

EFFECTS OF PHENOLIC COMPOUNDS EXTRACTED FROM *SALVIA FRIGIDA* ON INDUCED HYPERURICEMIA IN MICEMUHAMMAD ABDULHUSSEIN AL-DABBAGH^{1*}, SHIHAB ABDULRAHMAN SHIHAB¹, ENAS JAWAD KADHIM²¹Department of Pharmacology, College of Medicine, Al-Nahrain University, Baghdad, Iraq. ²Department of Pharmacognosy, College of Pharmacy, University of Baghdad, Baghdad, Iraq. Email: mhmd_aldabbagh@yahoo.com

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ABSTRACT

Objective: The aim of this study was to investigate effects of phenolic compounds rich fraction extracted from aerial parts of *Salvia frigida* on serum uric acid (SUA) and xanthine oxidase (XOD) in hyperuricemic mice model.

Methods: A total of 40 albino male mice were equally divided into four groups. The blank group was given vehicles only. The model of hyperuricemia was induced by single intraperitoneal injection of UA (250 mg/kg). *S. frigida* group was pretreated orally with phenolic compounds rich fraction (300 mg/kg) for 6 days and one final oral dose was given directly after the UA injection on the 7th day as an acute treatment and the same was applied to allopurinol (20 mg/kg) which served as a positive control. 1 h later blood was withdrawn to be tested for SUA, XOD, and creatinine levels. Furthermore, the body weights were daily documented before each administration.

Results: The results revealed a highly comparable significant reduction in SUA and XOD in both *S. frigida* and allopurinol groups without significantly affecting serum creatinine compared to model group, also both modestly decreased the body weights of mice.

Conclusion: *S. frigida* significantly reduces SUA in the hyperuricemic model and may also have a promising effect on weight management.

Keywords: Uric Acid, Xanthine Oxidase, Urate Lowering Therapy, Flavonoids, Quercetin, Allopurinol.

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INTRODUCTION

Hyperuricemia defined as the presence of elevated serum UA (SUA) concentration. UA is the end product of purine metabolism in human [1]. El-Yassin *et al.* (2012) found that 19% of a sample of Iraqi subjects from Karbalaa city has elevated levels of SUA [2]. Hyperuricemia complications can lead to gout and nephrolithiasis and increase the risk of cardiovascular diseases, metabolic disorders, and chronic kidney disease [3-9].

There are currently few approved urate-lowering therapies (ULTs) available in clinical practice at the same time they have several drug interactions, contraindications, side effects, and sometimes unfortunate severe adverse effects. The global rising burden of hyperuricemia together with the increased evidence that linked hyperuricemia to the pathogenesis of other diseases and the lack of the perfect agent to treat it further emphasizes the importance of the urgent need for developing new ULTs.

Natural plant-derived phenolic compounds, especially flavonoids have showed potential SUA lowering effect by xanthine oxidase (XOD) inhibitory action [10,11] and/or uricosuric effect [10,12] in addition to antioxidant and anti-inflammatory effects [10]. The dual effect as a XOD inhibitor (XOI) and a free radical scavenger, suggests new promising therapeutic perspective to prevent hyperuricemia and its complications, as UA can induce oxidative stress, inflammation, and tissue injury [10].

Salvia frigida Boiss. (Family: Lamiaceae) exhibited *in vitro* inhibition to XOD [13]. To the best of our knowledge, no *in vivo* study is available to assess its effects on model of hyperuricemia. Accordingly, this study has explored *S. frigida* Boiss. aerial parts ethanolic extract; phenolic compounds rich fraction, for potential promising urate lowering effect in hyperuricemic mice model.

MATERIALS AND METHODS**Materials**

Allopurinol tablets (Aspen, Germany), carboxymethyl cellulose sodium salt (CMC-Na) (Panreac, Spain), methanol high-performance liquid chromatography (HPLC) grade 99.9% (GCC, UK), myricetin, quercetin, and rutin standards (Chengdu Biopurify, China), UA powder (Downs development, England), and XOD ELISA kit for mice (Al-Shkairate establishments, Jordan) were used. All other chemicals are of the highest purity obtained from reputed sources. Animal feeding needle curved 20G×1" (2 mm tip diameter) Reusable (Petsurgical, India), Cobas c 111 analyzer (Roche, Switzerland), high performance liquid chromatography (HPLC) (Waters, Germany), Rotatory evaporator (Buchi, Switzerland), Soxhlet (Labtech, Korea).

