Protective effect of Worm-wood (Artemisia absinthium L.) and Lion's foot (Alchemilla vulgaris L.) Against L-Arginine- Induced Chronic Renal Failure in Rats and their Fortified to Hamburgers.

By

Rania Shams El Deen Fakher El Deen

Home Economics Dept., Faculty of Specific Education,
Tanta University, Egypt.

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Protective effect of Worm-wood (Artemisia absinthium L.) and Lion's foot (Alchemilla vulgaris L.) Against L-Arginine- Induced Chronic Renal Failure in Rats and their Fortified to Hamburgers.

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Abstract:

Background: The prevalence of chronic kidney disease (CKD) has dramatically increased in the past two decades and has become a significant public health problem globally. The present work aimed to highlight the protective effect of dried Lion's foot (Alchemilla vulgaris) and wormwood (Artemisia absinthium) and their mixture against L-arginine- induced chronic renal failure. Also, the preparation of hamburgers that are fortified with lion's foot and worm-wood powders as an available product rich in bioactive components to help kidney patients. Material and methods: Forty-eight rats were divided into 8 groups (6 rats each) as follow: negative control (G1): fed on the basal diet, positive control (G2): a fed basal diet containing 2% L-arginine to induce chronic renal failure, G (3 and 4): same positive control + 5% of A. vulgaris and A. absinthium, respectively, G (5 and 6): same positive control + 10% of A. vulgaris and A. absinthium respectively, G (7 and 8): same positive control + mixture of (A. vulgaris and A. absinthium) at 5 and 10%, respectively. Results: showed that administration of A. vulgaris and A. absinthium and their mixture at all dosages significantly improved, rats' body weight gain percentage, feed intake, reduced the elevated serum levels of liver functions (ALT, AST, ALP, albumin and total bilirubin), and kidney functions (creatinine, urea and uric acid), potassium, while serum sodium levels increased compared to L-arginine group. These were associated with a significant increment of serum total antioxidant, SOD, GSH-RX and CAT meanwhile, a significant decrement of MDA oxidative stress biomarker was achieved. The histopathologic evaluation suggested that A. vulgaris and A. absinthium and their mixture decreased hepatic and renal necrosis induced by L-arginine.

* Home Economics Dept., Faculty of Specific Education, Tanta University, Egypt.

The panelists accepted hamburgers up to 5% lion's foot and only 2.5% for wormwood. Conclusion: It could be concluded that A. Vulgaris and A. absinthium showed promising renal and hepatoprotective activities against adversely L-arginine and these effects may be due to their antioxidant contents, providing scientific support for use of these plants in the treatment of kidney and liver disorders.

Keywords: Wormwood (Artemisia absinthium), lion's Foot (Alchemilla vulgaris), chronic kidney disease, Liver and Renal Function, Antioxidants status, Histopathological Evaluation.

Introduction

Chronic kidney disease (CKD) has been considered one of the most diseases that are responsible for the world population mortality over the last three decades **Foreman *et al.* (2018)**. CKD is defined as the presence of kidney damage or an estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 m^2, persisting for 3 months or more, irrespective of the cause. Moreover, the pathological abnormalities by renal biopsy or imaging studies, the urinary sediment abnormalities, or the elevation of albumin urinary excretion rate all refer to kidney damage **(Inker *et al.*, 2014)**. The symptoms of CKD include malnutrition, inflammation, cardiopulmonary, alteration of the autonomic and central nervous systems activities, vascular and bone disease **(Zoccali *et al.*, 2017)**. In addition, reduction of renal function results in hypertension, which can cause heart failure and death **(Zoccali *et al.*, 2015).** The most common pathological manifestation of CKD, regardless of the initiating insult or disease, is some form of renal fibrosis **Lu *et al.* (2015).** Vascular endothelium damage occurs early in CKD and develops along with the disease progression **(Roumeliotis *et al.*, 2020).**

Prevention and early detection of CKD, limiting the progression of the primary causes and attention to secondary factors that lead to persistent nephron loss are the main tools for managing patients with CKD. On the other hand, the cornerstones of treatment are control of blood pressure, inhibition of the renin-angiotensin system, and disease-specific interventions **(Romagnani *et al.,* 2017)**. It has been shown that the intake of exogenous antioxidants prevents inflammation, atherosclerosis and oxidative stress in CKD patients. Furthermore, some antioxidants have been suggested to exert uric acid- lowering properties. Dietary polyphenols and flavonoids reduced uric acid levels, suppressed oxidative stress, and protected from kidney damage in multiple animal studies **Roumeliotis *et al.* (2019). Cicero *et al.* (2017)** reported that treatment with polyphenols significantly reduced circulating uric acid. Also, there is a growing body of evidence showing that flavonoids exert significant antioxidant and hypouricemic activities in vitro and in vivo **Pauff and Hille (2009)**.

