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Xanthine Oxidase Inhibitory Activity and Antigout of Celery Leek Parsley and Molokhia

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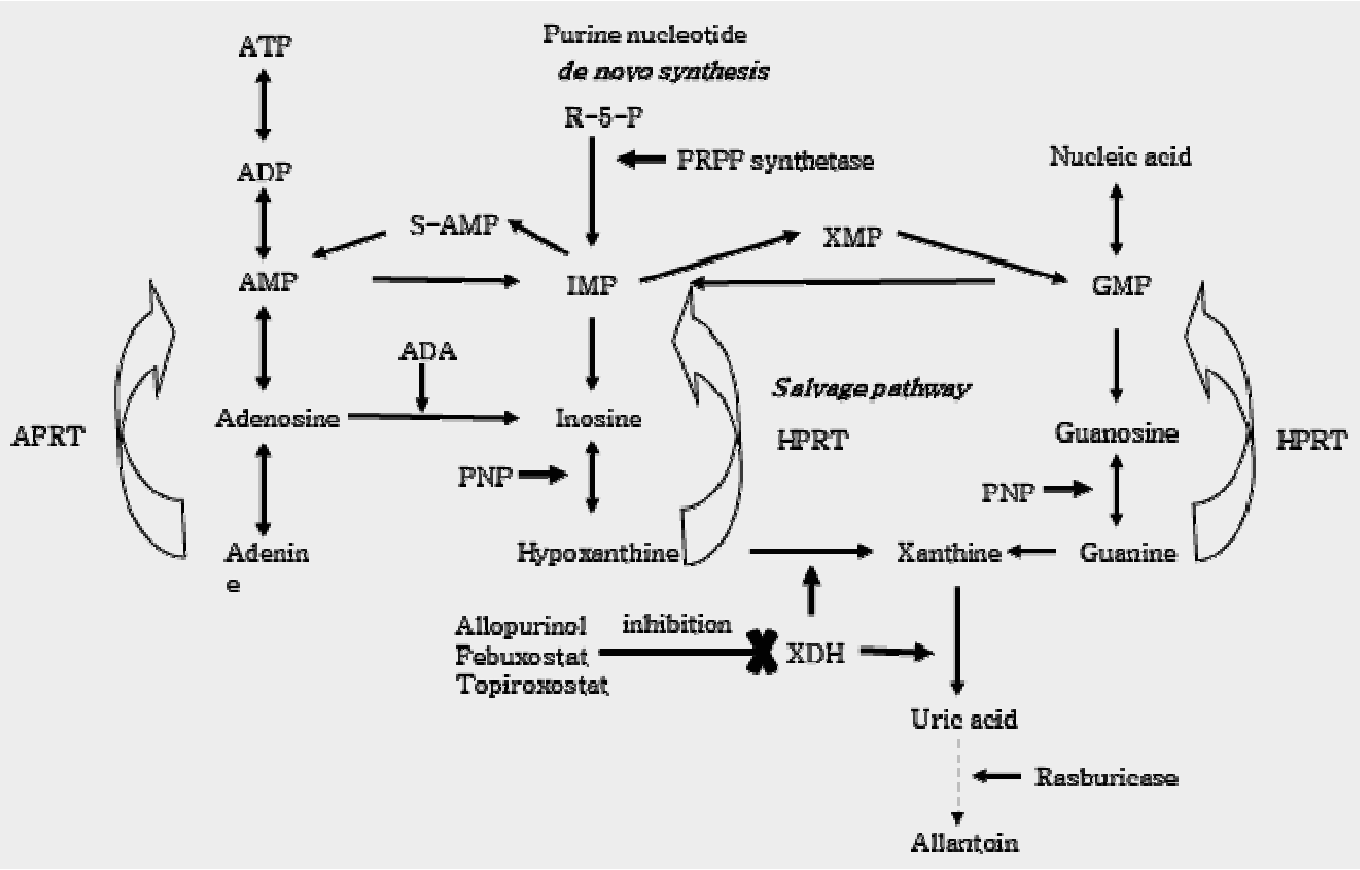
Abstract: The present study was aimed at investigating *in vitro* xanthine oxidase inhibitory (XOI) and *in vivo* antigout activity extracts of celery, leek, parsley, and molokhia. The degree of XO inhibitory activity was determined by measuring the absorbance spectrophotometrically at 295 nm, which is associated with uric acid formation which is linked to gout. Our preliminary screening study had employed the use of distilled water, and absolute ethanol to determine XOI from celery, leek, parsley, and molokhia. In general, our study showed that the ethanolic extracts were found to be more active than the aqueous extracts. Further *in-vivo* antigout was studied gout induced in rats by potassium oxonate. A total of 36 male *albino* rats were randomly divided into 6 equal groups. Group 1 negative control given only standard diet, and group 2-6 given Potassium oxonic acid (250 mg/kg, *i.p.*), Potassium oxonate an uricase inhibitor was used to induce gout. Oral administration (G3, G4, and G5) of celery, leek, parsley (5 g/Kg), and (G6) molokhia (4.8 g/Kg) showed a significant decrease in uric acid, and Creatinine levels in the gouty rats. All extracts (celery, leek, parsley, and molokhia) have shown significant decrease in level of Malonaldehyde (MDA) and increase in activity of antioxidant enzyme level, comparable to positive rat (G2). No significant changes between all extracts used and negative control in gain weights and organic phosphorus was noticed. The results showed that increasing serum total calcium level with extracts of celery, leek, parsley, and molokhia in comparison to positive control. The celery, leek, parsley, and molokhia extracts have some protective effects on the gout.

