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Composés bioactifs

Olea europaea leave extracts and allopurinol alleviate oxidative stress in liver rats induced by oxonate

L'extrait des feuilles d'*Olea europaea* et de l'allopurinol atténue le stress oxydatif du foie des rats induit par l'oxonate

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Résumé ***Introduction.*** Face aux limites thérapeutiques des médicaments chimiques, le développement de la recherche sur les plantes médicinales a été orienté vers l'obtention de phytomédicaments. ***Objectif***. Ce développement constitue une étape indispensable pour évaluer l'effet de la protection d'*Olea europaea* et de l'allopurinol sur les changements biochimiques et histologiques du foie des rats rendus hyperuricémiques par l'oxonate. ***Matériel et Méthodes.*** Des rats témoins ont reçu pendant 7 jours une injection intrapéritonéale (i.p.) quotidienne de NaCl à 0,9%. Un groupe de rats a reçu une injection i.p. d'oxonate de potassium (300 mg/kg poids corporel (PC) (oxonate de potassium) et traité soit par l'allopurinol 10mg/kg PC (oxonate de potassium+allopurinol), soit par l'extrait d'*Olea europaea* à une dose de 1500 mg de feuilles fraîches/kg PC (Oxonate de potassium+Olea). L'acide urique, l'aspartate et l'alanine amino transaminases et la phosphatase alkaline, le taux de peroxydation lipidique, l'activité antioxydante de la catalase, superoxyde dismutase et glutathion peroxydase ainsi que l'histologie hépatique ont été étudiés.

Résultats. Chez les rats hyperuricémiques traités par l'allopurinol ou par la décoction de feuilles d'olive, une réduction est notée au niveau de la peroxydation des lipides, de l'aspartate, de l'alanine amino transaminases, de la phosphatase alcaline et des changements histopathologiques ont été observés. ***Conclusion.*** Une amélioration plus prononcée est observée chez les rats traités avec la décoction d'*Olea europaea* que ceux traités avec de l'allopurinol.

Mots-clés: *Rats hypoeuricémiques, Stress oxydatif, Foie, Feuilles d'Olea europaea, Allopurinol*

Abstract ***Introduction.*** Facing the limits of therapeutic chemical drugs, the research development on medicinal plants has been directed towards obtaining herbal medicines. ***Objective.*** This development was an essential step in assessing the protection effect of *Olea europaea* and allopurinol on biochemical and histological changes in the liver of hyperuricemic rats induced by oxonate. ***Material and Methods.*** A control group received daily an intraperitonealy injection (i.p.) of 0.9% NaCl for seven days. Another group received i.p. injection of potassium oxonate (300 mg/kg body weight (BW) (potassium oxonate) and either treated by allopurinol (10mg/kg BW (potassium oxonate + allopurinol) or fresh leaf *Olea europaea* extract at a dose of 1500 mg /kg BW (potassium oxonate+Olea). Uric acid, aspartate and alanine amino transaminase and alkaline phosphatase, lipid peroxidation, antioxidant activities of catalase, superoxide dismutase and glutathione peroxidase, and liver histology were assayed. ***Results.*** Our results showed a reduction of lipid peroxidation, aspartate, alanine transaminase, alkaline phosphatase and histopathological changes in hyperuricemic rats treated with allopurinol or decoction of olive leaves. ***Conclusion.*** There is a more pronounced improvement in rats treated with decoction of *Olea europaea* than those treated with allopurinol.

Keywords: *Hyperuricemic rats, Oxidative stress, Liver, Olea europaea leaves, Allopurinol*

Introduction

Hyperuricemia is one of the risk factors causing gout. In addition, it has been demonstrated that hyperuricemia is closely associated with other life style related diseases. [1]. Uric acid is the final metabolite of endogenous and food-derived purine bases in humans.

Diet may play an important role in the development of hyperuricemia and gout. However, the association between dietary factors and hyperuricemia remains unclear, and few studies have investigated direct links between food intake and hyperuricemia. Villegas et al. have shown an association between high purine-content foods and protein intake with the uric acid level increase [2]. Hyperuricemia is one of the most common and extensive metabolic diseases in populations, characterized by high uric acid levels in the blood, causing deposition of urate crystals in the joints and kidneys. It is well known as an important risk factor for gout arthritis, nephrolithiasis, cardiovascular and renal diseases, leading to hypertension [3].

