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Research Article

Egyptian Chicory (Cichoriumintybu)vsJordian One in Treatment of Liver of MSU-Induced HyperUricemia Experimental Rats

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Abstract

Introduction: Goutis one of the oldest joint diseases known to human caused by the chronic elevation of serum uric acid levels above the normal level

Aim: to investigate use of Egyptian and/or Jordanian chicory in treatment of gout and to see its effect on liver function.

Materials and Methods: Sixty adult male Sprague-Drawly Albino rats weighing about (140±10 g) were taken and divided into 10 groups, each with six rats. The first group is the negative control (-) and fed on normal diet. The other groups received injections with Mono-Sodium Urate (MSU) crystal and fed different dose of chicory. The experiment lasted for 8 weeks.

Results: The results showed that the positive control group (+)had a significant increase in serum liver function test as compared to the negative control group (-). However the other groups that fed on different ratios of chicory showed improvement inliver function compared to the positive control group (+) and this improvements was seen also in liver sections of the treated groups.

Conclusion: Chicory can be used in the management of hyperuricemia and gout

Keywords: Chicory, MSU, Albino rats, Liver functions, Histopathology

Introduction

Gout is one of the oldest joint diseases known to human caused by the chronic elevation of serum uric acid levels above the saturation point for monosodium urate (MSU) crystal formation [21]. The joints and its membrane linings become infected and polluted with great pain (gouty arthritis), irritation, and joint redness are caused by immune system replication as it begins to produce itself [23]. Purine in the form of uric acid is the product of protein metabolismandis regulated by the liver. Due to the high level of uric acid above normal level kidneys are no longer able to get rid of it leading to manifestation of gouty symptoms [21,48]. stated that individuals suffering from gout often have a complex profile of comorbidities. According to [50] gene/genetics role in gout is still unclear. Factors as obesity, high blood pressure, a high protein diet can trigger gout [36]. The incidence of gout is raising due to the ageing population and lifestyle changes.Prevalence of gout

increased by 11-13% each decade of life, and incidence increasing to 0.4% in people older than 80 years [34]. The prevalence of gout is 5.68 % in Jordan; 8.42% in Saudi Arabia; 0.9% -2.5% in Europe; 3.9% in the USA and over 6% in some Oceanic-Pacific ethnic groups [9]. The prevalence of gout in Egypt was reported to be 1-4% of the general population. This agrees with the worldwide prevalence of gout which was recorded in the range of 1-4% with higher incidence range 0.1-0.3%. Men has higher incidence of gout than women by 2:1 to 10:1 [34] withincreasing comorbidities [16].

Medicinal drugs as non-steroidal anti-inflammatory (NSAIDs), corticosteroids and colchicines used for urate reduction have side effects as inducing gastro-duodenal ulcers [13]. As a result, there is an urgent requirement for alternative treatments for gouty arthritis. Herbal medications are generally considered to be safe and possess fewer adverse effects [51]. Of these herbal, we aimed to investigate using chicory as herbal treatment. Chicory contains many compounds that are considered functional food polyphenols, inulin, oligofructose and sesquiterpene lactones [37].

The population of Jordan had reached [11] million (516) thousand; (6,097) million of them are males, and (5,419) million are females until the end of the year (2023). There are also [12] administrative governorates in Jordan, and (42%) of the total population is concentrated in the capital, Amman, and the rest are distributed among the other remaining governorates. There are [11] governorates [5].

Materials and Methods

Materials

Chicory leaves was obtained from Egypt and Jordan. Monosodium Urate Crystals (MSU) was obtained from SIGMA pharmaceutical industries, Nasser city. Sixty male albino rats of Sprague Dawley strains (60 rats) weighing (140 ±10g) were obtained from the Animal House Colony of the National Research Center, Dokki, Cairo, Egypt.Basal diet of rat was prepared according to Reeves., *et al.,*1993.

Preparation of extract

The chicory leaves were washed and dried in an oven at 60 °C for 8 h [39]. Dried chicory plant (1 kg) was grounded into powder and extracted with water (10 L) by heating with reflux for 1 hour a time. Then, the decoction was filtered and concentrated under low pressure [51].

All rats were anesthetized with 2.5% isoflurane, followed by injection of 50 µL MSU crystals (25 mg/mL) or normal saline into the medial side of the right ankle of each rat to further establish the model of acute gouty arthritis with hyperuricemia according to [52].