Lion's foot (*Alchemilla Vulgaris L.*) which is widely known as lady's mantle, is traditionally used for the treatment of women's illnesses, wounds **(Said *et al.,* 2002; Tasic, 2012** and **Mills and Hutchins 2013),** upper digestive tract and diarrhea cause of its strong anti-inflammatory and antimicrobial activities **(Falchero *et al.,* 2009** and **Kaya *et al.* 2012)**. It has been used to relieve stomach and intestine pain, asthma, and obesity (**Afifi and Abu-Irmaileh 2000** and **Aburjai *et al.,* 2007),** for the treatment of menstruation ailments and headache **(Saric-Kundalic *et al.,* 2011)** and skin diseases **(Saad *et al.,* 2005)**. It possesses astringent, antioxidative, antimutagenic, anticancer properties **(Vlaisavljevic *et al.,* 2019),** antidiabetic **(Özbilgin *et al.,* 2019).** It was used against hormonal imbalances **(Lans *et al.,* 2006),** reproductive disorders **(Viegi *et al.,* 2003)** and to inhibit the improvement of cardiovascular diseases and cystic fibrosis **(El-Hadidy *et al.* 2019)**. Furthermore, *A. Vulgaris* extract has been demonstrated to have vasorelaxant and hypotensive properties **(Takir *et al.,* 2015)**. **Blumenthal *et al.* (1999)** stated that the official recommended dosage for the lion's foot is 5-10 g/day of the dried plant**. El-Hadidy *et al.* (2018)** attributed the antioxidants activities of *A. Vulgaris* to its high polyphenols, flavonoids, tannins and saponins contents. Also, **Mradu *et al.* (2012)** recorded that *A. vulgaris* has antioxidant and anti-inflammatory properties due to its high concentrations of catechin, catechol, salicylic acid, benzoic acid, ellagic acid and vanillic acid.

Artemisia absinthium L is a perennial herb belonging to the Asteraceae family, commonly known as wormwood. The beneficial effects of *A. absinthium* have been demonstrated in folk medicine and clinical trials **(Batiha *et al.*, 2020).** It is an antioxidant-rich plant that can be consumed as a part of the daily diet **(Craciunescu *et al.*, 2012** and **Lee *et al.*, 2013)**, which exhibited several pharmacological activities, such as antimicrobial **(Juteau *et al.*, 2003),** antifungal activity **(Saban *et al.*, 2005)**, antiviral, hypoglycemic **(Moslemi *et al.*, 2012)**, anthelmintic **(Meschler and Howlett, 1999),** wound healing, anti-inflammatory, and cardiovascular diseases **(Ahamad *et al.*, 2019 and Sultan *et al.*, 2020),** provide hepatoprotective **(Amat *et al.*, 2010)**, nephroprotective effects (**Bora and Sharma, 2010)**. *A. absinthium* has nephroprotection against immunoglobulin A nephropathy **(Krebs *et al.*, 2010).** Furthermore, by regulating oxidative stress *A. absinthium* reduced renal toxicity induced by azathioprine administration in rats **(Farzaneh *et al.*, 2015).** It has shown antioxidant, antitumor and anticancer activities **(Krebs *et al.*, 2009; Shafi *et al.*, 2012; Koyuncu, 2018; Ali and Abbasi 2020** and **Nazeri *et al.*, 2020)**. Its phytochemicals may protect against nephrotoxicity induced by cisplatin **(Mukhopadhyay *et al.*, 2012).** *A. absinthium* contains many phytochemical compounds: lactones, terpenoids, essential oils, organic acids, resins, tannins, and phenols **(Omer *et al.*, 2007)**. It also contains flavonoids (e.g., quercitin), flavonoid glycosides and phenolic acids which contribute to the free radical scavenging mechanism **(Kordali *et al.*, 2005)**.

MATERIALS AND METHODS

Materials:

- *Alchemilla Vulgaris, Artemisia absinthium,* red beef, animal fats and other ingredients used to prepare burgers were purchased from the local market in Tanta City, Egypt.
- Casein, minerals, vitamins mixture and L-arginine were obtained from El- Gomhoria Company.

- Adult of Sprague Dawley albino male rats were obtained from The Animal Colony, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

Methods:

Burger preparation

Meat and fat were grounded separately in a meat grinder by passing meat through a plate having 6 mm holes. Burgers were formulated according to **Aleson-Carbonell, *et al.*, (2005)** containing the following ingredients: 60% red beef meat, 20% animal fat, 1.5% salt, 0.2% cumin, 0.3% ground black pepper, 0.2% red pepper and 18% (w/w) ice water. The burger formula was divided and treated with two concentrations of *Alchemilla vulgaris, Artemisia absinthium* and their mixture (by 2.5 and 5%), each. Control without any additions was used in all assays. The beef burgers were frozen at -18 ºC.

Thermal treatment

The frozen burgers were thawed at 4°C for 12 hours, then it was cooked for 4 minutes for each side of the burger (8 min each sample of burgers) at 220°C using an electric grill (Genwex GW-066) and the distance between the samples and the heat source was 4cm **(Oliveira *et al.*, 2016)**.

Sensory evaluation:

Beef burgers were assessed for a ban umber of sensory characteristics by fifteen members of the department's staff of home economics, Faculty of Specific Education, Tanta University. Panelists were instructed to evaluate color, texture, flavor, odor, hardness, juiciness, and overall acceptability. The panelists were chosen based on previous experience in evaluating burgers according to **Yi *et al*. (2012).**

Biological evaluation

A total of (48) rats weighing 110 ± 5g were housed in cages and kept under normal healthy conditions. All rats were provided water and a standard diet ad-libitum for 7 days as an adaptation period, then they were divided into 8 groups (6 rats each) as follows:

Group (1): fed on a standard diet *(Negative control).*

Group (2): fed on a standard diet containing 2% L-arginine to induce chronic renal failure **(Yokozawa *et al*., 2003)** *(positive control).*

Groups (3 and 4): As group 2 + 5% and 10% of *Alchemilla vulgaris*, respectively.

Groups (5 and 6): As group 2 + 5%and 10% of *Artemisia absinthium*, respectively.

Groups (7 and 8): As group 2 + mixture of (*Alchofemilla vulgaris* and *Artemisia absinthium)* at (5 and 10%) for 4 weeks.

During the experimental period, rats were weighed each week and feed intake was recorded daily. Bodyweight gain percent (BWG %) was determined **(Chapman *et al*., 1959).** Rats fasted overnight then were sacrificed, collected blood and left to clot into a satirized centrifuge tube. The blood samples were centrifuged at 3000 rpm for 15 minutes, neatly serum was separated and frozen at - 20 C in the plastic tube till analysis.