Despite advances in the use of anti-hyperuricemic agents for the treatment of hyperuricemia and

gout, allopurinol a compound used frequently as a xanthine oxidase (XOD) inhibitor could cause in 2% of the users a severe hypersensitivity, an agranulocytosis and aggravate renal toxicity by impairing pyrimidine metabolism [4]. Therefore, the available anti-hyperuricemic agents are needed, especially medicinal plants [5].

The olive tree *Olea europaea*, belonging of Oleaceae family, has been cultivated for more than a thousand years in the Mediterranean regions. Olive oil has been used for medicinal purposes [6]. In many countries, they are known as a folk remedy for hypertension and diabetes [7]. It has been traditionally used to cure rheumatic and neuralgic diseases in Lebenan [8] and also to alleviate muscle and joint pain in some regions of Iran [6].

Olive leaf extract could exert antinociceptive effects on chemical and thermal models of pain and influence morphine analgesic/hyperalgesic properties in rats. Despite the number of papers published on olive leaf extracts and the effects of their constituents, there are no reports focused on their effects on nociceptive threshold and their analgesic activity. Previous studies have demonstrated that olive leaf extracts have analgesic property in chemical and thermal nociceptive tests in rats [6].

It is well known that oleuropein, hydroxytyrosol, tyrosol and caffeic acid are the main compounds of olive leaves, which are considered to be responsible for their pharmacological effects. Furthermore, olive leaves contain p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside [9-11].

Recent studies suggested that olive leaf was a significant source of bioactive phenolic compounds compared to olive oil and fruits. Identifying appropriate extraction methods were thus an important step to increase the yield of such bioactive components from olive leaf [12].

Therefore, the aim of the present study was to find a scientific basis that could support the use of Olea europaea in medicine. In this study, the effects of Olea europaea decoction were compared to allopurinol, a standard non-steroidal anti-inflammatory drug, in oxonate induced uricemic rats.

Materials and methods

Experimental design

Adult male Wistar rats, weighing 250±5 g, were purchased from the Central Pharmacy (The Society of Pharmaceutical Industries, Tunisia). They were housed at ambient temperature 22±3°C in a 12 hour light/dark cycle and a minimum relative humidity of 40%. The animals had free access to commercial pellet diet (Industrial society optimized packaging, ISOP Sfax, Tunisia) and water. The general guidelines for the use and care of living animals in scientific investigations were followed [13]. The handling of the animals was approved by the Tunisian Ethical Committee for the Care and Use of Laboratory Animals.

Rats were randomly divided in to four groups (n=6). The control group received daily for seven days i.p. injection of 0.9% NaCl. The (potassium oxonate) group was intraperitoneally given potassium oxonate at a dose of 300 mg/kg BW, as reported by Liu et al. [14], then either treated by allopurinol (10mg/kg BW) (potassium oxonate + allopurinol) group or *Olea europaea* extract (1500 mg fresh leaf/kg BW) (potassium oxonate + Olea).

Allopurinol, an analog of hypoxanthine and a common remedy to treat hyperuricemia was used in our study in order to compare its effect with those of Olea europaea extract.

Blood and liver samples

After 7 days of treatment, 6 fasted rats of each group were sacrificed under anaesthesia by i.p. injection of chloral hydrate. Serum samples were drawn from blood after centrifugation at 3000 x g for 15 min at 4°C.

The liver was removed, rinsed with ice-cold saline and kept at − 20◦ C until analysis. Some samples were homogenized in phosphate buffer (pH 7.4) and centrifuged. The resulting supernatants were used for biochemical assays. Other samples were immediately fixed in Bouin solution for histological studies.

Biochemical assays

Estimation of uric acid concentrations

Uric acid levels in serum and liver samples were measured by the uricase colorimetric test using a commercial reagent kit (Biomaghreb Diagnostic

Ariana, Tunisia).

Biomarkers of liver injury

Serum aspartate and alanine amino transaminases (AST and ALT) and phosphatase alkaline (PAL) activities were measured using commercial kits (Biomaghreb Diagnostic Ariana, Tunisia).