Plants

Salvia frigida Boiss. was provided by the Herbarium of Department of Biology, College of Science, University of Baghdad.

Animals

A total of 40 apparently healthy albino male mice weighing between 25 and 30 g were included in the study and were randomly divided into four groups (10 mice each). The mice were obtained from the National Center for Drug Control and Research/Ministry of Health/Baghdad. Each group was kept in a separated plastic cage, had free access to water and pellet diet, maintained at 12 h light/dark cycles with room temperature (22–25°C), and habituated for a week before starting with the *in vivo* study.

Approval of methods

The study protocol was approved by the Institutional Review Board at College of Medicine, Al-Nahrain University. Plant extraction and HPLC analysis were conducted at Pharmacognosy Department, College of Pharmacy, University of Baghdad.

The *in vivo* study was conducted at the laboratories of the Pharmacology Department/College of Medicine/Al-Nahrain University. The study was started during May 2018 and the thesis was completed in December 2018.

Extraction of plants

Dry powdered aerial parts plant (250 g) was defatted with hexane for 24 h then allowed to dry at room temperature. The defatted plant materials were extracted with 80% ethanol (1 l) in Soxhlet apparatus until complete exhaustion.

The alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40°C to give a residue designated as a crude fraction.

Crude fraction was acidified with hydrochloric acid (5%) to pH 2 and partitioned (three times) with an equal volume of ethyl acetate to get two layers (aqueous acidic layer and ethyl acetate layer).

The ethyl acetate layer designated as fraction 1 was separated and evaporated to dryness under reduced pressure and basified with 300 ml of sodium hydroxide 5% to pH 10 and extracted with chloroform in the separatory funnel to get two layers, the aqueous basic layer and chloroform layer.

The aqueous basic layer designated as fraction 2 was separated, evaporated to dryness and acidified with 5% hydrochloric acid to pH 2 then extracted with ethyl acetate to get the final fraction designated as fraction 3 (F-3) which after being subjected to evaporation process represents the phenolic compounds rich fraction that was used for formulating oral formula for *in vivo* administration. The previously mentioned procedure is according to Kadhim (2013) [14].

Preliminary qualitative phytochemical analysis

Test for flavonoids

- NaOH test: To 5 ml from the crude extract was treated with aqueous NaOH and HCl, looking for the formation of yellow-orange color [15].
- Lead acetate test: To a 5 ml of F-3 fraction, 1 ml of 10% lead acetate solution was added. The formation of a yellowish-white precipitate indicates a positive for flavonoids [15].

Test for phenolic compounds

Ferric chloride test: A little of F-3 fraction was dissolved in distilled water, 2 ml of 5% ferric chloride solution was added to it. Formation of violet, blue or green color indicates the presence of phenolic compounds [16].

Flavonoids identification using HPLC technique

The identification of flavonoids was done with HPLC. The identifications were made by comparing the retention times obtained at identical chromatographic conditions between extract fraction and standards. 1 mg from the extracted fraction was dissolved in 1 ml ethyl acetate, and 20 µl from this solution was injected into the HPLC. The standards were prepared as solutions with a concentration of 1 mg/ml for each. The solvent used for dissolving the standards was methanol. 20 µl from each solution was injected into the HPLC for each run in HPLC.

The experimental conditions of HPLC were:

- Mobile phase: Mixture of methanol:water (70:30) [17].
- Column temperature 25°C.
- Column ODS C18 (250 mm×4.6 mm, 5 µm particle size).
- Flow rate 1 ml/min.
- Detection wavelength 265 nm [18].

Experimental design

The induction of hyperuricemia model was done by a single intraperitoneal (i.p.) injection of UA, at a dose of 250 mg/kg [19] in 0.5% w/v CMC-Na/normal saline, to be given on the 7th day of the study right before the oral administration of either tested *S. frigida* F-3 fraction, vehicles, or allopurinol.