Keywords: Celery, Leek, Parsley, Molokhia, Gout, Xanthine Oxidase Inhibitory

1. Introduction

Gout is a multi-factorial disease affecting the flexibility of joints. It is usually characterized by re-current attacks of acute inflammatory arthritis-a red, tender, hot, swollen joints leading to bursitis (1). It is a serious disease that has been growing in prevalence during the past several years in Western civilizations (2). Gout is characterized by abnormally high levels of uric acid in the body, resulting in the formation and deposition of urate (as monosodium urate-monohydrate) crystals, generally known as tophi crystals in joints, tendons and surrounding tissues, characterized by hyper-uricemia and in chronic stage, may lead to renal failure(3).These crystals cause an acute inflammatory response and can induce a permanent tissue damage which is characterized by the appearance of ulceration of the joint cartilage, marginal osteophytosis, geodic and erosive lesions and chronic inflammation of synovial membrane (4 and 5).This is partly a reflection of changes in diet, increases in longevity, hypertension, metabolic syndrome, and advanced renal disease, and the broad use of diuretics in clinical practice. Management of gout in the elderly, in organ transplant recipients, and in patients with renal insufficiency and allopurinol intolerance can be particularly challenging (6).Uric acid is the end product of purine metabolism in humans, and its overproduction by xanthine oxidase (XOD) from purine compounds or under excretion can lead to hyperuricemia as gout. Enzymatic degradation of hypoxanthine and xanthine leads to the production of uric acid (7 and 8). The major site of purine synthesis is in the liver. The synthesis of uric acid occurs along two pathways, referred to as the *de novo* and the *salvage pathways*. Synthesis of the purine nucleotides begins with the formation of phosphoribosyl pyrophosphate (PRPP) by PRPP synthetase and leads to the first fully formed nucleotide, inosine 5'-monophosphate (IMP). IMP is converted into either adenosine 5'-monophosphate (AMP) or guanosine 5'-

monophosphate (GMP) through two distinct reaction pathways. Catabolism of the purine nucleotides leads ultimately to the production of uric acid (Figure 1) (9).



Abbreviations: ADA, adenosine deaminase; APRT, adenosine phosphoribosyl transferase; HPRT, hypoxanthine phosphoribosyl transferase; PNP, purine nucleoside phosphorylase; XDH, xanthine dehydrogenase

Fig. (1). *Purine metabolic pathway in humans.*

XOD (EC 1.17.3.2) is a rate-limiting enzyme in the biosynthesis of uric acid and catalyzes the oxidation of hypoxanthine and xanthine to uric acid (8), which is responsible for the medical condition leading to painful inflammation called gout (10). XOD is distributed most abundantly in the liver and intestine (11), situated at the end of a catabolic sequence of the purine nucleotide metabolism in humans and few other uric species (12). XOD also serves as an important biological source of oxygen-derived free radicals that contribute to oxidative damage to living tissues involved in many pathological processes such as inflammation, atherosclerosis, and cancer. *In-vitro* bioassays are used to examine test material for XOD inhibition, as inhibitors of XOD may be potentially useful for the treatment of gout or other XOD induced diseases (13). Therefore, XOD inhibitors can be potent therapeutic agents for the prevention of hyperuricemia by inhibition of uric acid biosynthesis (12). The treatment of gout entails the use of the therapeutic agents such as xanthine oxidase inhibitors (XOI) (14 and 12). XOI acts by blocking the biosynthesis of uric acid from purine in the body (12) and it is believed that either by increasing the excretion of uric acid or reducing the uric acid production helps to reduce the risk of gout (15).Current treatments to gout includes Non-steroidal anti-inflammatory drugs (NSAIDS) such as ibuprofen, naproxen, indomethacin, aspirin, etoricoxib (cox-2 selective inhibitors); corticosteroids such as prednisone; allopurinol, probencid, colchicines (to decrease severity of episodes). Although these agents are generally effective, they generates superoxide (16) and lead to several side effects such as skin allergies, fever, rash and diarrhoea progressively developing leukocytosis, eosinophilia, vasculitis, aseptic meningitis, nephritis and renal dysfunction, and hepatic dysfunction (17 and 18). Allopurinol is an XOD inhibitor used clinically for the treatment of gout. However, it can have side effects, such as hypersensitivity reaction, Stevens - Johnson syndrome, renal toxicity, and even fatal liver necrosis (19). So, there is a need of herbal extracts with antioxidant property to nullify oxidative and inflammatory response produced by xanthine oxidase. It is believed that XOD inhibitors from natural sources can be used as alternatives to allopurinol because of fewer potential adverse side effects (20). Some tropical plants and their phytochemicals are worth to be explored as potential XOI as they are already used as food or food supplements for many years and found safe for

human bodies (21). Polyphenols, flavonoids, coumarins, ellagic acid, and valoneic acid dilactone (VAD) have been reported to be potent plant-based XOI (22; 23; 24; 25 and 12).