Lipid peroxidation assay

Liver lipid peroxidation was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) which were expressed in terms of malondialdehyde content according to Yagi [15]. Supernatant (125µL) from liver homogenate was mixed with 125µL of trichloroacetic acid in order to precipitate proteins and after centrifugation at 1000g for 10 min at 4°C, 200 µL of the new supernatant was mixed with 40µL 0.6 M HCl and 160 µL of 20% thiobarbituric acid in Tris buffered Saline. The mixture was heated at 80°C for 10 min, and after cooling, the absorbance was read at 530 nm. The amount of TBARS was calculated by using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Antioxidant enzyme activities

Superoxide dismutase (SOD) activity was estimated according to Beyer and Fridovich [16]. The reaction mixture contained 50 µL of tissue homogenate in potassium phosphate buffer (pH 7.8), 0.1 mM ethylene diamine tetraacetic acid (EDTA), 13 mM methionine, 2 mM riboflavin and 75 mM Nitro Blue Tetrazolium (NBT). The developed blue color in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as units/mg protein. Catalase (CAT) activity was assayed by the method of Aebi [17]. Enzymatic reaction was initiated by adding an aliquot of 20 µL of the homogenized tissue and the substrate (hydrogen peroxide, H_2O_2) to a concentration of 0.5 M in a medium containing 100 mM phosphate buffer, pH 7.4. Changes in absorbance were recorded at 240 nm. The enzyme activity was expressed as µmol H_2O_2 consumed/min/mg protein. Glutathione peroxidase (GPx) activity was measured according to the method of Flohe and Gunzler [18]. The enzyme activity was expressed as µmol of GSH oxidized/min·g protein. Protein content in the supernatant was determined according to Lowry *et al*. [19]

Histopathological study in liver

Some portions of liver tissues were fixed for 48 h in Bouin solution, dehydrated in an ascending graded series of ethanol, cleared in toluene and embedded in paraffin. Sections of 5–6 µm thickness were made by using a rotary microtome and stained with hematoxylin and eosin (H&E) for microscopic observations Gabe [20]. Six slides were prepared from each group. All sections were evaluated for the degree of liver injury. Each liver slide was examined and assigned for severity of changes using scores on a scale of none (-), mild (+), moderate (++) and severe (+++) damages.

Statistical analysis

Results were reported as means ± standard deviation (SD) for at least 6 determinations throughout the study. The results were analysed by one-way ANOVA followed by the Duncan multiple range test. The Statistical Package for the Social Sciences (SPSS 11.0 for Windows) software was used for statistical evaluation. $P \leq 0.05$ was considered significantly different.

Results

Serum and liver uric acid levels

Serum and liver uric acid concentrations were increased significantly by 36 and 129 % respectively in potassium oxonate rats compared to controls ($P<0.05$) (Fig. 1). Allopurinol treatment of hyperuricemic rats restored partially serum uric acid level. Interestingly, a similar effect was observed in rats receiving *Olea europaea* extract. Moreover, the hypouricemic effect was more pronounced in serum than in liver content.

Biomarkers of liver injury

The activities of AST, ALT and PAL, increased significantly by 26, 25 and 20 % respectively in hyperuricemic rats compared to controls ($P<0.05$) (Fig. 2). After treatment of rats by Allopurinol or *Olea europaea* extract, these activities were significantly decreased by 54, 48 and 36 % and by 20, 18 and 11%, respectively as compared to those of potassium oxonate rats.