The study groups were:

- Group 1: Normal control group (blank group) was given vehicle "0.5% CMC-Na/Distal water" 0.2 ml/20 g/day orally for 7 days + single i.p. injection of vehicle "0.5% CMC-Na/normal saline" 0.2 ml/20 g on the 7th day of the study.
- Group 2: Induced non-treated control group (model group) was given vehicle "0.5% CMC-Na/Distal water" 0.2 ml/20 g/day orally for 7 days + single i.p. injection of UA 250 mg/kg on the 7th day of the study.
- Group 3: The positive control group (allopurinol group) was given allopurinol 20 mg/kg/day orally for 7 days + single i.p. injection of UA 250 mg/kg on the 7th day of the study.
- Group 4: *S. frigida* treatment group (*S. frigida* group) was given phenolic compounds rich fraction "F-3" of *S. frigida* extract 300 mg/kg/day orally for 7 days + single i.p. injection of UA 250 mg/kg on the 7th day of the study.

The oral vehicles, *S. frigida* extract, and allopurinol were given daily, every 24 h for 7 consecutive days. The preparation of CMC-Na 0.5% w/v colloidal solution was based on Kamal *et al.* (2017) [20] and the United States Pharmacopeia weight variation test was done on allopurinol tablets to ensure proper allopurinol weighing. All the medications were freshly prepared and were given according to the animal body weight which was measured directly before each administration.

Blood collection

On the 7th day, 1 h after the last administration, blood samples were collected by cardiac puncture under anesthesia using chloroform. Blood samples were allowed to clot for 1 h at room temperature, then were centrifuged to obtain serum for measuring UA and creatinine by cobas c 111 analyzer; furthermore, 150 µl of serum were stored at -80°C (until testing it later on for XOD by following the kit instructions).

Statistical analysis

The statistical analysis of this study was performed with the statistical package for the social sciences (SPSS) version 23.0 and Microsoft Excel 2016. Numerical data were expressed as mean ± standard deviation (SD) and were analyzed using independent Student's t-test (unpaired t-test) for comparison between two groups. Pearson correlation was done between SUA and XOD and expressed as r (correlation coefficient) and its p value. Paired t-test was used to compare body weight at day 7 (d7) and day 1 (d1) within each study group. For all of the previously mentioned statistical tests, it was considered significant if p<0.05 and highly significant if p<0.001.

RESULTS

The mean±SD for SUA, creatinine, XOD, and the difference in body weight between d7 and d1 were measured for the blank group, model group, allopurinol group, and *S. frigida* group.

Analysis of unpaired t-test revealed a highly significant increase (p<0.001) in the level of UA in model group in comparison with the blank group. Furthermore, a statistically significant increases (p<0.05) in XOD in model group compared to blank group. While no statistically significant difference regarding other parameters; almost no change in creatinine levels and very close weight gain occurred in both groups (Table 1).

The results showed a highly significant reduction (p<0.001) in UA level in both allopurinol group and *S. frigida* group compared to the model group. On the other hand, no significant change in creatinine levels for both groups in comparison with the model group. Serum XOD appeared to be statistically significant (p<0.05) lower in *S. frigida* group and highly significant (p<0.001) lower in the allopurinol group compared to the model group. There were statistically significant (p<0.05) decrease in body weight in both *S. frigida* and allopurinol groups compared to the model group. Numerical results are reviewed in Table 2.

Pearson correlation between SUA and XOD revealed that only the allopurinol group appears to have a statistically significant ($p < 0.05$) positive correlation between SUA and XOD. The resultant correlation coefficients and their significance for each group are shown in Table 3.

The mean \pm SD for body weight at d1 and d7 were measured for each study group, illustrated in Fig. 1. Analysis of paired t-test revealed a modest decrease in body weight in both allopurinol and *S. frigida* group while statistically significant ($p < 0.05$) weight gain in both blank and model group were documented.

Preliminary qualitative phytochemical analysis results

Phytochemical screening was done using colors and precipitates formation after following standard tests for detecting the phytochemicals of interest: Phenolic compounds and flavonoids. *S. frigida* achieved positive tests for NaOH (yellow-orange), lead acetate (yellow precipitate), and ferric chloride (dark green) tests.

