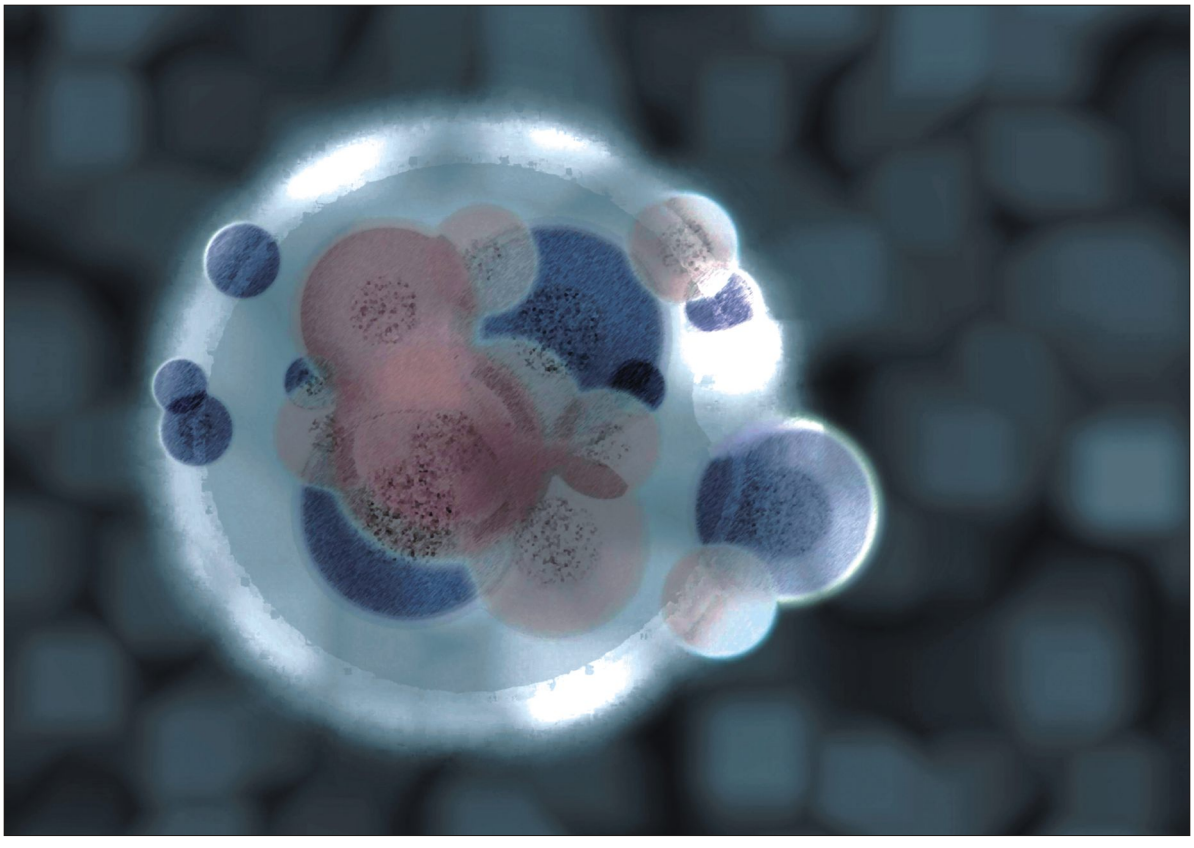

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Research Article
Anti-hyperuricemic, Uricosuric and Xanthine-oxidase Inhibitory Activities of Watermelon Powder in a Rat Gout Model