Chemical analysis

Inulin was isolated from chicory according to [25]. Moisture, ash, crude protein, carbohydrate, fat and inulin were determined according to [6,7]. Antioxidant activity measurement was carried out as DPPH (α, α-diphenyl-β-picrylhydrazy) according to [12]. Total Flavonoids and total phenolic content was estimated quantitatively using the method described by [29].

Biological experiment

Sixty male albino rats of Sprague Dawley strains weighing (140-10g) were kept in aerated wire cages under hygienic conditions. All rats were fed on basal diet for one week before starting of the experiment.Groups 2-10 were injected with 50 µl Monosodium urate crystals (25mg/ml) to induce gout, while control negative group was injected normal saline into the medial side of the right ankle once daily for 7 days.

- **Group 1:** Control (-): fed basal diet.
- **Group 2:** Control (+): Injected with MSU and fed basal diet.
- **Group 3:** Fed basal diet + extract of chicory from Egypt (5g/kg body weight).
- **Group 4:** Fed basal diet. + extract of chicory from Egypt (15g/kg body weight).
- **Group 5:** Fed basal diet. + extract of chicory from Egypt (20g/kg body weight).
- **Group 6:** Fed basal diet. + extract of chicory from Jordan (5g/kg body weight).

- **Group 7:** Fed basal diet. + extract of chicory from Jordan (15g/kg body weight).
- **Group 8:** Fed basal diet. + extract of chicory from Jordan (20g/kg body weight).
- **Group 9:** Fed basal diet. + Mix of chicory extracts from the two sources (7.5 from Egypt +7.5 from Jordan) g/kg body weight
- **Group 10:** Fed basal diet. + Mix of chicory extracts from the two sources (10 from Egypt +10from Jordan) g/kg body weight.

Diet and water were given ad libituum. Rats and diets were weighed every week. Body weight gain percent was calculated according to the method of [14].

At the end of the experiment, animals were anesthetized by ether then scarified. Blood samples were collected form orbital sinus veins by non-heparinized capillary tubes (1.5ml) [10] to determine serum glutamic pyruvic transaminase (ALT, GPT) and (AST, GOT) according to [45]. Organs as liverwas dissected at the end of the experimental period, then removed, washed with saline solution, dried between filter paper then weighed [51].

Histopathological examination

Histopathological examination by light microscope was carried out according to the method described by (10) and stained with Haematoxylin and Eosin.

Statistical analysis

To ascertained the significance among means of the treatment Duncan's multiple range test at significant level of ($P < 0.05$) was applied, using the SPSS statistical program SAS (1996). One way ANOVA followed by post Duncan test was also used [46].

Nutrient, mineral and vitamin composition of dried chicory leaves

Nutrient content

For thousands of years, medicinal plants have been used to treat various diseases. Shaimaa., *et al.,* (2024).Nutrient compositions of chicory are shown in Table 1. It was obvious from table 1 that protein content of Jordanian chicory leaves were lower than that of the Egyptian (15.15 vs 21.17 g/100g) respectively. This result agrees with [53] who mentioned that dry chicory leaves contains protein (15.02 g/100g). The highest ash, fat and protein contents of chicory were from the Egyptian (30.89, 21.17 and 1.3 g/100g, respectively). However the plant from Jordan has highest inulin content (10.1g/100g) whichincreases relatively in cold areas [28,30] found that the carbohydrates content of chicory leaves was 50.99g/100g and this result was close to the result of the Jordanian herb because of the long period of cultivation due to the cold weather, which leads to the decomposition of starch and the formation of carbohydrates.High carbohydrates content in the Jordanian chicory were an indicator of the increase in inulin. Inulin is a polysaccharide and a commercial source of the sugar fructose [38].

[49] found that the gut symbiosis sustained by inulin supplementation among other dietary fibers exerts preventive and/or therapeutic options for many metabolic disorders including obesity, type 2 diabetes mellitus, cardiometabolic diseases, kidney diseases and hyperuricemia.

The addition of 10 g inulin to the daily diets of subjects with moderately high blood lipids significantly reduced insulin levels, triacylglycerol and uric acid concentrations. Inulin has been reported to enhance colonic functions and systemic functions and reduce disease risk [16,17].