HPLC results

The phenolic compounds of *S. frigida* F-3 fraction were identified by HPLC method by comparing them with standard compounds retention times obtained at identical chromatographic conditions of the analyzed samples

and authentic standards. HPLC retention times for matching flavonoids were 2.48, 3.11, and 4.48 for rutin, quercetin, and myricetin standards, respectively. The information obtained from the HPLC method of analysis revealed that *S. frigida* is very rich in quercetin. Other flavonoids; rutin and myricetin, were also detected still the highest peak was not identified. The peaks extent of *S. frigida* components is shown in Fig. 2.

DISCUSSION

Hyperuricemia prevalence has increased dramatically throughout the past century reaching to the current dates. It is now a major concern not only because of risking for gout emergence but also it has been recently linked to developing chronic kidney diseases, cardiovascular diseases, and metabolic disorders. Very few ULTs are available with various contraindications and unfortunate adverse effects. In recent years, natural phenolic compounds; especially flavonoids and phenolic acids have been found to possess a promising inhibitory effect on XOD and can decrease UA level with the favorable safety profile and additional antioxidant effect.

This study revealed for the first time major flavonoids constituents of *S. frigida*. The Preliminary analysis results showed that flavonoids class is the richest component of the phenolic compounds content of

Table 1: Comparison between Blank group (Gp1) and model group (Gp2) in relation to serum uric acid, creatinine, xanthine oxidase, and the difference in body weight by unpaired t-test

Parameters	n=10	Blank (Gp1) vehicles only	Model (Gp2) UA 250 mg/kg
Serum uric acid (mg/dl)	Mean \pm SD	2.05 \pm 0.21	3.63 \pm 0.23
	P		<0.001***
Serum creatinine (mg/dl)	Mean \pm SD	0.18 \pm 0.04	0.18 \pm 0.06
	P		1.000
Serum XOD ¹ (pg/ml)	Mean \pm SD	490.25 \pm 167.71	615.05 \pm 76.07
	P		0.046*
difference in d7 and d1 weight ² (g)	Mean \pm SD	1.41 \pm 0.85	1.12 \pm 0.89
	P		0.469

The hyperuricemic mice model was induced by a single intraperitoneal injection of UA on the 7th day of the study while injecting vehicle to the Blank group. Both groups were given oral vehicles throughout the 7 days. ¹Xanthine oxidase, ²difference in body weight between d7 and d1, *denotes significant difference at $P < 0.05$, ***denotes highly significant difference at $P < 0.001$. UA: Uric acid, d7: Day seven, d1: Day one, SD: Standard deviation

Table 2: Comparison between model group (Gp2), allopurinol group (Gp3), and *Salvia frigida* group (Gp4) in relation to serum uric acid, creatinine, xanthine oxidase, and the difference in body weight by unpaired t-test

Parameters	n=10	Model (Gp2) UA 250 mg/kg	Allopurinol (Gp3) 20 mg/kg+UA	<i>Salvia frigida</i> (Gp4) 300 mg/kg+UA
Serum uric acid (mg/dl)	Mean \pm SD	3.63 \pm 0.23	1.12 \pm 0.21	1.13 \pm 0.32
	P		<0.001***	<0.001***
Serum creatinine (mg/dl)	Mean \pm SD	0.18 \pm 0.06	0.2 \pm 0.05	0.2 \pm 0.05
	P		0.433	0.433
Serum XOD ¹ (pg/ml)	Mean \pm SD	615.05 \pm 76.07	431.1 \pm 62.52	473.2 \pm 92.11
	P		<0.001***	0.001*
Difference in d7 and d1 weight ² (g)	Mean \pm SD	1.12 \pm 0.89	-0.75 \pm 1.18	-0.52 \pm 1.18
	P		0.001*	0.002*

The hyperuricemic mice model was induced by a single intraperitoneal injection of UA on the 7th day of the study while giving oral vehicle throughout the 7 days. Allopurinol and *Salvia frigida* (phenolic compounds rich fraction) were given orally for 7 days (+ the UA injection on the 7th day) to Gp3 and Gp4 mice groups, respectively. ¹Xanthine oxidase, ²difference in body weight between d7 and d1. *Denotes significant difference at $P < 0.05$ compared to model group, ***Denotes highly significant difference at $P < 0.001$ compared to model group. UA: Uric acid, d7: Day seven, d1: Day one, SD: Standard deviation

