Traditional Uighur Medicine Karapxa decoction, inhibits liver xanthine oxidase and reduces serum uric acid concentrations in hyperuricemic mice and scavenges free radicals in vitro

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Abstract

Background: Karapxa decoction (KD) is a Traditional Uighur Medicine used for hepatitis, cholecystitis, gastralgia, oedema, gout and arthralgia. Because of its purported effect in gout, its effects were tested in hyperuricemic mice models induced by yeast extract paste or potassium oxonate, as well as its capacity to scavenge free radicals in vitro.

Methods: Hyperuricemia was induced in mice by yeast extract paste or potassium oxonate. KD was given orally for 14 days at 200, 400 and 800 mg/kg/day, with Allopurinol 10 mg/kg/day as positive control. Serum uric acid (UA), and liver xanthine oxidase activity (XO) were measured. Scavenging activity of KD on 1, 1-diphenyl-2-picrylhydrazyl radicals (DPPH), nitric oxide (NO), superoxide (O2•−), efficiency against lipid peroxidation, and XO inhibition were determined in vitro.

Results: KD inhibited liver XO activity and reduced serum uric acid in hyperuricemic mice. KD also showed noticeable antioxidant activity, scavenging free radicals (DPPH, NO and O2•−). It was effective against lipid peroxidation and inhibited XO in vitro.

Conclusions: This study supports the traditional use of Karapxa decoction to treat hyperuricemia and gout.

Keywords: Karapxa decoction, Serum uric acid levels, Xanthine oxidase activities, Antioxidant, Potassium oxonate, Traditional Uyghur medicine

Background

Hyperuricemia and gout are metabolic disorders associated with abnormal amounts of uric acid in the body and uric acid crystals deposition or mobilisation in joints [1]. Hyperuricemia is considered a risk factor for gout, cardiovascular and many other diseases [2]. Uric acid, which is poorly soluble and deposits in articular and renal tissues, is the result of purine metabolism, [3] and especially of xanthine by xanthine oxidase (XO). XO inhibitors such as allopurinol are available to block the final step in uric acid synthesis, reducing the production of uric acid [4]. Control of XO is a key factor in the prevention and treatment of uric acid-related diseases [5,6]. Gout is mostly manifest as painful swelling of digital joints, and can be quite incapacitating. Being both highly painful and visible, it is a prime target of traditional therapy. However gout is but the most visible aspect of uric acid excess: hyperuricemia and uric acid tissue deposition are associated with chronic inflammation, and increased risk of cardiovascular diseases, diabetes mellitus, decreased cognition in the elderly, among others. Decreasing uric acid production may therefore have further benefits than just relieving the painful symptoms of gout [7].
The Traditional Uighur Medicine (TUM) herbal formula, *Karapxa decoction* (KD), composed of seven herbal ingredients (Table 1), including seed, leaves or roots of celery, chicory, fennel and dodders, has long been used for gout and arthralgia in addition to other symptoms such as Hepatic coldness, adiposis hepatica, jaundice, hepatitis, cholecystitis, gastralgia [8]. KD is recorded in the State Pharmacopoeia of People’s Republic of China in the Uighur Medicine volume. The seeds and roots of *Cichorium glandulosum* Boiss. et Huet (Chicory) serve as an important ingredient in KD. Previous studies have shown that extracts of *Cichorium glandulosum* Boiss. et Huet decrease serum uric acid and triglyceride concentrations in animal models [8-10], and may also decrease hyperuricemia in hypertriglyceridemia models [11]. Chicory is also commonly cited on websites for “natural” treatment of gout. Other components of KD also have effects, such as the hepatoprotective effect of *Cuscuta chinensis* against liver toxicity of acetaminophen and other drugs [8,12,13]. It is not clear however whether KD can actually reduce serum uric acid levels in hyperuricemia models and inhibit XO activities. The aim of the present study was to evaluate the effects of KD in vivo on reduction of serum uric acid level and XO activity in hyperuricemic mice and to measure XO inhibition and free radical scavenging activity in vitro.