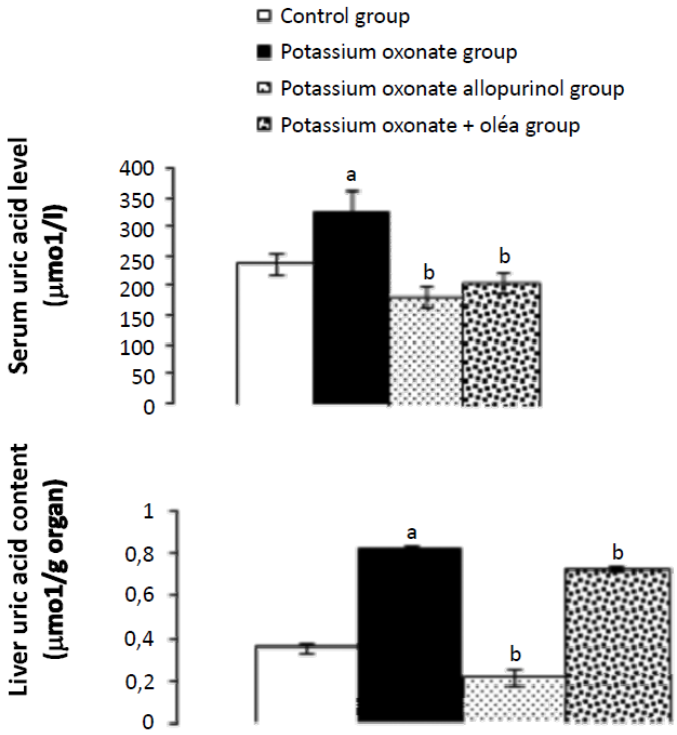

Fig. 1. Uric acid levels in serum and liver tissue after 1 week of allopurinol or *Olea europaea* extracts treatment in hyperuricemic rats

Values are means ± SD of 6 rats. Comparison of means was performed by Duncan multiple range test with a significant difference at $p < 0.05$: a : as compared to controls; b as compared to potassium oxonate rats.

Liver lipid peroxidation and antioxidant enzyme activities

Liver TBARS values were significantly increased by 78% in potassium oxonate-induced hyperuricemic rats when compared to controls ($P<0.05$). The administration of Allopurinol or *Olea europaea* significantly reduced the level of liver TBARS in hyperuricemic rats (Fig. 3).

In potassium oxonate rats, antioxidant enzyme activities of SOD, CAT and GPx increased, respectively by 62, 45 and 62% when compared to controls (Fig. 4). Allopurinol or *Olea europaea* leaves decreased these enzyme activities. Indeed, SOD, CAT and GPX activities were lowered by 49, 47 and 53% in uricemic rats treated with Allopurinol and by 121, 91 and 90% when treated with *Olea europaea* compared to those of untreated hyperuricemic group.

Liver histopathology assessment

In control group, normal liver histological aspect, with distinct hepatic cells and sinusoidal spaces, was observed (Fig. 5.A). Hyperuricemic rat developed a significant hepatic damage as compared to controls. Potassium oxonate intoxication exhibited, in hepatocytes of dams, several histopathological changes, such as marked leucocytes infiltration, sinusoidal dilatation (moderate peliosis) and granuloma inflammatory disorders surrounded by few necrotic cells (Fig. 5.B).

The administration of allopurinol showed no significant effect on reducing the injuries of liver (Fig. 5.C), whereas improved effect was obtained in potassium oxonate-induced hyperuricemic rats and tretaed with *Olea europaea* compared to the control group (Fig. 5.D). The histopathological changes are summarized in the Table.