Celery (*Apium graveolers dulce*) is a biennial plant, belongs to the Umbelliferae family. The celery plant cultivated in the Mediterranean region and its Arabic name is Karafs. Celery seeds contain several substances including volatile oils; flavonoids, antioxidants that give plants their colors and may protect cells from damage; coumarins, chemicals that help thin the blood; and linoleic acid, an omega-6 fatty acid. Celery seed is used for treating arthritis and gout, and to help reduce muscle spasms, calm the nerves, and reduce inflammation (26 and 27). Leeks (Allium porrum or A. ampeloprasumvar. porrum), sometimes called "the gourmet's onion". The thick leaf bases and slightly developed bulb look like a giant green onion, and are eaten as a fresh vegetable. Leeks contain saponins and the major flavonoid in leeks is kaempferol, with only a small amount of quercetin, carotenoids and chlorophyll mainly in the green tops(28 and 29).Parsley (*Petroselinum crispum*) is a member of Apiaceous family that has been employed in the food, pharmaceutical, perfume, and cosmetic industries (30). In folk medicine, parsley is used to treat a wide variety of conditions (31). Phytochemical screening of parsley has revealed the presence of several classes of flavonoids (32). Flavonols (kaempferol and quercetin) and flavones (apigenin and luteolin), which occur as glycosidic form in nature, are major flavonoids found in parsley (33).

Molokhia *Corchorus olitorius* (Tiliaceae) is an annual herb whose leaves and roots are used as herbal medicine and eaten as vegetable by local people in East Malaysia, India, Egypt, and Philippines (34).Traditionally, its leaves are used in the treatment of pain, fever, chronic cystitis and tumors (35). Molokhia abundantly contains 5-caffeoylquinic acid, 3, 5-dicaffeoylquinic acid, quercetin 3-galactoside, quercetin3-glucoside, quercetin 3-(6-malonylglucoside), quercetin3-(malonylgalactoside), ascorbic acid, α-tocopherol, and chlorophyll, etc., and the content of quercetin glycosidesis remarkable (36).

Therefore, the present study was carried out to evaluate the xanthine oxidase inhibitory potential of some plants (celery, leek, parsley, and molokhia) and *in vivo* anti-inflammatory in experimental gout model in rats.

2. Materials and Methods

2.1. Reagents and Chemicals

Potassium oxonate, allopurinol, xanthine and xanthine oxidase (buttermilk) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Dimethylsulphoxide (DMSO), hydrochloric acid (HCl), absolute ethanol, and other reagents of analytical grade were obtained from Merck (Darmstadt, FR, Germany). Potassium di-hydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were of the highest purity. All other reagents were purchased from Merck (Darmstadt, Germany). The reagents used were from of analytical grades.

2.2. In-Vitro Xanthine Oxidase Inhibitory Activity

2.2.1. Preparation of Crude Extracts

The plants were washed and oven-dried for 72 h at 40°C. The dried plant materials were grounded using domestic blender to small particle size. All plant materials were subjected to a standard procedure of solvent extraction process (37). 1 g of each of the dried powdered plant material was added into 10 ml of extraction solvent and all experiments were conducted in triplicate. Two extraction solvents were employed, namely, absolute ethanol and distilled water. The mixture of the ground sample and solvent were capped with aluminum foil, and placed in an incubator shaker. The agitation speed of the incubator shaker was set at 100 rpm and ran for 6 h at 30°C. Each mixture of plant material and extraction solvent was filtered using Whatman No. 1 filter paper and the filtrate was collected, concentrated by vacuum rotary evaporator and dissolved in Di-Methyl Sulfoxide (DMSO) (100%). Then, it was subjected to XO inhibitory activity assay spectrophotometrically at 295 nm to determine the XOI properties.

2.2.2. Xanthine Oxidase Inhibitory Activity Assay

The crude extract was used for the analysis of XO inhibition under in vitro assays. The inhibitory effect on XO was measured spectrophotometrically at 295 nm under aerobic condition, with some modifications, following the method reported by Unno *et al*., (12) and Umamaheswari *et al*., (15). A well-known XOI, allopurinol (100 µg/ml) was used as a positive control for the inhibition test. The reaction mixture consisted of 300 µl of 50 mM sodium phosphate buffer (pH 7.5), 100 µl of sample solution dissolved in DMSO 100 µl of freshly prepared enzyme solution (0.2 units/ml of xanthine oxidase in phosphate buffer) and 100 µl of distilled water. The assay mixture was pre-incubated at 37°C for 15 min. Then, 200 µl of substrate solution (0.15 mM of xanthine) was added into the mixture. The mixture was incubated at 37°C for 30 min. Next, the reaction was stopped with the addition of 200 µl of 0.5 M HCl. The absorbance was measured using UV/VIS spectrophotometer against a blank prepared in the same way but the enzyme solution was replaced with the phosphate buffer. Another reaction mixture was prepared (control) having 100 µl of DMSO instead of test compounds in order to have maximum uric acid formation. The equation reported by Naseem *et al*., (38) was used to evaluate the degree of XO inhibitory activity. Thus, XOI activity was calculated using Eq.1, in which α is the activity of XO without test extract and β is the activity of XO with test extract. % XO inhibition = $(1 - \beta/\alpha) \times 100$ (1)