Mineral content

Chicory leaves were rich in some important minerals (Table 1) such as Ca, K, Mg, Na, Zn, Fe and Mn. The difference in mineral content between Egyptian and Jordanian may be due to soil characteristics, pH, and the presence of organic matter and the ability of plants to selectively accumulate some of these elements. The results presented in table 1 shows that Egyptian chicory had highest Na, Mg, Ca, Mn, Fe and Zn while Jordanian chicory had highest content of K. These results agree with [26,35]. who concluded that chicory leaves are valuable sources of several nutrients, minerals and vitamins and therefore could be regarded as healthy foods in

well-balanced diets, while Czaban et al., 2023showed that chicory is rich in zinc. It disagrees with [1] where they reported higher Ca content (250 mg/100g).

Vitamin content

Chicory leaves were rich in some important vitamins (Table 1) such as A, C, β-carotene and α-tocopherol. The results presented in table 1 shows that Egyptian chicory had highest content of vitamins. These results disagree with [3].

Total phenols, total flavonoids and antioxidant activity of chicory leaves powder from Egypt and Jordan.

Results shown in table 2 reveal that leaf extract was found to show comparatively low value of IC50 for DPPH inhibition and high reducing power. Due to good biochemical, phytochemical and antioxidant composition, *Cichorium*intybus leaves would be valuable candidate in pharmaceutical formulations and play an important role in improving the human health by participating in the antioxi-

Nutrient composition g/100g			Mineral content mg/100g			Vitamin content		
	Egyptian	Jordan ain		Egypt	Jordan		Egypt	Jordan
protein	21.17	15.15	Sodium (Na)	75.65	60.4	Vitamin A IU	5,717	4,875
Fat	1.3	1.1	Potassium (K)	191.63	211.4	Vitamin C mg/100g	24.0	20.0
Ash	30.89	21.56	Magnesium (Mg)	135.33	120.1	β-carotene mcg/100g	950.25	900.2
Moisture	5.7	6.6	Calcium (Ca)	235.32	213.4	α-tocopherol mg/100g	0.71	0.64
Carbohydrates	44.46	55.54	Manganese (Mn)	1.05	.90			
Inulin	8.9	10.1	Iron (Fe)	7.75	5.94			
			Zinc (Zn)	1.82	1.14			

Table 1: Nutrient, mineral composition of dried chicory leaves.

dant defense system against endogenous free radicals [44]. Leaves are considered to contain high levels of total phenolic and total flavonoid content as compared to other parts of chicory plant [27]. Our results agree with [1].

Body weight, EEED intake, liver weight and relative organ weight

Results are presented in table 3. Body weight change is often a very sensitive indicator of animal well-being. It is noted that all groups were increasing at the same rate, and the increase was higher in the Jordanian groups, except for group 4. It is noted that the Jordanian groups or groups receiving mix of both herbs approached the negative group, and this is due to the increase in the concentration of carbohydrates in the Jordanian herb. This result agrees with [31,51]. who found a significant increase in the final body weight of the rat and this might be due to using chicory to induce hyperuricemia**.** The results showed that the rats with gout had significantly lower weight.

Liver function of examined group ALT, AST

Plasma enzyme levels have been used as markers for monitoring chemically induced tissue damages. The ALT and AST are important enzymes that are often employed in assessing liver injury [4]. Attention has been developed on the protective biochemical function of the natural antioxidants contained in the dietary plants that are candidates for prevention or protection of oxidative damage caused by free radicals' species for liver [8].

Antioxidant activity	Egypt	Jordan
Total flavonoids mg/g	9.50	7.1
Total phenols mg/g	26.4	23.2
Antioxidant activity (DPPH inhibition %)	47.4	44.5

Table 2: Total flavonoids and antioxidant activity of chicory leaves powder from Egypt and Jordan.

Groups	Feed intake (g//day/rat)	FBW g	Liver (g)	Relative Organ weight (%)
G1: Control (-)	14.33 ± 0.69 [a]	195.2 ± 15.0 [a]	5.9 ± 0.63 [c]	3.03 [c]
G2: Control (+)	9.19 ± 0.46 [c]	174.3 ± 6.6 [d]	7.54 ± 0.47 [a]	4.33 [a]
G3: (5g Egypt)	10.62 ± 0.25 [b]	190.0 ± 4.5 [b]	7.48 ± 1.2 [a]	3.94 [b]
G4: (15g Egypt)	9.00 ± 0.244 [c]	168.8 ± 15.4 [e]	7.1 ± 0.31 [a]	4.21 [a]
G5: (20g Egypt)	9.15 ± 0.18 [c]	182.2 ± 6.1 [c]	6.18 ± 0.44 [b]	3.39 [b]
G6: (5g Jordan)	13.5 ± 0.99 [a]	189.8 ± 5.9 [b]	6.75 ± 0.52 [b]	3.56 [b]
G7: (15g Jordan)	8.98 ± 1.2 [d]	175.0 ± 5.73 [d]	7.17 ± 0.51 [a]	4.10 [a]
G8: (20g Jordan)	9.6 ± 0.23[c]	196.0 ± 3.1 [a]	6.9 ± 0.56 [b]	3.52 [b]
G9: (7.5 E & 7.5 J)	10.0 ± 0.07 [b]	194.0 ± 4.5 [a]	6.64 ± 0.82 [b]	3.42 [b]
G10: (10 E & 10 J)	10.26 ± 0.53 [b]	189.8 ± 5.9 [b]	5.75 ± 0.62 [c]	3.03 [c]