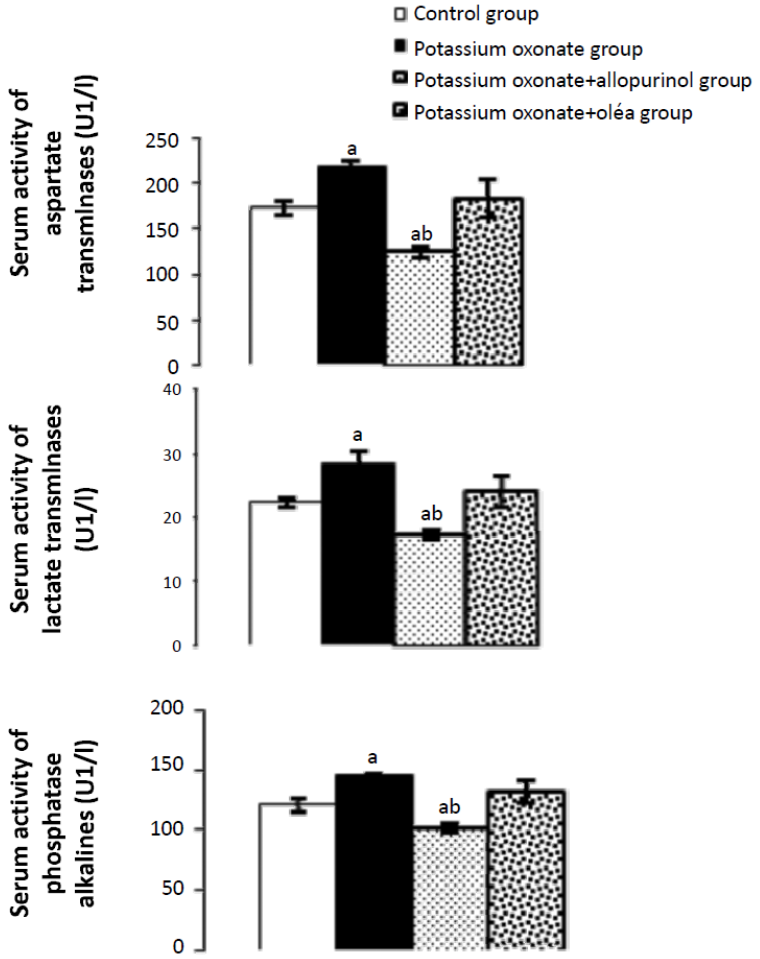

Fig. 2. Aspartate and alanine amino transaminases and phosphatase alkaline after 1 week of allopurinol or *Olea europaea* extracts treatment in hyperuricemic rats

Values are means ± SD of 6 rats. Comparison of means was performed by Duncan multiple range test with a significant difference at p<0.05. a: as compared to controls; b: as compared to potassium oxonate rats.

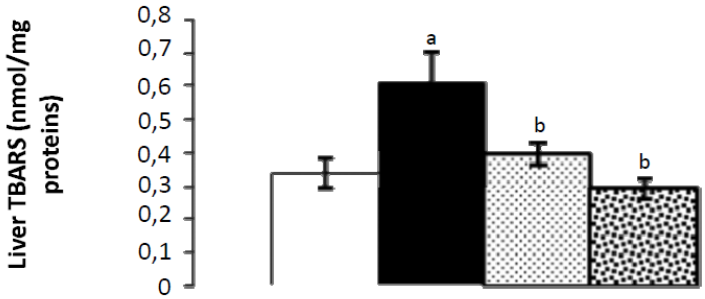

Fig. 3. Liver lipid peroxidation after 1 week of allopurinol or *Olea europaea* extracts treatment in hyperuricemic rats

Values are means ± SD of 6 rats. Comparison of means was performed by Duncan multiple range test with a significant difference at p<0.05. a: as compared to controls; b: as compared to potassium oxonate rats.

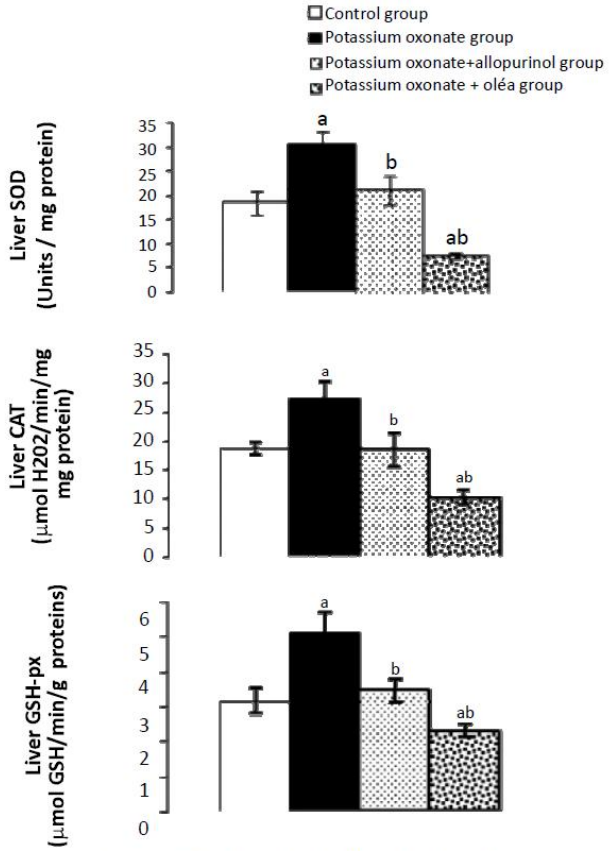

Fig. 4. Antioxidant enzyme activities after 1 week of allopurinol or *Olea europaea* extracts treatment in hyperuricemic rats

Values are means ± SD of 6 rats. Comparison of means was performed by Duncan multiple range test with a significant difference at p<0.05. a: as compared to controls; b: as compared to potassium oxonate rats.