Biochemical analysis:

Estimation of serum urea was carried out as outlined by **Henry *et al.,* (1974),** uric acid was measured by **Haisman and Muller (1977)** method and creatinine **(Bartels and Bohmer 1971).**

The activities of L- Alanine aminotransferase (ALT**)** and L-Aspartate aminotransferase (AST) were determined as the described method of **(Reitman and Frankel 1975)**, alkaline phosphatase (ALP) and serum albumin were estimated by **Tietz *et al*., (1999)** and **(Webster, 1974)** methods, respectively.

Serum potassium (K) and sodium (Na) were measured colorimetrically (**Henry *et al.,* 1974** and **Henry, 1964,** respectively).

Determination of serum malondialdehyde (MDA) was as the described method of (**Ohkawa *et al.,* 1979)**, glutathione reeducates (GSH-RX) (**Ellman, 1959)**, the activities of catalase (CAT) and superoxide dismutase (SOD**)** were measured by **Aebi (1984)** and **(Kakkar *et al.,* 1984)** methods, respectively.

Histopathological examination:

Rat's organs (liver and kidney) were subjected to histopathological assay at *the Histological lab, Faculty of Veterinary Medicine, Cairo University* according to **Bancroft et al. (1996).**

Statistical analysis:

All data were subjected to one–way analysis of variance (ANOVA) and represented as mean ± SD and the data normality was conducted by Kolmogorov-Smirnov test. The comparisons among different groups were performed using Tukey post hoc **(Snedecor and Cochran, 1967).**

RESULTS AND DISCUSSION

Effect of wormwood, lion's foot, and their mixture on nutritional parameters

Results from Table (1) showed that all animals under this study had similar initial body weights and at the end of the experiment, all groups gave positive BWG. It could be noticed that rats administrated with 2 % L-arginine showed a significant decrement in feed intake, FBW and BWG %; meanwhile, showed a significant increment in organ weight/BW. ratio in comparison to all groups. However, the treatment with worm-wood, lion's foot, and their mixture result in marked improvement in the rats feed intake, FBW and BWG%, on the other side decreased organs weight/BW ratio significantly, especially lion's foot at the level of 5 and 10 % which recorded the best mitigating ability against arginine toxicity followed by the mixture of worm-wood and lion's foot at the level of 5%.

These results are in accordance with **El-Said (2008); Kabil (2011); Shahat (2007) and Yokozawa *et al.* (2003)** who confirmed that administrated rats with 2% L-arginine led to a significant reduction in feed intake and BWG however, it results in a significant elevation in liver and kidney weights comparison with the negative control group. However, **El-Hadidy *et al.* (2018)** found that *A. vulgaris* whether powder or extract led to a significant elevation in BWG % and feed intake compared with CCL_4-induced toxicity rats. These improvements were adapted by **Al-Qarawi *et al.* (2002)** who stated that flavonoids might be improved the metabolic rate, regulate digestive enzymes and metabolic stimulation. Also, *A. vulgaris* improved the body weight of rats administrated with ZnSO4 **(Mohammed, 2020).**

Table (1): Effect of wormwood, lion's foot, and their mixture on nutritional parameters

Parameters / Groups	Bodyweight		Organs weight/body weight %		Bodyweight Gain %	Feed Intake (g/d)
	Initial weight (g)	Final weight (g)	Kidney	Liver		
G1: -ve control	112.0 ± 2.10	236.7 [a] ± 3.01	0.53 [e] ± 0.02	2.44 [e] ± 0.07	111.39 [a] ± 5.64	20.17 [a] ± 0.98
G2: +ve control	112.83 ± 1.33	170.67 [f] ± 5.13	0.93 [a] ± 0.03	5.42 [a] ± 0.06	51.27 [f] ± 4.81	13.33 [d] ± 1.75
G3: Lion's foot 5%	113.5 ± 1.87	221.33 [b] ± 2.80	0.73 [b] ± 0.04	3.00 [b] ± 0.07	96.71 [b] ± 6.82	18.00 [a,b] ± 1.41
G4: Worm-wood 5%	114.17 ± 1.17	203.17 [d] ± 5.98	0.63 [c,d] ± 0.04	3.01 [b] ± 0.08	77.98 [d, e] ± 5.90	16.33 [b,c] ± 1.21
G5: Lion's foot 10%	111.83 ± 1.72	213.50 [c] ± 2.51	0.66 [b,c] ± 0.07	2.79 [c] ± 0.10	90.93 [b,c] ± 2.94	17.83 [a,b] ± 1.47
G6: Worm-wood 10%	112.33 ± 1.21	195.00 [e] ± 3.46	0.58 [c,d,e] ± 0.04	2.63 [d] ± 0.07	73.59 [e] ± 2.18	15.83 [b,c] ± 1.33
G7: Lion's foot 5% + Worm-wood 5%	113.83 ± 1.47	212.50 [c] ± 1.87	0.57 [d,e] ± 0.04	2.88 [b,c] ± 0.03	86.71 [c,d] ± 3.55	17.00 [b] ± 1.26
G8: Lion's foot 10% + Worm-wood 10%	113.33 ± 2.25	208.67 [c,d] ± 2.73	0.55 [e] ± 0.03	2.49 [d, e] ± 0.16	84.17 [c,d] ± 4.13	14.17 [c,d] ± 1.17

Mean values in each column having different superscripts (a, b, c, d, e) are significant.

Means with the same letter are insignificantly different.