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Abstract

Background and Objective: Gout is a common metabolic disorder around the world. It characterized by elevation of uric acid levels in the blood, leading to increase the deposition of urate crystals in the joints and kidneys. The current study was carried out to investigate the efficacy and mechanism action of watermelon powder as antihyperuricemic agent. **Materials and Methods:** Enzyme assay was done by using bovine milk xanthine oxidase (XO). The XO inhibitory activity *in vitro* was performed by using different doses of watermelon powder and the degree of XO inhibition was expressed as IC_{50}. The antihyperuricemic and uricosuric activity of watermelon were tested in the potassium oxonate-induced hyperuricemic rats for seven consecutive days of oral treatment of 25, 50 and 100 mg kg^{-1} doses. **Results:** The results of the study revealed that the watermelon has a moderate activity of XO inhibition with IC_{50} =95.24 μg mL^{-1}. In addition, these results showed that all doses of watermelon powder were able to significant reduce serum uric acid levels in the hyperuricemic rats. Moreover, the results of uricosuric activity assay showed that the watermelon significantly increased the urinary excretion of uric acid. **Conclusion:** The watermelon powder showed significant effects on the evaluated models and therefore it may be promising agent for the treatment of gout since it possesses a moderate xanthine oxidase inhibitory and a potent of both antihyperuricemic and uricosuric effects.

Key word: Watermelon powder, xanthine oxidase, antihyperuricemic, uricosuric, gout, hyperuricemic, urate crystals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gout is a widespread metabolic disorder around the world, characterized by elevation of uric acid levels in the blood, leading to increased accumulation of needle-like urate crystals in the joints and kidneys[1]. The accumulation of urate crystals in the tissues activates the inflammatory sequence, followed by acute inflammatory response such as swelling, warmth and quite pain[2,3]. In humans, the uric acid is the ultimate product of metabolic pathway of purines. Most of the uric acid pool attributed to the breakdown of endogenous purines while the remaining quantity resulted from dietary (exogenous) purines[4]. Unfortunately, human is the only being of the rest of the other mammals cannot convert uric acid to a more soluble substance, allantoin because of the absence of the uricase enzyme[5]. Consequently, the altitude of uric acid levels in the blood due to the hepatic overproduction of uric acid or renal underexcretion of it or both will lead to the occurrence of hyperuricemia and gout respectively[6].

There are two types of urate-lowering drugs in the gout treatment: Xanthine oxidase inhibitors and uricosurics. The first type of drugs such as allopurinol is indicated for the patients who suffer from the increased in the uric acid production, whereas the uricosurics drugs such as febuxostat are indicated for the patients who are suffering from the underexcretion of urates. The mechanism action of xanthine oxidase inhibitors through inhibition the action of xanthine oxidase, which is responsible on the conversion of xanthine into hypoxanthine and of hypoxanthine to uric acid[6,7]. The action of uricosurics drugs via the enhancing the renal excretion of uric acid by inhibition of set of proteins involved in the renal tubules are called urate transport-related proteins[8]. However, the use of these drugs will not be without serious health complications such as hepatotoxicity, renal toxicity and severe allergic reactions and sometimes may lead to renal failure[9,10]. Therefore, the search for new antihyperuricemic drugs, including more safety and efficacy would be very useful in treatment of the gout and related diseases[11].

The use of herbal plants is earning new interest in the treatment of diseases due to their low sensitivity and side effects[12]. Plants contain a large amount of potential antioxidants and bioactive substances, making them a target for the search for new drugs[13]. It has been demonstrated that the many plant-derived antioxidants can be regulated uric acid through either increasing uric acid excretion (uricosuric activity) or by reducing the production of uric acid (inhibition of xanthine oxidase) to reduce the risk of gout[14].

Watermelon (*Citrullus lanatus*) commonly consumed in the summer as a refreshing fruit all over the world and in the Middle East specifically due to the very hot climate[15,16]. Watermelon consists of more than 90% water and hence it is called watermelon. Watermelon is a rich natural source for many bioactive materials such as A, C, E and B-complex vitamins and it contains amino acid citrulline, cucurbitacin, triterpenes, alkaloids and many different minerals[17-19]. The red colour of watermelon flesh is as result of an excellent quantity of the potent antioxidant carotenoid, lycopene. Many studies have shown that watermelon juice has numerous beneficial therapeutic effects such as potent antioxidant, anti-inflammatory, antimicrobial, laxative, anti-giardial and anti-hyperlipidemic activity[20-22]. The protective effect of watermelon for several different tissues such as liver, kidneys and nerves have been reported[23, 24]. The fruit is very effective in the treatment of, dropsy and infections and stones of urinary tract[25]. The present study was designed to evaluate the anti-hyperuricemic activity of *Citrullus lanatus* (watermelon) and investigation the mechanism action of urate-lowering effect through either by increasing of uric acid excretion and/or by decreasing of uric acid production via the inhibition of xanthine oxidase enzyme.

