017) Evaluation of Hepatoprotective and Antidiabetic Activity of *Alchemilla mollis*. *Biomedicine and Pharmacotherapy*, **86**, 172-176. https://doi.org/10.1016/j.biopha.2016.12.005

[10] Slinkard, K. and Singleton, T. (1997) Total Phenolic Analyses: Automation and Comparison with Manual Method. *American Journal of Enology and Viticulture*, **28**, 49-55.

[11] Chen, J.J. and Li, X.G. (2007) Hypolipidemic Effect of Flavonoids from Mulberry Leaves in Triton WR-1339 Induced Hyperlipidemic Mice. *Asia Pacific Journal of Clinical Nutrition*, **16**, 290-294.

[12] Taira, S. (1995) Astringency in Persimmon. In: Linskens, H.F. and Jackson, J.F., Eds., *Fruit Analysis*, Springer, Germany, 97-110.

[13] Anhawange, A.A., Ajibola, V.O. and Oniye, S.J. (2004) Chemical Studies of Seeds of *Moringa oleifera* (Lam.) and *Detarium microcarpum* (Guill and Sperr). *Journal of Biological Sciences*, **4**, 711-715. https://doi.org/10.3923/jbs.2004.711.715

[14] Goupy, P., Hugues, M., Boivin, P. and Amiot, M.J. (1999) Antioxidant Compounds of Barley (*Hordeum vulgare*) and Malt Extracts. *Journal of the Science of Food and Agriculture*, **79**, 1625-1634. https://doi.org/10.1002/(SICI)1097-0010(199909)79:12<1625::AID-JSFA411>3.0.CO;2-8

[15] Mattila, P., Astola, J. and Kumpulainen, J. (2000) Determination of Flavonoids in Plant Material by HPLC with Diode-Array and Electro-Array Detections. *Journal of Agriculture and Food Chemistry*, **48**, 5834-5841. https://doi.org/10.1021/jf000661f

[16] Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995) Use of Free Radical Method to Evaluate Antioxidant Activity. *Food Science and Technology*, **28**, 25-30.

[17] Reeves, P.G., Nielsen, F. and Fahey, G. (1993) AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition AdHoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. *Journal of Nutrition*, **123**, 1939-1951.

[18] Campbell, J.A. (1963) Methodology of Protein Evaluation.

[19] Jayasekhar, P., Mohanan, P. and Rahinam, K. (1997) Hepatoprotective Activity of Ethyl Acetate Extract of Acacia Catechu. *Indian Journal of Pharmacology*, **29**, 426-428.

[20] Chapman, D., Castilla, R. and Cambell, J. (1959) Evaluation of Protein in Foods: A Method for the Determination of Protein Efficiency Ratio. *Canadian Journal of Biochemistry and Physiology*, **37**, 697-686. https://doi.org/10.1139/y59-074

[21] Tietz, N.W., Burtis, C.A., Ashwood, E.R. and Saunders, W.B. (1999) Textbook of Clinical Chemistry. 3rd Edition, W. B. Saunders Co., Philadelphia, 676-684.

[22] Burtis, C.A., Tietz, N.W., Ashwood, E.R. and Saunders, W.B. (1999) Textbook of Clinical Chemistry. 3rd Edition, W. B. Saunders Co., Philadelphia, 1429-1431.

[23] Tietz, N.W. and Saunders, W.B. (2012) Textbook of Clinical Chemistry. 5th Edition, W. B. Saunders Co., Philadelphia.

[24] Fossati, P., Prencipe, L. and Berti, G. (1980) Use of 3,5-Dichloro-2-Hydroxybenzenesulfonic Acid/4-Aminophenazone Chromogenic System in Direct Enzymatic Assay of Uric Acid in Serum and Urine. *Clinical Chemistry*, **26**, 227-231.

[25] Young, D.S. and Friedman, R.B. (2001) Effects of Disease on Clinical Lab. Tests, 4th Edition, AACC.

[26] Bancroft, D., Stevens, A. and Turne, R. (1996) Theory and Practice of Histological Techniques. 4th Edition, Churchill Living Stone, Edinburgh.

[27] Armitage, P.G., Berry, S. and Matthews, J.M. (2002) Statistical Methods in Medical Research. 4th Edition, Blackwell Science Ltd., Hoboken. https://doi.org/10.1002/9780470773666

[28] European Pharmacopoeia (2008) 6.0 (Vol. 2) Alchemillae Herba.01/2008:1123.

[29] Gupta, M., Sasmal, S., Majumdar, S. and Mukherjee, A. (2012) HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants. *International Journal of Pharmacognosy and Phytochemical Research*, **4**, 162-167.

[30] Al-Qarawi, A.A., Al-Damegh, M.A. and El-Mougy, S.A. (2002) Effect of Freeze Dried Extract of *Olea europaea* on the *Pituitary thyroidaxis* in Rats. *Phytotherapy Research*, **16**, 286-287. https://doi.org/10.1002/ptr.855

[31] Shrivastava, R., Cucuat, N. and John, G. (2007) Effects of *Alchemilla vulgaris* and Glycerine on Epithelial and Myofibroblast Cell Growth and Cutaneous Lesion Healing in Rats. *Phytotherapy Research*, **21**, 369-373. https://doi.org/10.1002/ptr.2060

[32] Ergene, B., Acikara, O., Bakar, F., Saltan, G. and Nebdoglu, S. (2010) Antioxidant Activity and Phytochemical Analysis of *Alchemilla persica rothm. Journal of Faculty of Pharmacy*, **3**, 145-154.