Effect of lion's foot, wormwood, and their mixture on renal functions:

Table (2) presented the changes in serum urea nitrogen, creatinine, and uric acid as a result of administration of L-arginine to experimental diets. From the obtained results, it could be observed that G2 (positive control) showed severe impairment in renal functions, manifested by increased serum levels of urea nitrogen, uric acid and creatinine in comparison to G1 (normal control) and all other treatment groups. The treatment with lion's foot, wormwood and their mixture reversed the effects of L-arginine, as there were significant decreases in the levels of urea nitrogen, creatinine, and uric acid compared to the control positive group. The best results were recorded to the mixture of lion's foot and wormwood (G8 followed by G7) at the levels of 10 and 5%, respectively.

The results of renal functions support the reports of **Yokozawa *et al.* (2003)** who stated that feeding rats on 2%L-arginine for 30 days caused a significant increment in serum urea nitrogen and creatinine levels. Also, **Baylis (2006); El-Said (2008); Kabil (2011)** and **Shahhat (2007)** found that administration of 2% L-arginine for 4 weeks results in chronic renal failure which was identified by increased serum levels of urea, uric acid and creatinine. Supplementation with *A.vulgaris* restored the high levels of urea, uric acid and creatinine in CCL_4 treated rats **(Al-Asmari *et al.*, 2014)**. **El-Hadidy *et al. (*2018)** observed **s**ignificant reductions in serum levels of urea, uric acid and creatinine of rats fed on *A.vulgaris* powder or extracts in comparison to the positive control group and attributed these decrements to its antioxidants content. Dietary polyphenols and flavonoids reduced uric acid levels, suppressed oxidative stress and protected from kidney damage in multiple animal studies **(Roumeliotis *et al.*, 2019). Daradka *et al.* (2014)** showed that serum urea and creatinine were decreased significantly to almost normal levels in diabetic rats treated with *A. absinthium* ethanol extract after alloxan treatment. Also, *A. absinthium* extracts treatment groups significantly decreased the levels of urea and creatinine compared to the diclofenac control group **(Guarniz *et al.*, 2020).** Furthermore, shikimic acid isolated from A. absinthium had renoprotective effects identified by decreasing the high level of serum creatinine and confirmed by the histological kidney recovery in mice with kidney injury induced by cisplatin **(Lee *et al.*, 2020).** Shikimic acid is a natural phenolic compound and possesses antioxidant activity **(Al-Malki, 2019)**.

Table (2): Effect of lion's foot, wormwood and their mixture on renal functions.

Parameters / Groups	Urea (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)
G1: -ve control	47.50 [e] ± 1.69	0.57 [f] ± 0.12	1.75 [d] ± 0.10
G2: +ve control	251.62 [a] ± 15	5.32 [a] ± 0.48	4.44 [a] ± 0.36
G3: Lion's foot 5%	146.81 [b] ± 1.65	1.55 [b] ± 0.05	2.51 [b] ± 0.32
G4: Worm-wood 5%	143.28 [b,c] ± 1.77	1.43 [b,c,d] ± 0.05	2.09 [c,d] ± 0.12
G5: Lion's foot 10%	143.62 [b,c] ± 3.23	1.48 [b,c] ± 0.13	2.15 [b,c] ± 0.28
G6: Worm-wood 10%	148.42 [b] ± 2.01	1.00 [d,e,f] ± 0.33	1.93 [c,d] ± 0.10
G7: Lion's foot 5% + Worm-wood 5%	134.18 [c,d] ± 2.49	1.03 [c,d,e] ± 0.30	2.13 [b,c,d] ± 0.12
G8: Lion's foot 10% + Worm-wood 10%	129.72 [d] ± 3.25	0.77 [e,f] ± 0.09	1.84 [c,d] ± 0.06

Mean values in each column having different superscripts (a, b, c, d, e) are significant.

Means with the same letter are insignificantly different.

Effect of lion's foot, wormwood and their mixture on serum potassium (K) and sodium (Na) levels:

The changes in serum potassium (K) and sodium (Na) values were summarized in table (3). It could be noticed that the L-arginine treatment group results in a significant reduction in the value of serum Na and elevated the K level significantly relative to the normal rats group and all other treatment groups. By the contrary, rats fed on diets containing worm-wood, lion's foot and their mixture reversed the effects of L-arginine as they increased Na serum levels significantly and decreased K serum levels significantly. According to data, groups G4, G6, and G8 recorded the most improvement concerning serum Na values however, groups G6 and G8 concerning serum K values without significant differences compared to the negative control, assuming a powerful action in the concern to the combination of lion's foot and wormwood.

These results are going along with **El-Said (2008)** and **Kabil (2011)** concluded that a 2% L-arginine induced a significant increase in serum potassium level and a significant decrease in serum sodium level in chronic renal failure rats as compared to the negative control. **Koo *et al.* (2018)** concluded that in patients with chronic kidney disease the higher the urinary Na/K ratio over 24 hours, the faster the deterioration of renal function. **Sarafidis *et al.* (2012)** reported that sodium imbalance can be induced in patients with CKD because the ability of kidneys to regulate dilution and concentration becomes impaired as the renal disease progresses. Moreover, chronic kidney disease has reported a significantly higher incidence of hyperkalemia **(Sarafidis *et al.*, 2012).** Also, an analysis of population-based registries found that second episodes of hyperkalemia were documented within 6 months of the initial occurrence in 40% of patients with CKD **(Adelborg *et al.*, 2019).** **Amin *et al.* (2020)** indicated that there is a strong association with the serum increased concentrations of MDA and reduced serum antioxidant, potassium and calcium in obese patients compared to control except serum sodium level increased.

Table (3): Effect of lion's foot, wormwood and their mixture on sodium (Na) and potassium (K).