**Methods**

**Chemicals**

Xanthine and XO were purchased from Sigma (St. Louis, MO, USA). Potassium oxonate was purchased from Aldrich Inc. 2, 2-diphenyl-1-picrylhydrazyl (DPP*), sodium nitroprusside, N-(1-Naphthyl) ethylenediamine dihydrochloride, phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), Ascorbic acid (AA) and thiobarbituric acid (TBA) were supplied by Sigma Co. (St Louis, USA). Assay kits for serum Uric Acid (UA) were obtained from Biosino Biotechnology Company Ltd. Assay kits for liver Xanthine oxidase (XO) were obtained from Nanjing Jiancheng Bioengineering Institute. All other chemicals were of analytical grade.

**Plant material**

KD is composed of air-dried powdered raw materials (Table 1) that were purchased from Xinjiang Autonomous Region Traditional Uighur Medicine Hospital (Urumqi, China) and authenticated by associate chief pharmacist Anwar Talip. The voucher specimens (NU-110108, NU-100908, NU-110123, NU-110113, NU-110128, NU-100111) have been deposited in the Xinjiang Autonomous Region Traditional Uighur Medicine Hospital (Urumqi, China).

**Preparation of the aqueous extract of KD**

According to the recipe of KD recommended by the State Pharmacopoeia of People’s Republic of China, all herbs were cut into pieces, then 1 kg herbs were marinated in 10 L of warm distilled water for 12 hours. The aqueous extract was then prepared by boiling for 30 min. The extract was filtered and concentrated under reduced pressure and temperature (60°C) on a rotary evaporator, dried in vacuum conditions and stored in the refrigerator. The yield of the extract was found to be 21.84%. The powder was suspended in 0.5% sodium carboxymethylcellulose (CMC-Na) solution before use.

**Animals**

Kunming mice weighing 18 ± 22 g were obtained from the Experimental Animal Centre of Xinjiang Medical University. The mice were housed in plastic cages at room temperature of 22 ± 1°C under a 12 h light–dark cycle, and provided with rodent chow and water ad libitum. All procedures were in strict accordance with the guidelines set of the Good Laboratory Practice centre at Xinjiang Autonomous Region Traditional Uighur Medicine Institute.

All experimental procedures used in the present study were approved by the Ethics Committee of the Xinjiang Medical University which has adopted the guidelines established by the Xinjiang Uighur Autonomous Region on Animal Care and Experimentation.

**Animal model of hyperuricemia in mice**

Two different in vivo hyperuricemia models were established using yeast-induced and potassium oxonate stimulated mice.

### Table 1 Medicinal plants contained in Karapxa decoction

| Botanical name | Common name | Uighur name | Family      | Part used | Quantities |
|---------------|-------------|-------------|-------------|-----------|------------|
| *Apium graveolens* L. | Celery | Karapxa uruki | Umbelliferae | Seed | 30 g |
| *Apium graveolens* L. | Celery | Karapxa yiltizi | Umbelliferae | Root | 30 g |
| *Cuscuta chinensis* Lam. | Dodders | Sirik yogay uruki | Convolvulaceae | Seed | 20 g |
| *Cichorium glandulosum* Boiss. et Huet. | Chicory | Kasin uruki | Compositae | Seed | 15 g |
| *Foeniculum vulgare* Mill | Fennel | Badranji buya yiltizi posti | Umbelliferae | Root | 30 g |
| *Cichorium glandulosum* Boiss. et Huet. | Chicory | Kasin yiltizi | Compositae | Root | 15 g |
with some modifications [14,15]. Yeast contains large amounts of purine and is used to induce hyperuricemia in mice. For yeast-induced hyperuricemic animal model experiments 60 mice were equally divided into 6 groups as shown in Table 2. The normal control group was given 0.5% CMC-Na orally for 14 days. All other groups of mice were given yeast extract paste (30 g/kg) in 0.5% CMC-Na, orally once per day for 14 days. Group 2 was the hyperuricemic animal model control. Groups 3, 4 and 5 were treated with KD (200 mg/kg, 400 mg/kg and 800 mg/kg) by gavage for 14 days. Group 6 were treated with allopurinol 10 mg/kg orally for 14 days.