Table 3: Organ's liver weight (g) of experimental rats treated with different ratio of chicory.

Column with different letters means that is significant difference at ($P \leq 0.05$)

Data presented as mean ± SE

As a result of infection of control group with the disease, the results were high, but less than the positive control group, as the Jordanian chicory gave slightly better results than the Egyptian chicory. Results of biochemical analysis for all tested groups were presented in table 4 and it showed the liver function test (ALT, AST) among experimented group. Group 3 has the highest ALT (85.4 ± 4.8) (u/ml) and highest AST in group 4 (108.0 ± 3.) (u/ml) which were significantly height than all other groups.Also we noticed that group 10 had the lowest liver function tests values ALT (54.8 ± 2.3) (u/ml), AST (62.6 ± 2.4 u/ml) as compared by negative control than all other intervention group.This group which was treated with (10g from Egypt +10g from Jordan) followed by group 9 ALT (65.5 ± 3.2) (u/ml) and AST (77.9 ± 2.0) (u/ml). The best results were recorded for mixing the two sources. This is confirmed by histopathology of liver of all treated groups (Table 5 and Figure 1-10).

Uric acid levels were found to be significantly associated with liver enzymes ALT and AST, AST/ALT ratio and total bilirubin (Table 2). Post hoc analysis showed high uric acid level was significantly associated with high ALT (≥30), AST (≥33) and total bilirubin levels (≥17).

Our results go with results of [20] who showed that uric acid levels were associated with liver enzymes more than two times higher odds of elevated ALT, AST ($p < 0.001$) in adults with hyperuricemia and gout and are most likely to develop liver dysfunctions and suffer associated morbidities. Such patients need to be appropriately monitored and managed for their liver functions and to prevent associated morbidities [24].

[33] indicate that ALT, AST were significantly reduced due to dietary chicory extracts supplementation. [18] found that uric acid and increased activity of the liver enzymes gamma-glutamyl transferase (GGT), aminotransferase (AST) and alanine aminotransferase (ALT) are also associated with development of the metabolic syndrome. Chicory was established as a hepatoprotective by reducing serum levels of alanine aminotransferase (ALT) and aspartate aminotrasferase (AST) [21].

Groups	ALT(u/ml)	AST(u/ml)
G1: Control (-)	35.2 ± 0.9 [f]	46.2 ± 0.7 [g]
G2: Control (+)	97.2 ± 2.0 [a]	119.5 ± 1.4 [a]
G3: (5g Egypt)	85.4 ± 4.8 [b]	95.1 ± 2.6 [c]
G4: (15g Egypt)	83.5 ± 4.0 [b]	108.0 ± 3.0 [b]
G5: (20g Egypt)	78.6 ± 2.4 [c]	96.8 ± 1.4 [c]
G6: (5g Jordan)	71.9 ± 2.0 [c]	84.3 ± 4.4 [d]
G7: (15g Jordan)	81.5 ± 3.4 [b]	94.3 ± 1.0 [c]
G8: (20g Jordan)	76.3 ± 2.6 [c]	87.5 ± 1.9 [d]
G9: (7.5 E & 7.5 J)	65.5 ± 3.2 [d]	77.9 ± 2.0 [e]
G10: (10 E & 10 J)	54.8 ± 2.3 [e]	62.6 ± 2.4 [f]

Table 4: Liver function for rats suffering from gout and treated with different doses of Egyptian and Jordanian chicory. Column with different letters means that is significant difference at ($P \leq 0.05$)

Data presented as mean ± SE

Histopathology of Livers

Results of liver histology are presented in table 5 and figure 1-10.