2.3. In-Vivo Antigout Activity

2.3.1. Preparation of Extract

The edible portions of fresh plant (celery, leek, parsley, and molokhia) which were purchased from local markets in

Cairo, Egypt. Celery, Leeks, and Parsley, leaves were carefully washed with water and left to dry at room temperature. Then they were weighted and completely blended in distilled water (1: 1 w/v) (39). Aqueous extract of molokhia were washed with water and dried (solar drying), they were weighted and completely blended in distilled water and boiling for 10 min. The extract was filtered to remove particulate matters, and then administered orally to rats at dose of 4.8 mg/kg body weight (40 and 41). All freshly prepared juicy samples were administrated to the corresponding groups by oral gavage once a day for 4 weeks.

2.3.2. Animals

A total of 36 male *albino* rats (body weights: 160-205 g) were used in the present study. The rats were kept under normal health laboratory conditions and fed on basal diet for one week. Water and basal diet were provided *ad libitum* for 30 days. All the experimental procedures were carried out in the Ophthalmology Research Institute, Giza, Egypt. The animals were observed daily for any signs of toxicity. Body weight was recorded at regular intervals throughout the experimental period.

2.3.3. Animal Model of Gout in Rats

Experimentally-induced gout in rats (due to inhibition of uricase with potassium oxonate) was used to study antigout (42). Briefly, 250 mg/Kg, uricase inhibitor, potassium oxonate (PO), dissolved in 0.9% saline solution was administrated intraperitoneally to each animal except group 1 (group 2-6), 1 h before oral administration of test juicy samples.

2.3.4. Experimental Design

Animals were divided into 6 groups of six rats each. Group 1 served as the normal control (negative control). Group 2 served as gouty control (positive control). Group 3, 4, 5, (gouty rats) received celery, leek, and parsley at the oral dose of 5 g/Kg extract (39).Group 6 (gouty rats) received extract of molokhia at the oral dose of 4.8 g/Kg (41).

2.3.5. Biochemical Evaluation

At the end of the treatment period, rats were weighed. All animals were fasted for 12 h, and then blood samples were collected from the animal's eye plexus under diethyl ether anesthesia. Serums were separated out by centrifugation at 3000 rpm for 15 min. After the collections of blood samples, animals were killed. Lipid peroxidation in plasma was estimated by the method of Ledwozy *et al*., (43). Malonaldehyde (MDA) produced during peroxidation of lipids served as an index of lipid peroxidation. MDA reacts with TBA to generate a colour product, which absorbs at 532 nm. Superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund (44)). The degree of inhibition of the auto-oxidation of pyrogallol at an alkaline pH by SOD was used as a measure of the enzyme activity. Catalase and Glutathione peroxidase activities were estimated by the method of Sinha (45) and Rotruck *et al.,* (46). The activity of Catalase was expressed as µg of H_20_2 consumed/mn/mg protein. Glutathione peroxidase was expressed as µg of glutathione utilized /minute/mg/ protein. The activity of acid phosphatase was assayed by the method of King (47).Alkaline phosphatase (ALP; EC 3.1.3.1) activity was measured at 405 nm by the formation of para-nitrophenol from para nitrophenylphosphate as a substrate using the method of Varley *et al.*, (48). Creatinine, and uric acid were determined by using the methods described by Larsen (49) and Caraway (50), total protein content was measured by the method of Lowry *et al*., (51). Total calcium and organic phosphourus was measured by the method of Ferguson *et al*., (52) and Fiske and Subbarow (53).

2.4. Statistical Analysis

The resulted data were subjected to statistical analysis using the standard analysis of variance (one-way ANOVA) as outlined by Snedecor and Cochran (54).and the differences among means dose of all extracts effects were tested for the least significance difference value (LSD) at 0.05 probabilities by using Duncan's multiple range tests by SPSS for Windows statistical package, version 10.0.