[33] Abdulrahman, K., Al-Asmari, A., Al-Elaiwi, A., Athar, M., Tariq, M., Al Eid, A. and Al-Asmary, S. (2014) A Review of Hepatoprotective Plants Used in Saudi Traditional Medicine. *Evidence-Based Complementary and Alternative Medicine*, **2014**, Article ID: 890842. https://doi.org/10.1155/2014/890842

[34] Salman, M.M., Kasem, N.R.A. and Saleh, N.H.M. (2016) Comparative Effects of Scorpion Venom and Aqueous Basil (*Ocimum basilicum*) Leaves Extracts on CCl_4-Induced Toxicity in Albino Rats. *Journal Liver*, **5**, 7.

تأثير رجل الأسد علي وظائف الكبد و الكلي في الفئران المعاملة برابع كلوريد الكربون

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يهدف هذا العمل إلى دراسة تأثير نشاط مضادات الأكسدة لنبات رجل الأسد (*Alchemilla vulgaris*) بتركيزات مختلفة لتوفير المزيد من الحماية ضد أمراض الكبد المزمنة. أظهرت النتائج أن أوراق رجل الأسد المجففة غنية في المحتوى الكلي من الفينولات العديدة والفلافونويدات (٣٩٥,٦٥ و ١٨٣,١٠ مللجرام / ١٠٠ جم على التوالي). وقد إنعكست تلك النتائج على نشاط مضادات الأكسدة النشاط (DPPH) حيث لوحظ أن نشاط مضادات الأكسدة في أوراق رجل الأسد المجففة كانت مرتفعة (١٣١,٧٤٪). وكانت مركبات الفينولات العديدة الرئيسية هي حمض البنزويك (١٠٨٤,٦٣ جزء في المليون) تليها حمض الإيلاجيك والكاتيكول والكاتشين (٦١٤,١٦ و ٥٨٠,٥٤ و ٥٦٦,٥٣ جزء في المليون على التوالي) ثم حمض السالسليك و حمض البروتوكاتشويك (٤٧٩,٧١ و ٤٤٤,٤٣ جزء في المليون على التوالي). أشارت نتائج المركبات الفلافونوية إلي أن أعلى محتوى كان في ليوتو-٦-أرابينوز-٨جلوكوز و أبجنين-٦-رامينوز-٨-جلوكوز والأسيتين و النارينجين و اللوتولين (٤٠,٠١ ؛ ١٥,٠٤ ؛ ٨,٠٧ ؛ ٦,٦٤ و ٦,٤٢ جزء في المليون على التوالي).

تم إستخدام ٥٦ من ذكور الفئران الألبينو في التجربة البيولوجية. تغذت الفئران على الوجبة الأساسية لمدة أسبوعين قبل بداية التجربة. في البداية، تم تغذية الفئران المقسمة إلى ثمانية مجموعات رئيسية على الوجبات الغذائية لمدة ٤٥ يومًا على النحو التالي: تم تغذية المجموعة الضابطة السالبة (المجموعة الأولى) على النظام الغذائي الأساسي. تم تغذية تسعة وأربعين فأر على النظام الغذائي الأساسي و المعاملة برابع كلويد الكربون في زيت البارافين (٥٠ ٪ ح / ح ، ٢ مل / كجم) مرتين بالحقن تحت الجلد لإحداث تلف الكبد المزمن ، ثم التقسيم إلى ٧ مجموعات مرقمة من المجموعة ٢ إلى المجموعة ٨. تغذت فئران المجموعة الضابطه الموجبة على النظام الغذائي الأساسي حتى نهاية التجربة (المجموعة الثانية). المجموعة ٣ و ٤ تم معاملة الفئران ٥٠ و ١٠٠ جزء في المليون من المستخلص الإيثانولي للأوراق على التوالي. أيضا المجموعة ٥ و ٦ تمت معاملة الفئران ٥٠ و ١٠٠ جزء في المليون المستخلص المائي للأوراق على التوالي. تم تغذية جميع المستخلصات عن طريق الفم كل يوم. بينما تعاملت الفئران في المجموعة ٧ مع أوراق رجل الأسد المجففة ١٪ و ٢٪. في نهاية فترة التجربة، تم جمع مصل الدم لتحديد وظائف الكبد ووظائف الكلى. تمت أخذ الكبد جراحياً للوصف التشريحي للأنسجة. كشفت النتائج أن التسمم برابع كلوريد الكربون تسبب في إختلال وظائف الكبد وذلك بارتفاع مستويات AST و ALT و ALP

والبليروبين الكلي في المصل، في حين لوحظ إنخفاض معنوي في الألبيومين في المصل في مجموعة رابع كلوريد الكربون.

تشريحيا تسبب رابع كلوريد الكربون في إحتقان الأوردة المركزية، تكون دهون بخلايا الكبد، وإلتهاب الخلايا البؤرية في الكبد. عند المعاملة برجل الأسد بأشكاله وتركيزاته المختلفة يخفف من هذه الآثار الضارة ويخفف بشكل ملحوظ التغيرات المرضية والكيميائية الحيوية الناجمة عن رابع كلوريد الكربون خاصة مع مسحوق الأوراق بتركيز ٢% و ١٠٠ جزء في المليون من المستخلص الإيثانولي. لذلك لخصت نتائج هذه الدراسة إلى أنه يمكن إقتراح رجل الأسد لحماية تسمم الكبد الناجم عن رابع كلوريد الكربون في الفئران. كشفت النتائج أيضًا إلي أن تأثير رجل الأسد لحماية الكبد يعزى إلى محتواه من مضادات الأكسدة وتأثيرها علي إلتقاط الشقوق الحرة.

الكلمات الدالة: رجل الأسد – الفينولات العديدة – الفلافونيدات – مرض الكبد المزمن – وظائف الكبد و الكلي – التقييم التشريحي.

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