All results were higher than the negative group and lower than the positive group.Liver sections from control (-) (Figure 1) revealed normal histologic structure, and the hepatocytes were or-

Group	Photo no	Notes
G1: Control (-)	1	It reveals normal histologic structure, and the hepatocytes were orderly arranged in normal lobular architecture with central veins and radiating hepatic cords. The portal triads showed normal histological structure containing branches of the hepatic artery, hepatic portal vein and bile duct.
G2: Control (+)	2	It shows multifocal inflammatory cells aggregations in the hepatic parenchyma associated with accumulation of eosinophilic and karyorrhectic tissue debris. Portal hepatitis was observed in some examined sections accompanied by portal fibroplasia.
G3: (5g Egypt)	3	It showed focal inflammatory cells infiltration (H&E)
G4: (15g Egypt)	4	It showed mild portal inflammation (H&E)
G5: (20g Egypt)	5	It showed portal fibroplasia with fewer inflammatory cells infiltration (H&E).
G6: (5g Jordan)	6	It showed sinusoidal dilation (H&E)
G7: (15g Jordan)	7	It showed portal hepatitis and severe fibrosis (H&E)
G8: (20g Jordan)	8	It showed portal fibrosis with fewer inflammatory cells infiltration (H&E)
G9: (7.5 E & 7.5 J)	9	It showed portal fibroplasia (H&E)
G10: (10 E & 10 J)	10	It showed portal fibroplasia with multifocal mononuclear cells infiltration (H&E)

Table 5: Histopathological Examination of Liver.

derly arranged in normal lobular architecture with central veins and radiating hepatic cords. The portal triads showed normal histological structure containing branches of the hepatic artery, hepatic portal vein and bile duct.

[32] found thaturic acid could potentiate the effects of high glucose resulting in augmented triglyceride accumulation. Thus, uric acid may be viewed as an amplifying pathway that may increase the biologic effects of glucose, and fructose to stimulate fatty liver. [43] found that increased accumulation of plasma, hepatic, and renal uric acid as well as, histopathological examinations revealed hepatic and renal architectural derangement and cellular necrosis and degeneration in MSU rats.

Chicory has a promising role and it worth to be considered as a natural substance for ameliorating the oxidative stress and hepatic injury induced by gout [42].

[4] found that the results of the serum biomarkers of chicory treated rats showed significant reduction indicating the effect of the plants leaves extract in restoring the normal functional ability of the hepatocytes.

This study recommends that dietary intake of chicory can be beneficial for patients with hypercholesterolemia and for the treatment of liver tissue damage [2].

Figures (1-10) figure micrographs of sections of rats livers from different groups stained with (H and E).

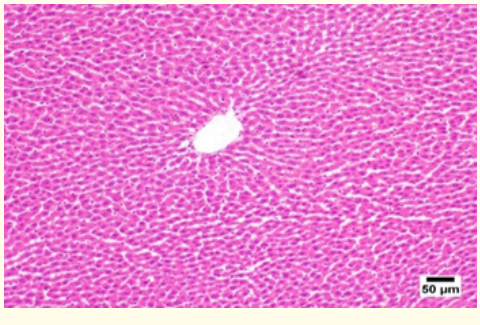

Figure 1: Normal renal cortex.

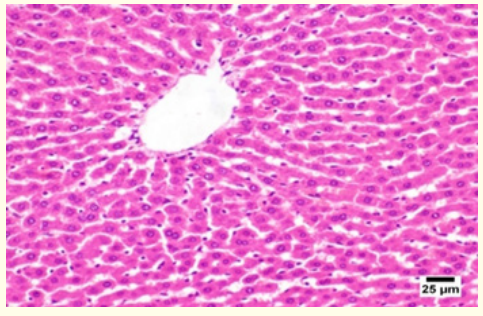

Figure 2: Perivascular edema and inflammatory cells infiltration.

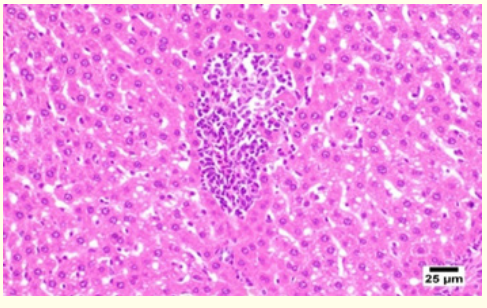

Figure 3: Congestion of the renal cortex.