Groups	NA	K
G1: -ve control	142.00 ± 1.79 [a]	4.41 ± 0.46 [d]
G2: +ve control	120.83 ± 3.13 [d]	8.90 ± 0.82 [a]
G3: Lion's foot 5%	134.67 ± 2.16 [c]	6.71 ± 0.13 [b]
G4: Worm-wood 5%	138.17 ± 3.06 [a,b,c]	5.83 ± 0.52 [b,c]
G5: Lion's foot 10%	137.17 ± 2.79 [b,c]	6.08 ± 0.41 [b]
G6: Worm-wood 10%	140.41 ± 0.46 [a,b]	4.80 ± 0.30 [d]
G7: Lion's foot 5% + Worm-wood 5%	136.95 ± 0.65 [b,c]	6.02 ± 0.49 [b]
G8: Lion's foot 10% + Worm-wood 10%	139.10 ± 0.66 [a,b]	5.04 ± 0.39 [c,d]

Mean values in each column having different superscripts (a, b, c, d, e) are significant.

Means with the same letter are insignificantly different.

Effect of lion's foot and wormwood and their mixture on liver functions:

Results in the table (4) showed that administration of L-arginine increased the value of serum liver enzymes GPT, GOT, ALP, Total bilirubin, and Albumin in comparison to the control negative group. Meanwhile, the treatment with lion's foot, wormwood and their mixture reversed the effect of L-arginine, as there was a significant decrease in levels of GPT, GOT, ALP, Total bilirubin, and Albumin in comparison to the control positive group. The best result was recorded for the mixture of lion's foot and wormwood (G8) at a level of 10% among the other herbs treated groups followed by (G6) worm-wood group at a level of 10%.

These results are in parallel with **Baylis (2006); El-Said (2008); Kabil (2011) and Shahat (2007)** who confirmed that L-arginine- induced chronic renal failure rats have an elevation in the liver enzymes levels. In addition, this finding was confirmed by other studies which reported that L-arginine administration induced albuminuria **(Huang *et al.*, 2021 and Peters *et al.*, 1999)**, as a result of kidney injury and/or protein reabsorption tubular blockade **(Bello *et al.,* 1999)**. On the other hand, *A. vulgaris* powder and extracts diminished the high level of ALT, AST, ALP, and bilirubin significantly, however, serum albumin significantly increased as compared to the CCl_4 treated group (**El-Hadidy *et al.,* 2018).** Also, *A. vulgaris* treatment showed significant reductions in AST and ALT in $ZnSO_4$ treated rats **(Mohammed, 2020).** The hepatoprotective properties of *A. vulgaris* extracts have been demonstrated against CCl_4-induced liver injury **(Bahadir *et al.,* 2017)**. Also, A. *absinthium* extracts treatment groups significantly decreased the levels of AST, ALT and ALP compared to the diclofenac control group **(Guarniz *et al.,* 2020)**. **Amat *et al.* (2010)** and **Ansari *et al.* (2018)** attributed the hepato-protective effect of *A. absinthium* to the decreasing of AST, ALT, and bilirubin levels. These liver function improvements maybe because of the antioxidants content (polyphenols, tannins, and flavonoids) of *A. vulgaris* and *A. absinthium*, which have anti-inflammation and wound healing activities **(Gilani and Janbaz, 1995; Shrivastava *et al.,* 2007; Ergene *et al.,* 2010; Al-Asmari *et al.,* 2014; Ansari and Maiti, 2018** and **Samani *et al.,* 2018)**. The important mechanism of hepatoprotective activity is related to its ability to transfer hydrogen to free radicals, activate antioxidant enzymes, and inhibit oxidases **(Huang *et al.,* 2018).**

Table (4): Effect of lion's foot, wormwood, and their mixture on liver functions.

Parameters / Groups	ALT (U/L)	AST (U/L)	ALP (U/l)	Total bilirubin (mg/ dL)	Albumin (g/dL)
G1: -ve control	34.98 ^{c} ± 0.89	60.96 ^{f} ± 1.61	63.69 ^{f} ± 0.61	0.45 ^{d,e} ± 0.03	3.63 ^{d,e} ± 0.22
G2: +ve control	70.77 ^{a} ± 5.21	108.82 ^{a} ± 2.41	125.42 ^{a} ± 4.54	0.99 ^{a} ± 0.09	6.26 ^{a} ± 0.47
G3: Lion's foot 5%	60.36 ^{b} ± 3.39	90.89 ^{b} ± 1.59	102.80 ^{b} ± 2.42	0.67 ^{b} ± 0.04	4.35 ^{b} ± 0.07
G4: Worm-wood 5%	46.84 ^{c} ± 4.46	82.75 ^{c} ± 3.54	87.32 ^{d,e} ± 5.44	0.58 ^{b,c} ± 0.04	3.59 ^{d,e} ± 0.17
G5: Lion's foot 10%	49.80 ^{c} ± 4.87	76.36 ^{d} ± 1.80	93.26 ^{c} ± 1.96	0.53 ^{c,d} ± 0.06	4.13 ^{b,c} ± 0.21
G6: Worm-wood 10%	35.45 ^{c} ± 2.60	72.82 ^{d} ± 2.44	83.05 ^{e} ± 2.52	0.43 ^{d,e} ± 0.04	3.48 ^{e} ± 0.07
G7: Lion's foot 5% + Worm-wood 5%	49.55 ^{c} ± 2.12	81.80 ^{c} ± 3.04	91.30 ^{c,d} ± 1.95	0.48 ^{c,d,e} ± 0.08	3.91 ^{c,d} ± 0.07
G8: Lion's foot 10% + Worm-wood 10%	36.63 ^{c} ± 2.13	68.22 ^{e} ± 0.80	82.13 ^{e} ± 1.72	0.41 ^{e} ± 0.05	3.70 ^{d,e} ± 0.06

Mean values in each column having different superscripts (a, b, c, d, e) are significant.