Table 3: Pearson correlation between serum uric acid and serum xanthine oxidase within all study groups

n=10	Serum uric acid (mg/dl)			
	Blank (Gp1) vehicles only	Model (Gp2) UA 250 mg/kg	Allopurinol (Gp3) 20 mg/kg+UA	<i>Salvia frigida</i> (Gp4) 300 mg/kg+UA
Serum XOD ¹ (pg/ml)				
r^2	-0.089	-0.081	0.688	0.597
P	0.807	0.815	0.028*	0.069

*Denotes significance at $P < 0.05$. The hyperuricemic mice model was induced by a single intraperitoneal injection of UA on the 7th day of the study while giving oral vehicle throughout the 7 days. Blank group mice (Gp1) were given vehicles only. Allopurinol and phenolic compounds rich fractions from *Salvia frigida* were given orally for 7 days (+ the UA injection on the 7th day) to Gp3 and Gp4 mice groups, respectively. ¹Xanthine oxidase, ²correlation coefficient. UA: Uric acid, d7: Day seven, d1: Day one, SD: Standard deviation

S. frigida. Quercetin is abundant in *S. frigida*; it appeared as the second highest peak. Still, the highest peak and several other peaks were not identified. Other detected flavonols in high concentrations were rutin and myricetin. The pharmacological activity of *S. frigida* phenolic compounds rich fraction (300 mg/kg/day) appears to be due to those three major flavonoids constituents (quercetin, rutin, and myricetin) in addition to the unidentified highest peak.

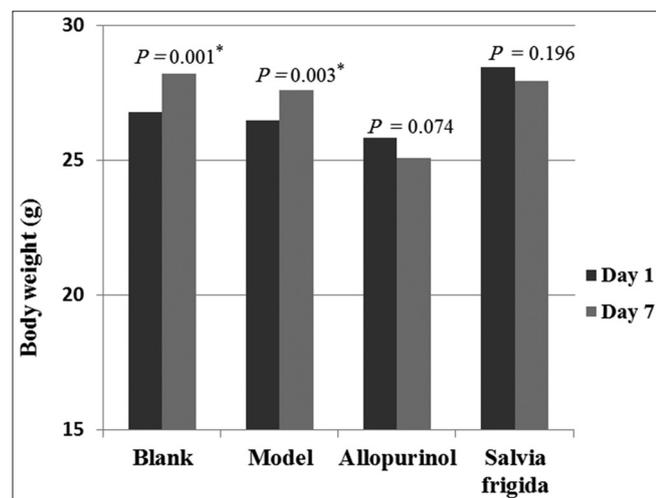


Fig. 1: Comparison between body weight at day 1 (d1) and at d7 within each study group by paired t-test. The hyperuricemic mice model was induced by a single intraperitoneal injection of uric acid (UA) 250 mg/kg on the 7th day of the study while giving oral vehicle throughout the 7 days. Blank group mice were given vehicles only. Allopurinol 20 mg/kg and phenolic compounds rich fractions (300 mg/kg) from *S. frigida* were given orally for 7 days (+ the UA injection on the 7th day) to their corresponding mice groups, respectively. Data represent mean \pm standard deviation for 10 mice. *Denotes significant difference at $p < 0.05$ compared with d1

In this study, single i.p. injection of UA (250 mg/kg) on the 7th day of the study to model group while giving vehicle orally throughout the previous days was resulted in marked hyperuricemia, being measured after 1 h of the last administration, compared to blank group, with a highly significant increase in SUA levels (Table 1). These findings are in agreement with reported protocol by Chen *et al.* (2006) who found a significant increase in SUA concentration even after 10 min from injecting the same dose into mice [19].