3. Results

3.1. In-Vitro Xanthine Oxidase Inhibitory Activity

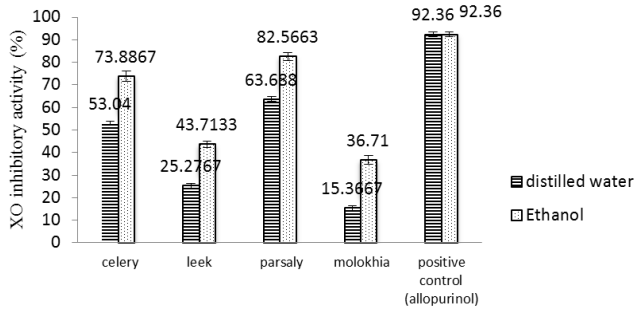


Fig. (2). *XO inhibitory activity (%) of extracts of celery, leek, parsaly, and molokhia using absolute ethanol and distilled water as the extraction solvent.*

The effectiveness of selected extraction solvents to extract bioactive compounds responsible for XO inhibitory activity was studied. The percentages of XO inhibitory activity of all crude extracts obtained by using distilled water and absolute ethanol were in figure 2. The comparison was also made between the plant extracts in two extraction solvents and the positive control (allopurinol), to determine the best extraction solvent. In general, the ethanol extracts were found to be more active than the aqueous extracts. The highest XOI activity was shown by ethanol extract both of parsley and celery with 82.5663% and 73.8867 %, respectively. While, the lowest value were 15.3667% and 25.2767% for aqueous extract of molokhia and leek, respectively. The results were compared with the standard drug allopurinol, which showed

90.36% inhibition.

3.2. In-Vivo Anti-Gout Activity

The ability of extracts (celery, leek, parsley, and molokhia) to inhibit uricase was investigated in this study. In the present study, no significant changes had been observed in gain weights (%) of the treated groups as compared with controls (Table 1). Administration of uricase inhibitor, potassium oxonate significantly affected various bio-chemical parameters on rat blood. As shown in Table 1, an increase was in the serum uric acid and creatinine levels in positive control (G2) when compared to the negative control (G1). Extracts of Leek (G4) and parsley (G5), significantly ($p<0.05$) reduce serum uric acid levels of gouty rats to values than that found in G2.

Administration of aqueous extract of molokhia at a dose of 4.8 g/kg was effectively reduced serum creatinine levels in rat compared to the gouty rat groups (G3, G4, G5), though still higher than the normal control level (G1).

As shown in Figure 3, significant decrease in serum total calcium concentrations in G2. No significant changes were observed in extracts of parsley (G5), molokhia (G6), and negative control (G1).

Oral administration extracts of celery, leek, parsley, and molokhia significantly reduced ($p < 0.05$) the serum organic phosphorus levels of gouty rats but not positive control (G2). The results also indicate that no significantly changes between all extracts used and negative control (Figure 4).

Table 1. *Effect of the orally administered of celery, leek, parsely, and molokhia on serum uric acid, creatinine, and gain weight in potassium oxonate -induced rat.*

Treatment	Uric acid (mg/dl)	Creatinine level (mg/dl)	Gain weight (%)
Negative control (G1)	2.9286 ± 0.2536^{a}	0.6855 ± 0.0697^{a}	16.886 ± 0.4640^{a}
Positive control (G2)	6.1402 ± 0.5783^{c}	1.3966 ± 0.04803^{c}	16.269 ± 0.6496^{a}
Extract of Celery 5 g/Kg (G3)	4.2531 ± 0.6665^{b}	1.2933 ± 0.14629^{bc}	15.804 ± 0.6692^{a}
Extract of Leek 5 g/Kg (G4)	3.4822 ± 0.2362^{ab}	1.1816 ± 0.0424^{bc}	17.216 ± 1.1514^{a}
Extract of Parsley 5 g/Kg (G5)	3.5704 ± 0.2149^{ab}	1.1520 ± 0.06612^{bc}	16.736 ± 1.1055^{a}
Extract of molokhia 4.8 g/Kg (G6)	4.1739 ± 0.1836^{b}	1.046 ± 0.0583^{b}	16.851 ± 0.49^{a}

All the values are expressed as the mean ±standard error of the mean. The mean difference is significant ($P < 0.05$) when compared with the positive control (gouty rat) group

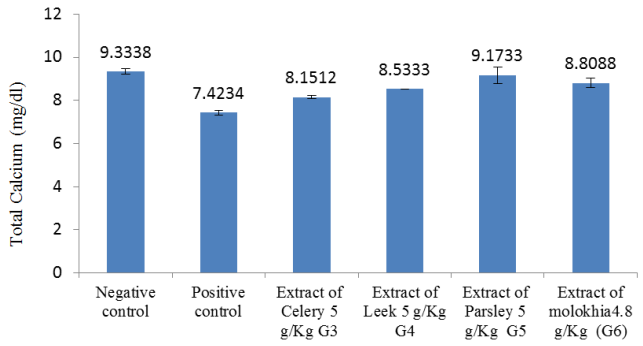


Fig. (3). *Total calcium (mg/dl) levels of aqueous extract of celery, leek, parsaly, and molokhia in normal and potassium oxonate-induced gout rats.*

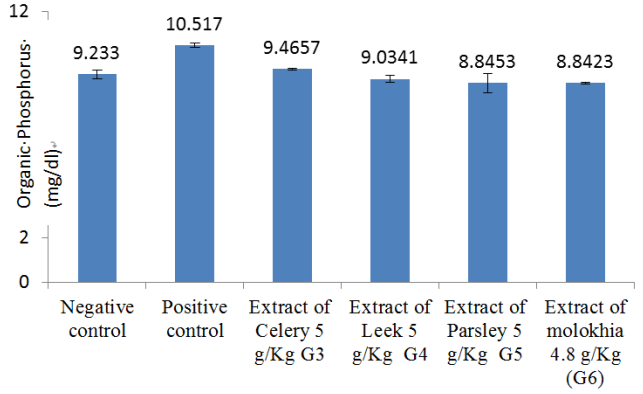


Fig. (4). *Organic Phosphorus (mg/dl) levels of aqueous extract of celery, leek, parsaly, and molokhia in normal and potassium oxonate-induced gout rats.*