Means with the same letter are insignificantly different.

AST: Aspartate transaminase ALT: Alanine transaminase ALP: alkaline phosphatase

Effect of lion's foot, wormwood, and their mixture on plasma oxidative/antioxidant biomarkers:

From the presented data in Table (5) it could be noticed that L-arginine administration caused a demotion in total antioxidants, glutathione reductase (GSH), superoxide dismutase (SOD) and catalase (CAT) levels, however, it showed an elevation of malondialdehyde (MDA) levels relative to the normal control group (-ve). On the other hand, the antioxidants parameters of total antioxidants, SOD, CAT and GR levels showed significant increases in rats fed on a diet containing lion's foot, wormwood and their mixture versus the corresponding positive group (+ve). It's clear that Treatment with lion's foot, wormwood and their mixture at all levels markedly reversed the alterations in biochemical parameters induced by L-arginine. G (8) which was treated with a mixture of lion's foot and wormwood at the level of 10% followed by G (6) which was treated by worm-wood group at the level of 10% had the best result in increasing total antioxidants, SOD, CAT and GSH levels and lowering the elevation of MDA level caused by administration of L-arginine without any significant changes compared to the negative control group (-ve).

These results are in accordance with **Czako *et al.* (1998)** who demonstrated that MDA level was significantly elevated after L-arginine administration however, superoxide dismutase (SOD) and catalase activities decreased significantly. On the other hand, **El-Hadidy *et al.* (2019)** reported that rats fed on diets containing lion's foot powder or extract were a highly significant increase in glutathione reductase meanwhile, recorded a significant decrease in the serum MDA level of CCL_4 treated rats. These improvements may be due to polyphenols and flavonoids content in the lion's foot which affects working as a scavenging free radical to prevent liver cell damage **(Jayathilake *et al.*, 2016)**. *A. vulgaris,* reduced serum MDA levels numerically and suppressed lipid peroxidation in a dose-dependent manner of high environmental temperature in broilers **(Köseman *et al.*, 2021)**. **Boroja *et al.* (2018); Kiselova *et al.* (2006)** and **Vlaisavljevic *et al.* (2019)** attributed the antioxidant properties of *A. vulgaris* to its phenolic compound, especially tannins. **Bora and Sharma (2010)** found that pre-treatment with *Artemisia absinthium* extract significantly decline GSH content, SOD and CAT activities in rats suffering from focal ischemia. *A. absinthium* extract reduced MDA levels in liver injury rats induced by CCL4 however, increased SOD and GSH levels **(Amat *et al.*, 2010)**. Also, **Bagheri *et al.* (2020)** showed that the *A. absinthium* extract significantly improved oxidative stress markers (SOD and MDA) in the kidney tissues of diabetic-treated rats. **Al-Malki (2019)** stated that shikimic acid isolated from *A. absinthium* acts as a strong antioxidant agent which increases SOD, GSH levels and decreases MDA in diabetic rats. In vivo, significant inhibition of oxidative stress was found in the central nervous system after oral administration of *A. absinthium* extract. The amount of TBARS also decreased and the concentrations of superoxide and glutathione dismutase increased, which indicates the possibility of using extracts of this plant as antioxidant agents **(Bora and Sharma, 2011 and Kamali, *et al.*, 2015)**, this effect may be related to the phenolic content **(Ali *et al.*, 2013)**, presence of naringenin and caffeic acid **(Hbika *et al.*, 2022)** and shikimic acid **(Saltveit, 2017).** The oxidative stress in kidney cells induced with cisplatin had been regulated using wormwood extract and shikimic acid by decreasing the reactive oxygen species (ROS) accumulation **(Lee et *al.*, 2020)**.

Table (5): Effect of lion's foot, wormwood, and their mixture on plasma oxidative/antioxidant biomarkers.

Parameters / Groups	Total antioxidants	SOD (U/mg tissue)	Catalase	GSH	MDA (nmol/g tissue)
G1: -ve control	1.38 ± 0.05 [d]	212.51 [a] ± 4.49	902.31 ± 10.08 [b]	1079.05 [a,b] ± 39.22	2.02 [d] ± 0.19
G2: +ve control	0.40 ± 0.02 [f]	151.22 [b] ± 3.92	710.74 ± 47.77 [c]	313.61 [e] ± 22.58	24.20 [a] ± 0.91
G3: Lion's foot 5%	1.75 ± 0.03 [c]	183.40 [c] ± 5.19	996.30 ± 7.19 [a]	764.68 [d] ± 8.82	7.51 [b] ± 0.45
G4: Worm-wood 5%	1.16 ± 0.06 [e]	189.20 [c] ± 4.80	979.85 ± 6.32 [a]	1038.36 [b] ± 44.96	4.40 [c] ± 0.65
G5: Lion's foot 10%	2.10 ± 0.07 [a,b]	190.25 [c] ± 1.81	1001.47 ± 8.39 [a]	929.90 [c] ± 35.77	4.01 [c] ± 0.07
G6: Worm-wood 10%	2.19 ± 0.18 [a]	190.57 [c] ± 4.87	993.58 ± 2.47 [a]	1128.04 [a] ± 25.15	2.79 [d] ± 0.16
G7: Lion's foot 5% + Worm-wood 5%	1.88 ± 0.07 [c]	199.60 [b] ± 4.03	985.96 ± 3.58 [a]	936.22 [c] ± 73.21	4.63 [c] ± 0.30
G8: Lion's foot 10% + Worm-wood 10%	2.04 ± 0.04 [b]	204.16 [b] ± 5.46	1002.90 ± 5.59 [a]	1075.05 [a,b] ± 16.22	2.58 [d] ± 0.08