MATERIALS AND METHODS

Reagents and chemical: Uric acid kit was purchased from Biolabs Company (Maizy, France). Xanthine oxidase from bovine milk (Grade I), xanthine and allopurinol were provided from Sigma-Aldrich (Dorset, England). All other chemicals were supplied from Merck (Darmstadt, Germany). All chemicals and reagents used in this study were of analytical grade.

Plant material and preparation of watermelon powder: Ripe fruit of red-fleshed watermelon (*Citrullus lanatus*) was purchased in May, 2018 from a local market of Basrah, Iraq. The fresh watermelon fruit was washed under tap water and the rinds were peeled by using a stainless knife, then the seeds were removed from the fruit flesh. The flesh was sliced using slicer and then the slices were dried by hot-air oven (Lab-Line, USA) at 40°C for two days. The dried watermelon slices were then grinded by an electric mill (Silver Crest, Germany) into a fine powder, kept in a sealed glass container and stored in the refrigerant before use[26]. This study was conducted during May-June of 2018 at the Faculty of Pharmacy, University of Basrah, Basrah, Iraq.

Animals: This study included the use of male Wistar rats (150-180 g), were supplied from the unit of animals' house at college of Pharmacy, Basrah University. The rats were separated into different groups (n = 6), then the animals were accommodated in isolated plastic cages and kept in the animal's room under a regulated condition at temperature 25±2°C and humidity 30±15% with 12-h dark/12-h light cycle for a week before being used for acclimatization. The animals were consumed standard chow and water ad libitum. Animal Ethics committee, University of Basrah, Iraq (No. 2013/32), authorized all of dealing procedures with animals that described in this study.

***In vitro* XO inhibitory activity:** The xanthine oxidase (XO) inhibitory effect of watermelon powder was assessed spectrophotometrically at 290 nm according to Sunarni *et al.*[27] and Yumita *et al.*[28] with minor changes. The mixture assay consists of 0.9 mL of 0.05 M sodium phosphate buffer (pH 7.5 at 25°C), 1 mL of watermelon powder (100 μg mL^{-1} in DMSO) and 0.1 mL of XO enzyme solution (0.1 unit mL^{-1} in phosphate buffer, pH 7.5) was prepared in cold buffer directly before using. After a 15 min pre-incubation at 25°C, then the reaction was allowed to start by addition of 2000 μL of freshly prepared solution of substrate (0.15 mM xanthine solution). Next, a further incubation process was achieved for the reaction mixture at 25°C for 30 min. After addition of 1 mL of 1N HCl solution into assay mixture for stopping the reaction, the absorbance was recorded at wave length 290 nm by using UV/Vis spectrophotometer (Chrom Tech, USA) against the blank which is prepared in the same procedure but with replacement of enzyme solution by phosphate buffer. The positive control solution was prepared by using allopurinol (100 μg mL^{-1}) in DMSO. The inhibitory activity against the XO was stated as the percentage of inhibition (%):

$$\text{XO inhibition (\%)} = \left(1 - \frac{\alpha}{\beta}\right) \times 100$$

where, α represents the activity of XO in absence of the tested substance (watermelon powder) and β is the activity of XO with presence of watermelon powder. Different concentrations of both watermelon powder and allopurinol (1, 2, 3, 4, 5, 10, 25, 50 and 100 μg mL^{-1}) were used for evaluation of XO inhibitory activities and then the dose-response logarithmic curve was applied to determine the median maximum inhibitory concentration IC_{50}.