The uricase inhibitor potassium oxonate was used to induce hyperuricemia in mice [16]. Sixty mice were equally divided into 6 groups: the normal control was given orally only 0.5% CMC-Na for 14 days. All other mice were injected intraperitoneally with potassium oxonate 250 mg/kg 1 h before drug administration. Group 2 served as hyperuricemic animal model control. Groups 3, 4 and 5 were treated with KD (200 mg/kg, 400 mg/kg and 800 mg/kg) for 14 days. Group 6 was treated with allopurinol 10 mg/kg orally for 14 days.

Sample collection and measurement of serum UA, liver XO activities

Whole blood samples were collected from mice 1 h after final administration by retro-orbital sinus puncture. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuged at 3500 × g for 5 min to obtain the serum. The serum was stored at −80°C until assayed. Mouse liver was excised, frozen immediately and stored at −80°C until used. Tissue sample was homogenized in 5 vol. of 50 mM ice-cold phosphate buffer (pH 7.5). The homogenate was then centrifuged for 10 min at 1500 × g at 4°C. The lipid layer was carefully removed and the resulting fraction centrifuged further at 10,000 × g for 30 min and the supernatant was used for assays. Serum UA and liver XO was determined using a commercial kit (Nanjing Jiancheng Biochemical Reagent Co) according to the manufacturer’s instructions. Liver XO activities were expressed as nmol/min per mg protein. Protein concentration was determined using a commercial kit (Nanjing Jiancheng Biochemical Reagent Co) according to the manufacturer’s instructions. The supernatant obtained after the last centrifugation was also used for the assays of XO activities.

**Assay of xanthine oxidase activity**

The XO activity was assayed spectrophotometrically under aerobic conditions as reported with minor modifications [17]. The assay mixture consisted of 1 ml of test solution, 2.9 ml of phosphate buffer (pH 7.5), and 0.1 ml of enzyme solution (0.01 units/ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After preincubation at 25°C for 15 min, the reaction was initiated by the addition of 2 ml of substrate solution (50 mM xanthine in the same buffer). The assay mixture was incubated at 25°C for 30 min. The reaction was then stopped by adding of 1 ml of 1 N hydrochloric acid and absorption was measured at 290 nm using a UV spectrophotometer. A blank was also prepared in the same way, but the enzyme solution was added to the assay mixture after adding 1 N

| Group    | Dose (mg/kg) | UA (μmol/L) | UA inhibition (%) | Liver XO (U/per mg protein) | Liver XO inhibition (%) |
|----------|-------------|-------------|-------------------|-----------------------------|-------------------------|
| Normal   | –           | 6.12 ± 2.12 | –                 | 1.84 ± 0.15                 | –                       |
| Model (YEP) | –         | 135.5 ± 29.2** | –                 | 3.59 ± 0.28**               | –                       |
| YEP + KD | 200         | 96.7 ± 27.8** | 28.6              | 3.05 ± 0.21*                | 15.0                    |
| YEP + KD | 400         | 81.8 ± 22.9** | 39.6              | 2.65 ± 0.13*                | 26.2                    |
| YEP + KD | 800         | 65.5 ± 15.9** | 51.6              | 2.15 ± 0.22**               | 40.1                    |
| YEP + AP | 10          | 15.0 ± 3.0**  | 88.9              | 1.28 ± 0.25**               | 64.3                    |
| Normal   | –           | 9.53 ± 1.42  | –                 | 1.62 ± 0.23                 | –                       |
| Model (PO) | –         | 243.1 ± 17.2** | –                 | 3.86 ± 0.31**               | –                       |
| PO + KD  | 200         | 196.3 ± 22.8** | 19.2             | 3.15 ± 0.31                 | 18.4                    |
| PO + KD  | 400         | 162.5 ± 21.0** | 33.2             | 2.81 ± 0.13*                | 27.2                    |
| PO + KD  | 800         | 135.5 ± 15.6** | 44.3             | 2.65 ± 0.22*                | 31.4                    |
| PO + AP  | 10          | 25.0 ± 19.0**  | 89.7              | 1.48 ± 0.15**               | 61.7                    |

**KD:** Karapxa decoction; **YEP:** yeast extract paste model; **AP:** allopurinol; **PO:** potassium oxonate model. Data represent mean ± S.E.M. of 10 animals.