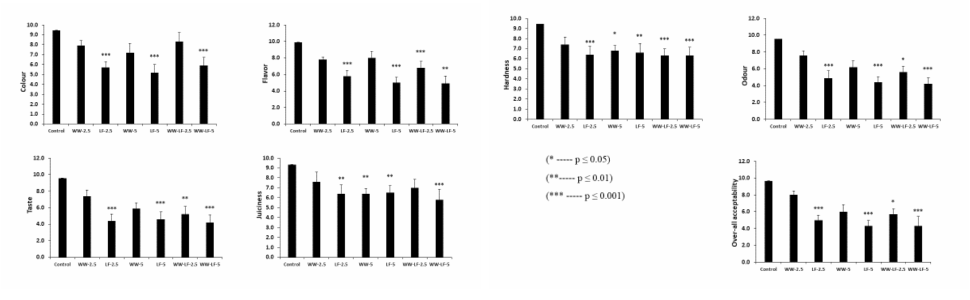
Mean values in each column having different superscripts (a, b, c, d, e) are significant.

Means with the same letter are insignificantly different.

SOD: Superoxide dismutase. GSH: Glutathione reductase. MDA: Malondialdehyde (MDA).

Sensory evaluation of hamburgers fortified with lion's foot, wormwood, and their mixture:

The statistical analysis revealed that sensory properties of hamburgers prepared with the addition of lion's foot (LF), wormwood (WW), and their mixture (WW-LF) at levels of 2.5% and 5% were significant differences for all parameters as shown in Fig. 1 and 2. The highest value was recorded for control burgers followed by lion's foot at a level of 2.5% for all parameters. Generally, a high percentage of wormwood was added, a low score of acceptability, taste, odor, and Flavor was achieved. It can be concluded that the panelists accepted hamburgers up to 5% lion's foot and 2.5% wormwood only. That indicates the lion's foot has a sweet, better taste which can be taken on its own or in an herbal mixture **(El-Hadidy *et al.*, 2019).** In small quantities, *A. absinthium* is recommended for seasoning meat, vegetable soups and fresh vegetables. It is also used as a dye and flavoring in the traditional Korean rice cake "green songpyeon", which is an integral part of the celebration of the "chuseok" thanksgiving festival. In Morocco, *A. absinthium* is added to mint tea **(European Food Safety Authority, 2020)**.



Histopathological examination:

Kidney: Rat's Kidneys in the normal group (G1) and groups (5, 6 and 8) rats showed normal histological tissues (Fig. 3). Meanwhile, the kidney of rats treated with 2% L-arginine alone (G2) revealed atrophy of glomerular tufts and vacuolization of epithelial lining renal tubules (Fig. 4).

Moreover, the kidney of (G3) lion's foot 5% and (G7) mixture of lion's foot and wormwood at the level of 5% showed congestion of kidney blood vessels (Fig. 5). However, the kidney of rats from the group (4) 5% wormwood treatment showed slight congestion of glomerular tufts (Fig. 6). Rat's kidneys treated with Wormwood 10%, lion's foot 10% and the mixture at the level of 10% didn't show any histological changes.

Liver: The liver of male rats in the normal group (G1) and groups (G 5, 6 and 8) didn't show any histopathological changes and showed normal architecture of the central vein and normal sinusoidal (Fig.7). On other hand, liver tissue in the L-arginine group (G2) that induced renal failure figure (8), showed necrosis of hepatocytes associated with inflammatory cells infiltration. But the result of histopathological changes in G (3 and 4) lion's foot 5% and wormwood 5% treatments have shown mild return normal hepatocyte with slight kupffer cells activation (mild response) figures (9 and 10), respectively. While the results in histopathological changes in G (7) mixture of lion's foot and wormwood at the level of 5% treatment has shown small focal hepatic necrosis associated with mononuclear cells infiltration figure (11). Livers tissues which treated with Wormwood 10%, lion's foot 10% and the mixture at the level of 10% showed normal parenchymal tissue with no inflammation figure (7). These results agreed with **Mohammed (2020)** who reported that *A. vulgaris* improved histological changes in ZnSO4 treated rats. Wormwood extract diminished the pathological changes of azathioprine may be due to its antioxidant activities (**Farzaneh *et al.*, 2015)**.

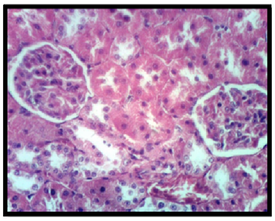


Fig. 3. **Kidney of rats from groups (1, 5, 6, and 8) showed no histopathological changes (H and E x400).**

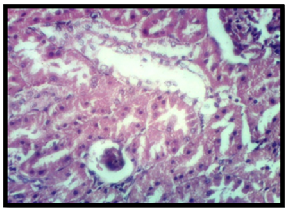


Fig.4. **Kidney of rat from positive control (G2) showing atrophy glomerular tufts and vacuolization of epithelial lining renal tubules (H and E x400).**

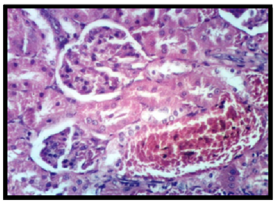


Fig. 5. **Kidney of rat from groups (3 and 7) showing congestion of renal blood vessel (H and E x400).**

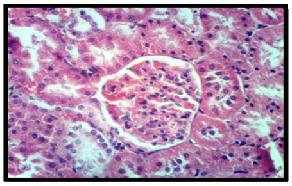


Fig. 6. **Kidney of rat from group 4 showing slight congestion of glomerular tufts (H and E x400).**