To the best of our knowledge, the current study is the first to report serum XOD concentration in mice under such conditions. It revealed that circulating XOD has mildly increased to a level that reaches statistical significance. The mildly elevated levels of mean serum XOD enzyme may indicate increased permeability, damage, or necrosis of tissues which is not only a marker of hepatic or intestinal injury but it can also result from a variety of remote organ damage as a consequence of oxidative injury [21,22]. This probably occurred in some, but not all ten animals as the results also showed a very weak negative correlation (though have not reached statistical significance) between SUA and XOD levels in both blank and model groups (Table 3). It has been established that UA acts normally as serum antioxidant [23]; nevertheless, UA may give pro-oxidant activity, mainly intracellularly [24]. It exerts pro-oxidant deleterious effect under several conditions; most importantly when presents at high levels even in the upper two-thirds of physiological ranges [25]. Furthermore, the pro-inflammatory action of UA may induce cell transformation through reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation. Besides, intracellular UA may contribute to decreasing the level of XOD expression [26]. Moreover, UA can actually inhibit XOD and thereby act as a feedback inhibitor of the enzyme; this effect is associated with increased ROS formation [21]. UA may exert a pro-oxidant activity within the cell mainly, possibly due to the free radicals derived from its reaction with ROS, RNS, and nitric oxide (NO), in particular in the presence of myeloperoxidase [24]. Hyperuricemia causes uncoupling of endothelial NO synthase which increases superoxide radical production [25]. UA-derived free radicals have been associated with several effects such as reduced availability of NO, cyclooxygenase-2 expression and up-regulation of the renin/angiotensin pathway, platelet activation, migration and proliferation of

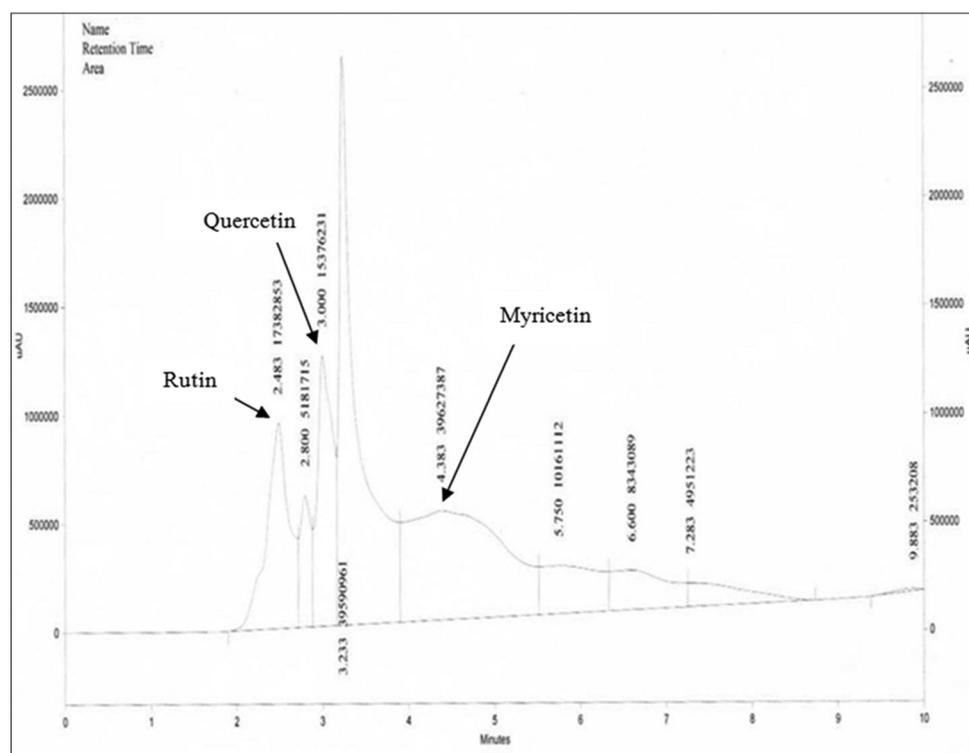


Fig. 2: High-performance liquid chromatography of *Salvia frigida*

vascular myocytes, and production of pro-inflammatory cytokines by macrophages [26].