The present study also investigated the efficacy of orally administered celery, leek, parsley, and molokhia extracts on blood biomarkers of oxidative stress (Malonaldehyde concentration) and lipid peroxidation levels in rats blood. In gouty control rats (G2), the levels of serum MDA, as a biomarker of lipid peroxidation, were statistically ($p \leq 0.05$) higher than normal rats (G1). Oral administration of celery, leek, parsley, and molokhia to gouty rats induced a significant reduction ($p \leq 0.05$) in these elevated levels of MDA, but could not yet reach these levels to the normal value G1 (Table 2). Potassium oxinate treatment increased MDA levels significantly at dose used (250 mg/Kg), reflecting the increase in lipid peroxidation. In contrast to these results, there was decrease in glutathione peroxidase (GPx) enzyme activity compared with the controls (G1). GPx enzyme activity levels were found to have increased in parsley (G5) the value was 3.0597 U/mg protein).

The effect extracts of celery, leek, parsley, and molokhia on the enzymatic antioxidant levels catalase (CAT) and superoxide dismutase (SOD) in experimental rat were tabulated in Table 2. CAT and SOD level was decreased significantly in positive control (G2) when compared to control group (G1). Administration of extracts to potassium oxonate-induced rat altered the above changes by regulating the CAT level to nearly that of normal levels. Molokhia and parsley (G5, G6) also significantly inhibited the decreased levels of MDA and increased the levels of SOD and CAT in gouty rats ($P < 0.05$).

Table 2. *MDA levels and antioxidant enzyme activities of groups.*

Treatment	MDA (nmol/mL)	Superoxide dismutase (SOD) (U/mg protein)	Catalase (CAT) (U/mg protein)	GPX (U/mg protein)
Negative control (G1)	3.0619±0.1197 [a]	3.6201±0.2451 [ab]	10.134±0.7023 [a]	3.8180±0.1499[a]
Positive control (G2)	5.2374±0.0808[c]	1.9656±0.06397[c]	5.9359±0.32497[e]	1.0732±0.0885[e]
Extract of Celery 5 g/Kg (G3)	4.1747±0.1528[b]	3.1081±0.1924[b]	7.2546±0.27389 [d]	2.0222±0.10558 [d]
Extract of Leek 5 g/Kg (G4)	4.0085±0.1939 [b]	3.3099±0.1937[ab]	8.3224±0.05065 [c]	2.5289±0.10609[c]
Extract of Parsley 5 g/Kg G5	3.839 ±0.4286 [b]	3.8174±0.1169 [a]	9.0268±0.100796 [b]	3.0597±0.08822 [b]
Extract of molokhia 4.8 g/Kg (G6)	3.5263±0.2626[ab]	3.252±0.1913[ab]	8.5267±0.39954 [bc]	1.9917±0.15365 [d]

All the values are expressed as the mean ±standard error of the mean. The mean difference is significant ($P < 0.05$) when compared with the positive control (gouty rat) group

There was a significant (P<0.05) increase in acid phosphatase (ACP) and alkaline phosphatase (ALP) enzyme activities in rat treated with the uricase inhibitor, potassium oxonate (G2) when compared to the normal control (G1). These were found to be reverted back in extracts of celery, leek, parsley, and molokhia treated animals. Treatment with extract of parsley significantly (P<0.05) decreased the ACP and ALP when compared to all extracts. On the other hand, the activity produced by extract of parsley (G5) was almost similar to that of the negative control (G1) (Table 3).

Table 3. *Acid phosphatase and Alkaline phosphatase levels of groups.*

Treatment	acid phosphatase (ACP)	Alkaline phosphatase (ALP)
Negative control (G1)	0.1108 ±0.1046 [a]	168.72±7.3169[a]
Positive control (G2)	0.2778 ±0.0078[c]	432.63±12.1662[d]
Extract of Celery 5 g/Kg (G3)	0.16114 ±0.02044 [b]	292.48±5.2898[c]
Extract of Leek 5 g/Kg (G4)	0.1915 ±0.00961 [b]	286.14±5.2285[c]
Extract of Parsley 5 g/Kg (G5)	0.11384±0.01347[a]	253.44±8.850[b]
Extract of molokhia 4.8 g/Kg (G6)	0.1613±0.016634 [b]	286.24±4.2929[c]

All the values are expressed as the mean ±standard error of the mean. The mean difference is significant ($P < 0.05$) when compared with the positive control (gouty rat) group