$P < 0.05$ compared to normal control group.

$P < 0.05$ compared to model control group.

$P < 0.01$ compared to model control group.
Antioxidant activity

Assay for DPP•-free radical scavenging activity

DPP• is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The model of scavenging the stable DPP• radical is widely used for relatively rapid evaluation of antioxidant activities. The free radical-scavenging activity of the extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable DPP• radical. DPP•'s purple colour shows a characteristic absorption at 517 nm. As antioxidants scavenge the free radical by hydrogen donation, the colour of the DPP• assay solution becomes light yellow resulting in a decrease in absorbance at 517 nm. Assay was performed in a 96-well microplate using the previously described modified method. Different concentrations of test sample and ascorbic acid were prepared in ethanol and 100 µl of the sample solution pipetted into each well and followed by 100 µl of 0.1 mM ethanolic DPP• solution. The reaction mixture was shaken vigorously and incubated at 37°C for 30 min. Absorbance was measured at 517 nm using a microplate reader. The percentage inhibition (%) of the DPP• radical by the samples was calculated using the following equation: % inhibition = (A_C - A_S) / (A_C) × 100, where A_C is the absorbance of the control and A_S is the absorbance of the sample. The concentration required to scavenge 50% DPP• free radicals was calculated. All determinations were performed in triplicate.

Lipid peroxidation assay

To test the in vitro inhibition of lipid peroxidation by the extracts, lipid peroxidation induced by Fe^{3+}/ascorbate system in mouse liver homogenate was used and thiobarbituric acid-reactive substances (TBARS) were measured with some modifications [20]. The reaction mixture contained mouse liver homogenate 0.1 ml (25%, w/v) in Tris–HCl buffer (20 mM, pH 7.0), KCl (150 mM), FeSO_4·6H_2O (0.8 mM), ascorbic acid (0.3 mM) and various concentrations of the extract in a final volume of 0.5 ml and was incubated for 1 h at 37°C. The incubated reaction mixture (0.4 ml) was treated with sodium dodecyl sulphate (0.2 ml, 8%) and thiobarbituric acid (1.5 ml, 20%). The total volume was then made up to 4 ml by adding distilled water and kept in a water bath maintained at 100°C for 1 h. After cooling, 1 ml of distilled water and 5 ml of n-butanol were added and shaken vigorously to separate the butanol fraction and measure TBARS formed at 535 nm. The percentage of inhibition of lipid peroxide formation was determined by comparing the absorbance of the treated extract and non-treated samples.
Statistical analysis
Values are presented as mean ± S.E.M. Analysis of variance (ANOVA) was used to test for differences among treated and controlled groups. Inhibitory concentration 50% (IC\textsubscript{50}) of each sample was calculated by linear regression analysis using SPSS 11.0 software programme.

Results
Effect on uric acid
The effects of KD on yeast extract paste (YEP) and uricase inhibitor potassium oxonate (PO) induced hyperuricemia in mice are shown Table 2. YEP and PO caused hyperuricemia in mice, with serum uric acid level increased to 135.5 ± 29.2 μmol/L and 243.1 ± 37.2 μmol/L, after 10 days oral YEP or 2 h after intraperitoneal PO, respectively. Fourteen days treatment with KD at 200, 400, 800 mg/kg effectively opposed the increase of serum UA concentrations in both models. Allopurinol significantly lowered serum uric acid concentrations in experimental animals, to values not different from normal controls.