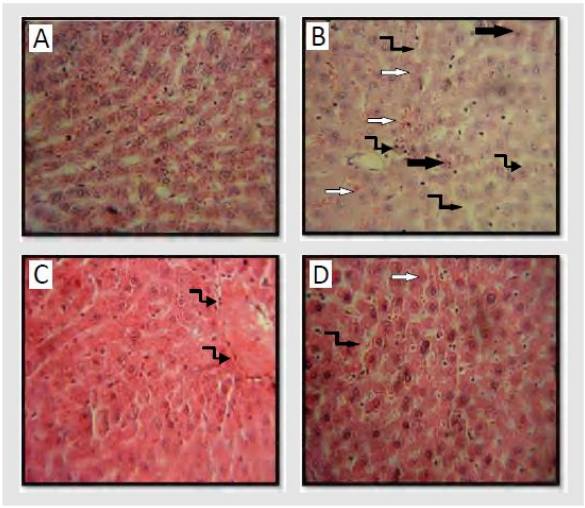

Fig. 5. Liver histological sections of adult rats: Controls (A), Potassium oxonate group (B), Potassium oxonate+allopurinol (C), Potassium oxonate+*Olea europaea* (D)

Optic microscopy; hematoxylin–eosin stain; magnification A, B, C, D: (x 400)

: Leucocytes infiltration; : sinusoidal dilatation; : granuloma inflammatory disorders; : necrotic cells.

Discussion

In this study, olive leaf extract were compared to Allopurinol treatment in potassium oxonate Wistar rats on oxidative stress and protective effects against hyperuricemia.

Potassium oxonate a selective competitive uricase inhibitor blocks the effect of hepatic uricase and produces hyperuricemia in rodents [21]. Indeed, potassium oxonate rats could serve as a useful animal model of hyperuricemia to evaluate drugs that affected serum uric acid levels and also to evaluate the possible therapeutic agents in certain disorders associated with abnormal uric acid levels. Uric acid was generated when purine catabolism of hypoxanthine and xanthine was catalyzed by xanthine oxidase.

In recent years, several data documented low rates of adherence to gout medication treatments. These facts suggest a major role for therapeutic education in patients with gout [22-23].

Allopurinol, often used in clinical drugs to decrease uric acid levels, was assayed in the present study to treat hyperuricemia in rats. The activity of xanthine oxidase was inhibited by flavonoids, leading to reduced synthesis of uric acid [24]. Despite advances in the use of anti-hyperuricemic agents for the treatment of hyperuricaemia and gout, allopurinol as a frequently used xanthine oxidase (XOD) inhibitor, could cause 2% of the users to induce severe hypersensitivity and agranulocytosis, and aggravate renal toxicity by impairing pyrimidine metabolism [4]. Therefore, it is for urgent need of available anti-hyperuricemic agents, especially medicinal plants [5,25]. Around the world, millions of people use medicinal plants as a part of traditional medicine for a large range of medical disorders [26]. Olive tree (*Olea europaea L.*) leaves have been widely used for traditional remedies in European and Mediterranean countries such as Greece, Spain, Italy, France, Turkey, Israel, Morocco, and Tunisia. They have been used in the human diet as an extract, an herbal tea, and a powder. These leaves also contain many potentially bioactive compounds that may have antihypertensive, anti-atherogenic, anti-inflammatory, hypoglycemic, hypocholesterolemic and antioxidant properties [11-12,27].

We demonstrated that in the hyperuricemic model, olive leaf extracts fully prevented the uric acid increase compared with the control group. This is most likely due to the characteristics of absorption or the metabolism of the compounds responsible for the effect after oral administration in vivo. Thus, the present study suggested that olive tree could be more useful to treat gout attacks, than allopurinol.