Recently, Wang *et al.* (2016) have found a significant increase in hepatic XOD activity and protein levels in hyperuricemic mice model [27] while Qin *et al.* (2018) have found a significant increase in both serum and hepatic XOD activities and hepatic XOD protein levels in hyperuricemic mice model [28]. Serum XOD enzyme activity has significantly increased in several studies using various hyperuricemic models in rodents compared to blank controls [19,29]. Increased XOD activity could induce oxidative stress through ROS and UA production. XOD pathway is an important path in the oxidative damage to tissues. The UA conversion from hypoxanthine and xanthine by XOD is accompanied by the generation of superoxide anion and hydrogen peroxide and eventually give rise to hydroxyl radical through the Haber-Weiss and Fenton reactions in the presence of transition metals [4,26,30-32]. These cytotoxic ROS are highly reactive and can directly oxidize macromolecules including DNA, protein, and lipids [30,31] which may result in cellular damage when produced in large amounts [32]. Furthermore, XOD activities may produce RNS, although other enzymes are implicated in the generation of these [26] as with highly reactive peroxynitrite (OONO-) [33,34]. Accordingly, in this study, lipid peroxidation mediated by ROS and RNS has probably caused cell membrane damage leading to the release of XOD into the serum.

Humans have only 1% activity of XOD compared with other mammals. The decrease in XOD activity is one likely evolutionary adaptation to uricase loss in humans [35] as the sudden knockout of uricase in mice appears to be fatal due to striking increase in SUA levels which cause acute urate nephropathy and renal failure [36]. No significant change in creatinine occurred in model group as the elevation in the serum levels of creatinine can be only noted after extensive kidney injury involving the damage of approximately half of the kidney nephrons [37].

As expected, changes in body weight after 1 week were kind of similar in both model and blank groups. This observation is consistent with Jiang *et al.* (2017) as they have injected mice intraperitoneally with potassium oxonate (300 mg/kg) to induce hyperuricemia on the 7th day of the study while giving normal saline to the blank group, noting no significant difference regarding weight gain between the two groups [38].

In the present study, mice were pretreated orally for 6 days with either phenolic compounds rich fraction extracted from *S. frigidula* (300 mg/kg) or allopurinol (20 mg/kg) and on the 7th day the mice were given, immediately after the single UA i.p. injection, one final oral dose as an acute treatment. The results revealed excellent hypouricemic effect exerted by *S. frigidula* as both *S. frigidula* and allopurinol accomplished quite similar highly significant SUA reduction after 1 h treatment compared to the model group (Table 2). As it has been previously reported that *S. frigidula* exert excellent *in vitro* inhibition to XOD (93.2%) compared to allopurinol (80.52%) [13].

The resultant *in vivo* hypouricemic effect that was achieved by *S. frigidula* is probably attributed to XO1 effect exhibited by its high flavonoids content. Quercetin probably contributes to the majority of the effect as it represents one of the major constituents of the *in vivo* tested F-3 fraction and to rutin and myricetin to a lower extent. These conclusions are consistent with several previous studies showing hypouricemic and XOD inhibitory effect exerted by those three flavonols [39,40].

Allopurinol results agree with Qin *et al.* (2018) as they have shown that allopurinol (20 mg/kg), being orally administered to mice for 7 days, results in significant reduction in SUA level compared to model group with significant inhibition to XOD activity [28].

Another hypothesis behind the hypouricemic effect is proposed by the current study which is a decrease in XOD level equals a decrease in its activity (one enzyme and one reaction) leading to further lowering in

UA production. Both *S. frigidula* and allopurinol significantly lowered XOD serum level compared to the model group.

In most biological environments, enzyme concentration is lower than the concentration of its substrate. As long as this is true, there is a directly proportional to relationship between enzyme concentration and its activity. In most situations, decreasing enzyme concentration has a direct influence on its activity since each enzyme molecule is able to catalyze only one reaction at a time. In other words, the rate of the reaction decreases as enzyme concentration decreases [41]. Furthermore, metabolic labeling studies indicate that H₂O₂ stimulate the conversion of xanthine dehydrogenase to XOD [42]. Hence, allopurinol's known XOD activity inhibition and *S. frigidula* probable XOD activity inhibition have resulted in decreasing H₂O₂ production thus decreasing XOD serum presence.

Our hypothesis may also be supported by the correlation results (Table 3) as it obviously showed a positive correlation between UA and XOD expression in both *S. frigidula* and allopurinol groups compared to negative ones in both blank and model groups. Although only allopurinol showed statistical significance still, *S. frigidula* showed very close results to reach significance which might only needed a couple of further doses. Still, further investigation is needed for confirming such effects and its extent and for revealing the mechanism behind it.