Drug administration: Allopurinol, benzbromarone and watermelon powder were suspended in 0.5% sodium salt of carboxymethylcellulose, CMC-Na (vehicle). Potassium oxonate, uricase inhibitor (250 mg kg^{-1}) was suspended in the solution of 0.9% sterile saline. All solutions were prepared freshly before use for *in vivo* experiments.

Evaluation of anti-hyperuricemic and uricosuric activity: The anti-hyperuricemic and uricosuric activity of watermelon powder was investigated by using the potassium oxonate-induced hyperuricemia in the rat's model according to Qin *et al.*[29] and Ferrari *et al.*[30] with modifications. Animals were fasted by withdrawing of food and water 2 h before drugs administration. Experimental animals (rats) were divided randomly into seven groups (n = 6). The uricase inhibitor (Potassium oxonate) at a dose of 250 mg kg^{-1} was injected intraperitoneally (i.p.) to rats of groups (2-7) in the 1st, 3rd and 7th days, of the experiment period. Rats groups were administered with oral treatments of the vehicle, allopurinol, benzbromarone and watermelon powder solutions by oral gavage 1 h. after the administration of potassium oxonate, once daily for seven consecutive days of experiment. Animals of normal control (group 1) and hyperuricemic control (group 2) were received only vehicle via oral administration. Allopurinol and benzbromarone groups (group 3-4) treated orally with allopurinol and benzbromarone in a dose (10 mg kg^{-1}) respectively. Sample groups (5-7) treated orally with watermelon powder at the doses 25, 50 and 100 mg kg^{-1}, respectively once a day, throughout the days of the experiment. At the 6th day of experiment, the animals were transferred to the metabolic cages for the urine collection over 24 h. The urine samples then centrifuged at 2000 rpm to get the supernatant for uric acid assay. The collection of whole blood samples from each rat was achieved by cutting tail vein 2 h. After last administration of tested drugs the blood samples were permitted for 0.5 h at room temperature for clotting and 5 min for centrifugation at 3500 rpm to get the serum. The sera and urine samples were stored at -20°C until the uric acid is assayed.

Uric acid assay: The enzymatic-colorimetric method was employed to determine the serum uric acid levels by using a standard diagnostic kit.

Statistical analysis: The results of all trials in this study are stated as Mean±SEM. Statistical analysis was carried out by one-way ANOVA pursued by the Dennett's t-test. The values of probability (P) less than 0.05 were considered as statistically significant.

RESULTS

***In vitro* XO inhibitory activity:** The inhibitory effects of watermelon and allopurinol for bovine milk xanthine oxidase

at different concentrations were represented in Table 1. Each has revealed more than 50% of XO inhibition at the concentration 100 μg mL^{-1}. At highest concentration 100 μg mL^{-1}, the watermelon resulted in 58% of XO inhibition activity, whereas the standard XO inhibitor, allopurinol demonstrated 95% of XO inhibition activity at the same concentration. The xanthine oxidase inhibitory effects for both watermelon and allopurinol were also stated in the term of IC_{50}, which is represent the concentration of standard drug or tested sample that is required for 50% inhibition of xanthine oxidase activity under the same experimental conditions. The IC_{50} values were calculated according to the dose-response logarithmic curve by using GraphPad Prism V 6.05 program (GraphPad Prism software, Inc., USA), Where the value was equal to 1.834 μg mL^{-1} for allopurinol and 95.243 μg mL^{-1} for watermelon respectively as shown in Fig. 1.