4. Discussion

One of the most sensitive and dramatic indicator of gout is neutrophil influx into the joint fluid. Neutrophils accumulate in both the joint fluid and the synovial membrane, where a small fraction of these cells actively phagocytose monosodium urate crystals and release mediators, that are chemotactic and amplify the inflammatory reaction (55). The enzyme XO catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid, which plays a crucial role in gout (56). XO is an important source of oxygen derived free radicals. The enzyme catalyzes reduce oxygen (during reperfusion phase), leading to the formation of superoxide anion radicals and hydrogen peroxide, as well as hydroxyl radicals (57). It has been proposed as a central mechanism of oxidative injury in some situations like gout, ischemia, renal damage, hypertension, diabetes, etc. (58, 16 and 59). Recent findings show that the occurrence of gout is increasing worldwide, possibly due to the changes in dietary habits like intake of high-purine foods viz., organ meats, yeast, beer and other alcoholic beverages (60 and 61). The main therapeutic approach for gout is the use of XOI such as allopurinol, which block the final step in the synthesis of uric acid from purines (62 and 63). An alternative to allopurinol is the use of medicinal plants which possess phytochemical constituents. We thus began our program to look for xanthine oxidase inhibitors of phytochemical origin from the extracts of celery, leek, parsley, and molokhia. Phytochemical screening of extracts revealed the presence of flavonoids, phenolics, and saponins accounting for its antioxidant property. Flavonoids are a group of polyphenolic compounds which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, antiulcer activities, etc. Also, the structure–activity relationship of different chemical classes of flavonoids have been reported as potential inhibitors of XOD (26, 27, 33, 28, 29, 22, 32and 36). Several *in-vitro* studies confirmed the xanthine oxidase to Xanthine dehydrogenase (XOD/XDH) inhibitory activity of some flavonoids. These compounds are structurally similar to XOD/XDH substrate and so can inhibit the enzyme activity (64 and 65). Celery, leek, parsley, and molokhia have also demonstrated the least XO inhibitory (XOI) activity probably due to limited bioactive compounds present. The extent of increasing in XOI activity elicited by allopurinol was much higher than that observed with the celery, leek, parsley, and molokhia in both extraction solvent. Similar results have been reported by others (66 and 39).In the present study, we noted Parsley and celery were the best source of raw material for obtaining the XOI compound as each exhibits more than 70% inhibition of XOD under all two extraction solvents. In fact, Parsley and celery under evaluation have shown considerable activity for XOD inhibition, substantiate the fact that secondary metabolites in the leaves contain diverse classes of bioactive phenolic compounds such as polyphenols, tocopherols and alkaloids (67, 68 and 32), which may act as XOI.

It is well known that XOD is an inducible enzyme. Oxonic acid and its salts are foreign substances that could interfere with some other metabolic systems in mice. It was found that oxonate was distributed to the intracellular sites of the small

intestine at a much higher concentration after oral administration. In addition, it was converted mostly to cyanuric acid in the gastrointestinal tract partly by XOD (69). Serum urate level is partly regulated by the kidney in rodents. Physiological and pharmacological studies have suggested that urate produced in liver is transported bidirectional in the proximal tubule. As oxonate is a competitive uricase inhibitor, it is likely that oxonate similarly competes with urate for binding at a specific site within urate transporter and permit efflux of urate subsequent to its intracellular production. Further, many studies confirmed that oxonate specifically inhibited renal electrogenic urate transport, and also blocks channel activity of urate transporter in mammalian (70, 71, and 72).The levels of uric acid, and creatinine were significantly elevated after oxonate treatment in our study. In the goutyrat, serum uric acid and creatinine levels reduced significantly after extracts of celery, leek, parsley, and molokhia administration. Similar results have been reported by others (20, 73, 74 and 75). Although the elevated levels of uric acid in the circulation could give rise to gout and possibly other pathological conditions (76), the antioxidant action of uric acid, particularly its ability to inhibit DNA damage, is also well documented (76 and 19). Parsley (*Petroselinum crispum*) as a dietary vegetable can be used safely long-term; this feature of parsley makes it a possible alternative for allopurinol, or at least in combination therapy to minimize the side-effects of allopurinol (39).XOD was found to be significant activity in liver. Uric acid synthesis appears to be mainly a hepatic process. Therefore, the gout effect of celery, leek, parsley, and molokhia could be explained, at least in part, by blocking of liver of XOD activities. The present study was the first account demonstrating the in vivo gout action of celery, leek, parsley, and molokhiaas well as their ability to decrease serum uric acid and creatinine levels in animals orally administered with our extracts. Duke (77) and Balch *et al.,* (78) recorded that the seeds and stalks of celery are known to reduce uric acid levels, relieving symptoms of joint pain and immobility. In one study, *Apium graveolens* has shown significant reduction in serum uric acid level in rats (79). *Apium graveolens* is used to treat fungal infections and tumors. *Apiumgraveolens* contains furocoumarins that are typically prescribed for their stomachic, carminative, diuretic and emmenagogue properties (80).