The current study revealed that *S. frigidula* and allopurinol probably prevented organs damage and consequent XOD leak to serum as they both significantly lowered serum XOD level compared to the model group. This may be attributed to *S. frigidula* significant antioxidant activity as shown by its remarkable scavenging effect against 2,2-diphenyl-1-picrylhydrazyl radical [13]. Likewise, quercetin and rutin have strong antioxidant activities [43]. While the mechanism behind allopurinol protection against UA-induced probable tissue damage is that allopurinol is not only capable of inhibiting XOD activity and reducing UA levels but also it was proven to significantly reduce serum malondialdehyde (MDA) hence further decreases oxidative stress [44].

Analysis of unpaired t-test showed significant growth restraint in both allopurinol and *S. frigidula* groups compared to the model group (Table 2). However, the analysis of paired t-test revealed a non-significant decrease in body weight within both allopurinol and *S. frigidula* groups (Fig. 1).

Emerging evidence and correlations have linked UA level with body weight. Uricase loss and the consequent increase in SUA level have been hypothesized to give another survival advantage to hominoids. The increased SUA levels may lead to obesity, fatty liver, metabolic syndrome, and diabetes. Hence, uricase knockout subjects could more easily overcome starvation periods [45]. In a clinical study, enrolling patients with cardiac diseases, a positive correlation was detected between the plasma level of XOD activity and B-type natriuretic peptide, as well as with body mass index [46].

In mice, adipose tissue has a very high comparable level of xanthine oxidoreductase (XOR) expression to that of the liver and small intestine. Furthermore, XOR activity is higher in obese mice fat tissue than in healthy controls [47]. Adipogenesis, XOR expression, and amount of UA were increased by subjecting human mesenchymal stem cell-derived adipocytes to fructose. Allopurinol inhibited those effects. Directly treating adipocytes with UA also resulted in enhanced adipogenesis [48]. XOR activity has been portrayed as involved in pre-adipocyte differentiation and adipogenesis in studies carried out in cultured cells and XOD gene knockout mice [49]. Negative effects such as adipogenesis, inflammation, and hypertension are induced by XOR activity on purine metabolism [26]. These results suggest a fundamental role for XOD activity-derived oxidative stress in UA-mediated adipogenesis. Interestingly, it has been hypothesized that XOR has a protective role in adipose tissue when the environment allows it to function as nitrate reductase as an adaptive response to obesity-

consequent hypoxia in fat tissue by activating the reprogramming that leads to browning of the white fat tissue and anti-hypertensive activity [26].

Rutin administration can reduce the mass of adipose tissue and body weight in an obese rat model. Rutin was found to increase mitochondrial DNA content, mitochondrial size, and gene expression related to mitochondrial biogenesis (peroxisome proliferator-activated receptor gamma coactivator-1 α , transcription factor A, nuclear respiratory factor-1, and nicotinamide adenine dinucleotide-dependent deacetylase) in skeletal muscle [50].

Allopurinol side effects include nausea, diarrhea, and gastrointestinal intolerance which might also contribute to the weight loss in allopurinol group. Allopurinol results in the present study are in agreement with a previous work that found 7 days allopurinol therapy to significantly inhibit weight growth in hyperuricemic mice model [38].

CONCLUSION

S. frigida Boiss. and allopurinol have achieved highly similar results with a highly significant reduction in SUA compared to hyperuricemic mice model; making *S. frigida* aerial parts phenolic compounds rich fraction an excellent candidate for further exploration in the goal of developing newer and possibly safer urate-lowering therapy. Furthermore, *S. frigida* mildly decreased body weight of mice; such modest action suggests a useful application in developing weight control therapy from *S. frigida* phenolic compounds content.

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AUTHORS' CONTRIBUTIONS

Muhammad A. Al-Dabbagh has provided the design, intellectual content, innovation, and protocol and has majorly conducted the practical work of the study, interpreted the results and drafted the manuscript. Shihab A. Shihab has helped in drug administration and blood withdrawal along with mentorship. Enas J. Kadhim has suggested the extraction procedure and helped in executing HPLC analysis. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest.

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