Table. Grading of the histopathological changes in liver sections of Controls, Potassium oxonate, Potassium oxonate+allopurinol, and Potassium oxonate+*Olea europaea rats*

Groups	Leucocytes infiltration	Sinusoidal dilatation (peliosis)	Granuloma inflammatory disorders	Necrosis
Control	-	-	-	-
Potassium oxonate	++	++	+++	++
Potassium oxonate + allopurinol	+	-	+	-
Potassium oxonate + *Olea europaea*	+	-	+	-

[Scoring was done as follows: none(–), mild(+), moderate(++) and, severe(+++) damage]

Drugs frequently used to treat gout, such as allopurinol, probenecid, benzbromarone, sulfinpyrazone, and colchicine, often caused secondary effects [28]. Therefore, such phytochemicals, containing flavonoids, may be considered as another choice to treat hyperuricemia and gout. found to inhibit xanthine oxidase activity, which reduced uric acid, but their side effects were not studied [29]. The present study demonstrated that *Olea europaea* effectively lowered serum uric acid level, which was the key factor for preventing gout. Flavonoids and other phytochemicals have been found to inhibit xanthine oxidase activity, which reduced uric acid, but their side effects were not studied [29]. The present study demonstrated that Olea europaea effectively lowered serum uric acid level, which was the key factor for preventing gout. Drugs reducing uric acid levels were agents usually: uricolytic which promoted the excretion of uric acid or uricostatic agents which arrested the synthesis of uric acid agents.

The bioactive compounds in traditional plant reduced uric acid through inhibiting xanthine oxidase. Here, *Olea europaea* inhibited the uric acid synthesis by a different mechanism than allopurinol. We found that *Olea europaea* significantly lowered uric acid involving uric acid excretion. This result seemed independent of the human mechanism to reduce uric acid and thus suggesting possible new treatment options for patients with gout [30]. *Olea europaea* may be able to prevent gout arthritis. Although previous studies have indicated that the flavonoids can inhibit xanthine oxidase activity, some in vitro experiments have found that flavonoids have minimal or no xanthine oxidase activity. They fail to reduce uric acid and may have limited effectiveness [31]. Antioxidants can potentially decrease serum uric acid levels [32].

We therefore postulate that olive leaf extract exerted an anti-toxicity effect. The olive (*Olea europaea*) plant belongs to the Oleracea family, and its leaves and oil are used for medicinal purposes. Furthermore, olive leaf extract has anti-oxidant and anti-inflammatory properties [33]. oxidant and anti-inflammatory properties [33]. Like many natural herbs, olive leaves are also known to be an antioxidant and contain some of the most powerful known antioxidants [34]. Olive leaf extracts were showin real promise in both of these areas. In animal and basic laboratory studies, olive leaf extracts and oleuropein have been found to lower blood sugar through several mechanisms [35-36]. Indeed, a slow digestion of starches in simple sugars, a slow absorption of these sugars from the intestine, and increased glucose uptake by tissues from the blood were observed [36]. Moreover, tissue protection from the oxidant damage caused when glucose binds to proteins in the process called glycation were also observed [35].

In our experimental conditions, hyperuricemia was accompanied by a high marked oxidative impact as evidenced by the significant increase of hepatic lipid peroxidation and antioxidant enzymes including SOD, CAT, and GPX activities and liver damage biomarkers. Relationship between hyperuricemia and biomarkers of liver damages, including aspartate, alanine amino transaminases and phosphatase alkaline was indicated.

Since, the olive leaves have been recommended in the literature as a remedy for the hyperuricemia treatment due to their antioxidant agents compounds. The high levels of TBARS suggested a cell aggression by free-radicals and reactive oxygen species resulting from uric acid toxicity and could be partly responsible for the oxidative stress and liver injury. The administration of Allopurinol or *Olea europaea* significantly decreased the levels of TBARS in the liver of the potassium oxonate-induced hyperuricemic rats. Studies demonstrated that plants reduced inflammation and aging, and inhibited free radical activity [27,37].

Our histological studies substantiated liver dysfunction. Nevertheless, rats treated with oxonate developed a significant hepatic damage as compared to controls. Free radical formation during the metabolism of uric acid by hepatic microsomes, caused lipid peroxidation of the cellular membrane leading to the necrosis of hepatocytes. An ameliorative effect was obtained in hyperuricemic rats treated with olive leaves decoction.

The present study is the first to demonstrate that *Olea europaea* has a significant antioxidant and anti-uricemic effects in oxonate-induced hyperuricemic rats. This study confirms the fact that olive leaves decoction is a basic component in

folk medicine. Further studies on this species may yield fruitful results and isolation of some active constituents may lead to the provision of new drugs for treatment of hyperuricemia and gout.

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