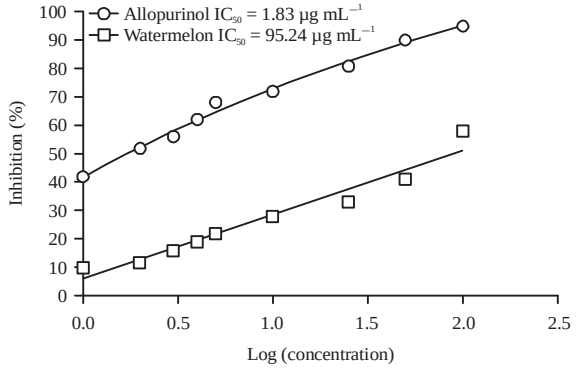

Fig. 1: Xanthine oxidase inhibitory activity and IC_{50} values of watermelon acid and allopurinol

Table 1: Xanthine oxidase inhibitory activity of watermelon and allopurinol at different concentrations

	XO inhibitory activity (%)	
Concentration (μg mL^{-1})	Allopurinol	Watermelon
100	95±1.5	58±2.2
50	90±0.6	41±0.7
25	81±1.1	33±1.8
10	72±2.1	28±1.4
5	68±2.0	22±0.3
4	62±0.4	19±0.2
3	56±0.6	16±1.1
2	52±1.6	12±0.8
1	42±1.2	10±0.7

Anti-hyperuricemic and uricosuric activity: To assess the existence of anti-hyperuricemic effect of the watermelon, the potassium oxonate-induced hyperuricemic rats' model was used in this study. As exposed in the Table 2, the intraperitoneal administration of uricase inhibitor, potassium oxonate (250 mg kg^{-1}) significantly raised ($p<0.001$) the uric acid concentrations in the serum of rats compared to healthy normouricemic control group. The administration of standard xanthine oxidase inhibitor, allopurinol and standard uricosuric drug, benzbromarone in a does (10 mg kg^{-1}, p.o), permitted to considerably reduction in the urate levels of hyperuricemic rats to values close of normal control. The consecutive 7 day treatment of rats with watermelon at the dose 25, 50 and 100 mg kg^{-1} significantly reduce the serum uric acid levels as compared with hyperuricemic control group in all doses above. Administration of potassium oxonate for rats caused a significant elevation in the output urine volumes, also treatments of rats with each of allopurinol, benzbromarone and the different doses (25, 50 and 100 mg kg^{-1}) of watermelon powder led to a significant raise in the volumes of urine output as compared with normal control. Treatments of animals with allopurinol and benzbromarone as well as the watermelon powder were able significantly ($p<0.001$) to increase the excretion of uric acid in the urine as compared towards the rats of normal control group.

DISCUSSION

Although, gout and hyperuricemia are widespread, the therapeutic agents for reduction the uric acid in the blood are very few in numbers at present and their use

Table 2: Effect of watermelon powder on urine output, urine uric acid and serum uric acid in hyperuricemic rats

Groups	Dose (mg kg^{-1})	Urine output (mL/24 h)	Urine uric acid (mg/24 h)	Serum uric acid (mg dL^{-1})
Normouricemic control	-	3.25±0.58	1.28±0.54	1.25±0.21
Hyperuricemic control	-	5.10±0.57[a]	4.86±0.28[b]	4.42±0.85[c]
Allopurinol	10	5.62±0.57[b]	6.37±1.24[b]	1.34±0.27[c]
Benzbromarone	10	7.21±0.68[b]	11.64±1.15[b]	1.65±0.41[c]
Watermelon	25	5.74±1.09[b]	7.23±0.95[b]	2.65±0.62[c]
	50	6.12±0.65[b]	8.65±0.85[b]	2.14±0.54[c]
	100	6.68±0.86[b]	9.94±0.79[b]	1.87±0.37[c]

Values are expressed as Mean±SEM for 6 rats, [a]$p<0.01$, [b]$p<0.001$, significant difference compared to normal control, [c]$p<0.0001$ significant difference compared to hyperuricemic control

is sometimes limited due to undesirable side effects. Therefore, natural products represent a potential source of novel anti-hyperuricemic agents[31,32]. This study is the first to investigate that the watermelon powder exerts antihyperuricemic effect in potassium oxonate-induced hyperuricemic rats. Furthermore, the underlying mechanism of watermelon powder might be through inhibiting of xanthine oxidase activity and of uricosuric activity, increasing of uric acid excretion in urine.