Our study indicates that by increasing serum total calcium level with extracts of celery, leek, parsley, and molokhia in comparison to positive control, symptoms of gouty arthritis are reduced. While comparing of organic phosphorus levels of Group 2 (positive control) with group 1 and gouty rat groups, highly significant correlation was observed ($P < 0.05$). It was due to high organic phosphorus levels of Group G2 (10.517 mg/dL) which is the highest of all these groups. Molokhia are edible and are used as an ingredient for popular food in Egypt. This plant is also rich in potassium, calcium, phosphorous, iron, ascorbic acid, and carotene (36). Celery provides an excellent source of vitamin B1, B2, B6, C and fiber. It's a very good source of folic acid, potassium, and calcium (81 and 82). Leeks are a good source of dietary fiber, folic acid, calcium, potassium, and vitamin C. Calcium in leeks is also used for the proper clotting of blood in the human body (83 and 84).

In recent years, there is increasing interest in free radicals; they have been shown to modify biological molecules, which may result in various pathological conditions. Thus additional natural products need to be evaluated for their antioxidant potential. Considerable evidence suggests that oxidative stress and reactive oxygen species (ROS) play significant roles in several aspects of acute and chronic inflammation (85). The increased lipid peroxide level noticed in potassium oxonate induced rat in our study (Group 2), due to its release from neutrophils and monocytes during inflammation (86). The result of the present study indicate that the antioxidant defense system is compromised in potassium oxante induced rat as evidenced by increased lipid peroxidation concentration and decreased activity of antioxidant enzyme. Our results show that the activities of Superoxide dismutase, Glutathione peroxidase and Catalase decreasing in potassium oxonate-induced animals which may be due to consequence of their increased consumption during oxidative stress and cellular lysis. All extracts of celery, leek, parsley, and molokhiahave shown significant decrease in level of MDA and increase in activity of antioxidant enzyme level, comparable to positive rat (G2).Thereby revealing that serum uric acid through lipid peroxidation, might be working towards the etiopathogenesis of oxidative stress diseases and its serum level may be a deciding factor for progression of the disease, and also our study showed that parsley (*Petroselinum crispum*) has a higher potential than celery, leek, and molokhia to increase glutathione peroxidase activity. Several studies have identified the active antioxidants within parsley (*Petroselinum crispum*) including flavonoids (32), carotenoids (87), ascorbic acid (88), tocopherol and coumarines (32). These phytochemicals improve total antioxidant capacity, suppress destructive oxygen free radicals and prevents oxidative stress damage (75 and 89). This was in agreement with Haidari *et al*., (39). Leeks are a good source of allyl sulfides and also rich in the flavonoid especially kaempferol. Allyl derivatives of leek oils stimulate the activity of GP_X and inhibited the decreased ratio of reduced to oxidized glutathione produced by 12-*O*-tetradecanoylphorbol-13- acetate in epidermal cells. Diallyldisulfide increase GP_X activity in animal tissues with increased the activity of glutathione reductase, and superoxide dismutase (90, 84 and 91). A phenolic extract of molokhia exhibited antioxidant activity through the radical generator-initiated peroxidation of linoleic acid (36).Al Batran *et al*., (92) showed that gastroprotective effect of an ethanolic extract of molokhia against ethanol-induced gastric ulcers in adult *Sprague Dawley* rats. SOD and MDA levels were significantly increased ($P \leq 0.05$) and reduced ($P \leq 0.05$), respectively pretreatment with molokhia. Oyedeji and Bolarinwa (93) found that aqueous extract of molokhia has beneficial potentialities on the blood chemistry of male albino rats. As in traditional medicine they were used for

management of gout it is thought that their anti-gout activity, at least by part, was related to this xanthine oxidase inhibitory activity. Gouty arthritis is metablic disorder that is characterized by deposition of uric acid into joints (94). Now there is new trend to use herbal medicine because of their potential to cure and fewer or no side effects. Medicinal plants having anti-inflammatory, uricosuric and xanthine oxidase inhibitory activities are used in gouty arthritis. Herbal medicines are comparably safe, and are useful in the management of gout. Although allopurinol is commonly used in the treatment of gouty arthritis but this has side effects such as skin rashes, nauseas and vomiting. The possible explanation can be drawn from number of studies showing that superoxide dismutase during catalyzing dismutation of $O_2$to H_2O_2 can form copper bound hydroxyl radical from hydrogen peroxide H_2O_2 (95). Hydroxyl radical when gets bounded to SOD, then it can attack adjacent histidine residue which is attached to copper resulting in inactivation of both SOD1 and SOD3 (96).

Conclusion, this study had established the xanthine inhibitory action of several plant extracts used in Egypt folk medicine. The extracts of celery, leek, parsley, and molokhia have been tested for xanthine oxidase inhibitory activity. The efficacy of distilled water as the extraction solvents was commendable because most of the plants used to treat gout were administered as decoctions and infusions, so the biologically active compounds were most likely water-soluble. In addition, the possibility of any harmful residue due to use of organic solvent was also avoided. The selection of tropical plants used in medicine and screening of their extracts for pharmacological activity may provide identification of newer medicaments for the treatment of various ailments especially gout. Further research on celery, leek, parsley, and molokhia have significantly reduced the serum uric acid, lipid peroxidation, and increase activity of antioxidant enzyme levels in gouty rats. This may be due to the inhibition of XO activity and the presence of phytochemical constituents.

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