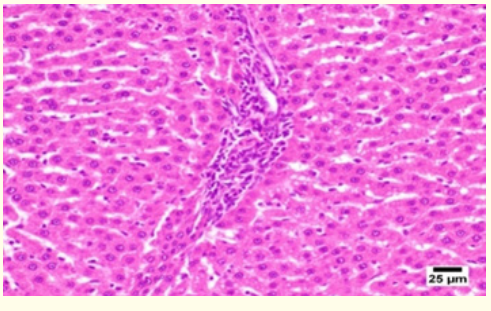

Figure 4: Mild necrobiotic changes in the renal cortex.

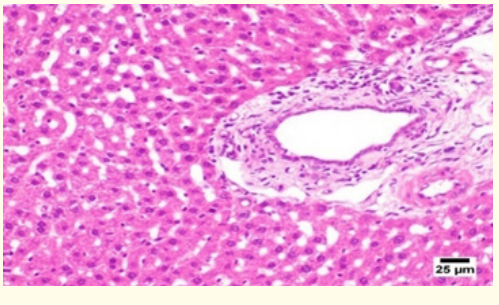

Figure 5: Congestion of the renal cortex.

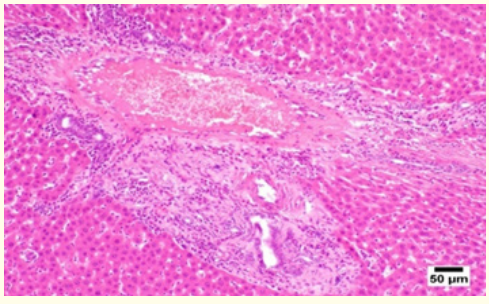

Figure 6: Apparently normal renal cortex.

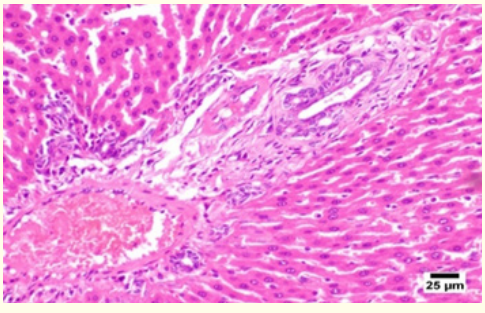

Figure 7: With swelling of some renal tubular epithelium.

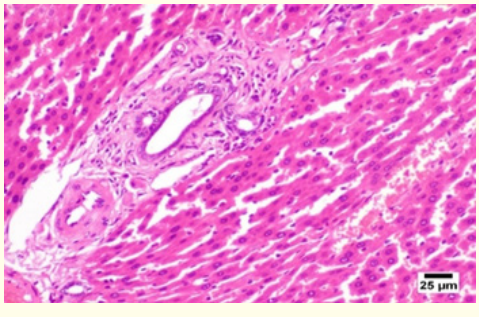

Figure 8: Swelling of the renal tubular epithelium with narrowing of tubular lumen.

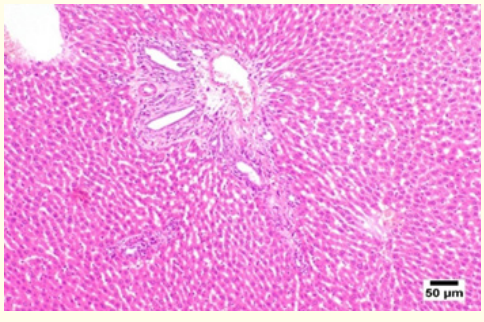

Figure 9: Mild vacuolation of renal tubular epithelium renal cortex.

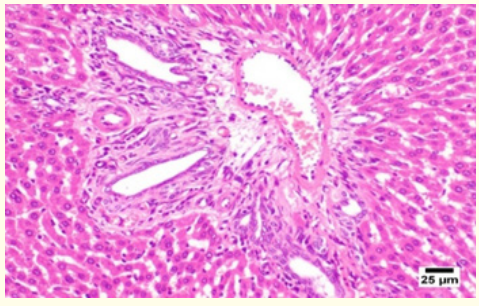

Figure 10: Vacuolar degeneration in the renal tubules of the renal cortex.

Conclusion

Chicory extract decreased serum levels of ALT and AST and suppressed gouty inflammation in experimental rats induced with MSU crystals, with the most significant Improvement in those treated with Mixed dose from both Egypt and Jordan (10gm/10gm).This mixture contains the highest antioxidant activities as well as highest inulin content.

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Author Contributions

All authors have accepted and shared responsibility for the entire content of this manuscript and approved its submission.

Competing Interests

Authors state no conflict of interest.

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