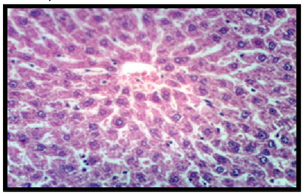


Fig. 7. **Liver of rat from groups (1, 5, 6, and 8) showing no histopathological changes (H and E x400).**

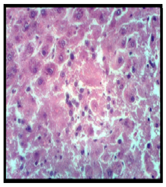


Fig. 8. **Liver of rat from group (2) showing necrosis of hepatocytes associated with inflammatory cells infiltration (H and E x400).**

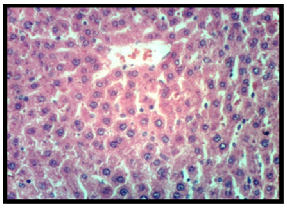


Fig. 9. **Liver of rat from groups (3) showing slight kupffer cells activation (H and E x400).**

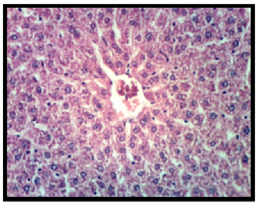


Fig. 10. **Liver of rat from group (4) showing slight kupffer cells activation (H and E x400).**

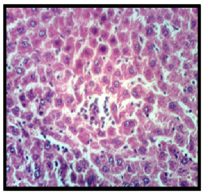


Fig. 11. **Liver of rat from group (7) showing small focal hepatic necrosis associated with mononuclear cells infiltration (H and E x400).**

Conclusion

From this study, it can be stated that lion's foot, wormwood and their mixture have Reno protective, hepatoprotective and antioxidants activities against adverse effects of L-arginine and these may be attributed to their antioxidant contents.

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التأثير الوقائي لرجل الأسد والدمسيسة للفشل الكلوي المزمن المستحث باستخدام *L-Arginine* واستخدامهم فى تدعيم الهامبرجر

رانيا شمس الدين فخر الدين *

الملخص العربي:

لقد زاد انتشار أمراض الكلى المزمنه (CKD) بشكل كبير في العقدين الماضيين وأصبح مشكلة صحية عامة على مستوى العالم. يهدف العمل الحالي إلى تسليط الضوء على التأثير االوقائى لعشبتى رجل الأسد (Alchemilla vulgaris) و الدمسيسة (Artemisia absinthium) وخليطهما ضد الفشل الكلوي المزمن المستحث باستخدام L-arginine. كما تم تحضير الهامبرغر المدعم بمسحوق رجل الأسد و الدمسيسة لتوفير منتج غني بالمركبات النشطة بيولوجيًا لمساعدة مرضى الكلى. حيث تم تقسيم ٤٨ فأر إلى ٨ مجموعات (٦ فئران) على النحو التالي: المجموعة الضابطة السالبة (G1): تغذت على الوجبة القياسية العادية، المجموعة الضابطة الموجبة (G2): تغذت على الوجبة القياسية العادية تحتوي على ٢٪ من L-arginine لاحداث الفشل الكلوي المزمن، المجموعتان (٣ و ٤): مثل المجموعة الضابطة الموجبة + ٥٪ من رجل الاسد و الدمسيسة على التوالي ، المجموعتان (٥ و ٦): مثل المجموعة الضابطة الموجبة + و ١٠٪ رجل الاسد و الدمسيسة على التوالي ، المجموعتان (٧ و ٨): مثل المجموعة الضابطة الموجبة + خليط من رجل الاسد و الدمسيسة بنسبة ٥ و ١٠٪ على التوالي. و لقد أظهرت النتائج أن إعطاء رجل الاسد و الدمسيسة ومخلوطهما في جميع الجرعات ادى الى تحسنا معنويا فى زيادة نسبة وزن الجسم لدى الفئران ، الغذاء المتناول، وخفض مستويات المصل الدم المرتفعة لوظائف الكبد (ALT، AST، ALP ، الألبومين والإجمالي. البيليروبين) ووظائف الكلى (الكرياتينين واليوريا وحمض البوليك) والبوتاسيوم فى حين رفعت مستويات الصوديوم في الدم مقارنة بمجموعة الضابطة الموجبة (L-arginine). ارتبطت هذا بزيادة كبيرة في المؤشرات الحيوية لمضادات الأكسدة في الدم (مضادات الأكسدة الكلية ، SOD، GSH و CAT) وفي الوقت نفسه تم تحقيق انخفاض معنوى كبير في العلامات الحيوية للإجهاد التأكسدي MDA. و بين التقييم الهستولوجى إلى ان رجل الاسد و الدمسيسة ومزيجهما يقللان من النخر الكبدي والكلوي الناجم عن L-arginine. وافق أعضاء اللجنة على الهامبرغر باضافة رجل الأسد حتى ٥٪ و ٢,٥٪ فقط من الدمسيسة. ويمكن الاستنتاج أن رجل الاسد و الدمسيسة أظهروا نشاط عالى في الوقاية من أمراض الكبد والكبد المستحدثة باستخدام L-arginine وقد تُعزى هذه التأثيرات إلى محتوياتهما من مضادات الأكسدة ، مما يوفر الدعم العلمي لاستخدام هذه النباتات في علاج امراض الكلى و الكبد.

الكلمات المفتاحية: رجل الأسد (Alchemilla vulgaris)، الدمسيسة (Artemisia absinthium) ، الفشل الكلى المزمن (CKD)، وظائف الكبد والكلى، مضادات الأكسدة ، تقييم الأنسجة.

* كلية التربية النوعية – جامعة طنطا