In the present study, hyperuricemia status was induced in rat's model by intraperitoneal administration of potassium oxonate in a dose (250 mg kg^{-1}) for 7 days. The induction of hyperuricemia in rats caused a significant increase in urine secretion and significant increase the concentration of uric acid in both blood and urine. These results confirm that the model used was effective in stimulating hyperuricemia and are in agreement with previous reports[29-33]. Administration of watermelon powder caused a significant reduction in the blood uric acid levels in hyperuricemic animals. Moreover, the same administration resulted in a significant increase the excretion and clearance of uric acid in hyperuricemic rats. Watermelon powder increased each of urinary excretion, clearance of uric acid and reduction of blood uric acid in a dose-dependent manner. Watermelon powder at 100 mg kg^{-1} dose manifested similar influence of both benzbromarone (10 mg kg^{-1}) and allopurinol (10 mg kg^{-1}), which indicated to the importance of watermelon as a potent antihyperuricemic and uricosuric agent[32,34].

The watermelon fruit is a rich source for many natural antioxidants such as vitamin C, lycopene, cucurbitacin, citrulline and other polyphenols[15,19-25]. The presence of such constituents, especially vitamin C, supports the hypothesis that the watermelon is an effective diet in the prevention and treatment of gout[35-38]. The previous studies has been reported that the vitamin C can reduce the uric acid in blood through either the direct uricosuric potential, which is due to a competitive inhibition of renal reabsorption of uric acid via an anion-exchange transport system at the proximal tubule in nephron or through enhancing the glomerular filtration rate. Moreover, as a potent antioxidant, vitamin C is able to diminish the oxidative stress and inflammation in the body cells, thereby reducing the synthesis and ultimately blood level of uric acid[39-41].

The uricosuric effect of watermelon powder may be of great interest the treatment of gout and related diseases, taking into account that the more than 90% of gouty patients are resulted from the underexcretion of uric acid[32,33]. Unlike standard uricosuric drugs, it was reported that the watermelon possesses hepato and nephroprotective effects[18,24]. The property of antiurolithic that caused by the use of watermelon exhibits a further advantage over uricosuric effect, which may reduce the risk of deposition of uric acid in the renal tubules that commonly occurred with use of uricosuric drugs[42].

Another probable mechanism that any material can reduce of uric acid in the blood is by inhibiting the activity of xanthine oxide enzyme, which in turn reduces the production of uric acid. The watermelon powder revealed a mild *in vitro* xanthine oxidase inhibitory activity. It has been reported that watermelon contain many bioactive materials like polyphenols. Previous studies have indicated that these substances showed a good inhibitory activity towards xanthine oxidase. The viewed xanthine oxidase inhibitory activity of watermelon powder may be attributed to the existence of these polyphenols at low concentrations[43-47].

This study has limitations that will be addressed in our next experiments. First, isolation, purification and identification of the active constituents of the watermelon powder in order to investigate their anti-hyperuricemic effect on potassium oxonate-induced hyperuricemic rats. Second, study the effect of watermelon powder on the inhibitory activity of xanthine oxidase *in vivo*.

CONCLUSION

Based on the findings of the present study, the watermelon powder significantly reduced the uric acid levels in the blood of the potassium oxonate-induced hyperuricemic rats via the synergistic effect of both xanthine oxidase inhibitory and uricosuric activities. The same findings clearly demonstrated that the antihyperuricemic activity of watermelon powder might be attributable mainly to the uricosuric nature and partially to the xanthine oxidase inhibitory activity.

SIGNIFICANCE STATEMENT

This study confirmed that the watermelon red-fleshed works to reduce the levels of uric acid in the blood through the synergistic effect of both increasing the uric acid excretion in the urine and inhibition of uric acid synthesis. The uric acid-lowering effect for melon may be due to the presence of many natural antioxidants, especially vitamin C. It is very important to increase the consumption of melon for gout patients as food and alternative treatment to promote human health and reduce the risk of gout and related diseases.

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