Liver XO inhibitory activity
The XO activities in normal groups were 1.84 ± 0.35 and 1.62 ± 0.23 U/per mg protein respectively. The liver XO activities were increased to 3.56 ± 0.28 and 3.86 ± 0.31 U/per mg protein in the model control mice (p < 0.05). KD inhibited YEP-induced XO activity by 15.0%, 26.2%, 40.1% in 200, 400, 800 mg XD-treated mice, respectively and PO-induced XO activity by 18.9%, 27.2%, 31.4% inhibition for 200, 400 and 800 mg/kg XD groups (p < 0.05 except 200 mg, NS). Allopurinol inhibited mouse model XO by 64.3% and 61.66% at the dose of 10 mg/kg (both p < 0.05), obviously more than KD (Table 2).

In vitro effects
Inhibition of xanthine oxidase
At a concentration of 250 μg/ml KD, uric acid formation was completely suppressed; the IC\textsubscript{50} value for KD inhibition was 25.8 μg/ml. Allopurinol had an IC\textsubscript{50} value of 6.28 μg/ml (Figure 1).

DPP• radical scavenging
The results concerning DPP• free radical scavenging effect of KD are indicated in Table 3. KD was a potent DPP• free radical scavenger. This activity was 31.1%, 45.2%, and 65.2%, respectively, for 25, 50, and 100 μg/ml of KD (all p < 0.05). The IC\textsubscript{50} value was calculated to be 55.6 μg/ml.

Nitric oxide (•NO) radical scavenging
The efficiency of KD to scavenge •NO free radical is presented in Table 3. KD was found to possess strong NO scavenging ability, showing concentration-dependent activity (p < 0.05). The IC\textsubscript{50} value was 31.4 μg/ml.

Superoxide anion radical scavenging
As shown in Table 3, KD exhibited significant and concentration dependent O\textsubscript{2}•− scavenging activity (5.8–83.5% inhibition, p < 0.05 for concentrations above 6.25 μg/ml) at 6.25–400 μg/ml. The IC\textsubscript{50} value was calculated to be 89.3 μg/ml for a scavenging effect at 49.7%.

![Figure 1](image-url) Concentration-response curves for in vitro Xanthine Oxidase inhibition (in %) by Xarapxa decoction (XD) or allopurinol (AP) at increasing concentrations.
Table 3 Effect of increasing concentrations of Karapxa decoction (KD) on radical scavenging with DPP+, Nitric oxide (-NO), Superoxide, and lipid peroxidation models, compared to ascorbic acid or butylated toluene controls

| Product | Concentration (µg/ml) | DPP+ scavenging (%) | -NO scavenging (%) | Superoxide scavenging (%) | Lipid peroxidation inhibition (%) |
|---------|----------------------|---------------------|--------------------|---------------------------|----------------------------------|
| KD      | 3.15 µg/ml           | 61.3 ± 1.23         | 13.3 ± 1.89        | 4.8 ± 0.4                 | 12.0 ± 2.65                      |
| KD      | 6.25 µg/ml           | 15.7 ± 1.46         | 31.2 ± 2.72        | 8.6 ± 4.23                | 21.2 ± 3.33                      |
| KD      | 12.50 µg/ml          | 31.1 ± 3.21         | 49.1 ± 2.84        | 23.0 ± 3.69               | 29.3 ± 2.15                      |
| KD      | 25.0 µg/ml           | 45.2 ± 2.56         | 64.2 ± 5.52        | 33.4 ± 4.12               | 43.4 ± 4.96                      |
| KD      | 50.0 µg/ml           | 65.2 ± 3.14         | 71.5 ± 4.82        | 49.7 ± 3.88               | 48.5 ± 4.48                      |
| KD      | 100.0 µg/ml          | 82.2 ± 3.67         | 83.3 ± 2.52        | 69.1 ± 5.19               | 65.7 ± 4.18                      |
| AA      | 50.0 µg/ml           | 84.3 ± 4.68         | 39.2 ± 3.39        |                           |                                  |
| BHT     | 50.0 µg/ml           |                     |                    |                           |                                  |

KD: Karapxa decoction; AA: Ascorbic acid; BHT: Butylated hydroxytoluene.

Inhibition of lipid peroxidation
KD displays dose-dependent lipid peroxidation inhibition, and this activity was 24.5%, 34.2%, 48.3%, and 63.5%, for 25 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml KD (all p < 0.05). The concentration of KD needed for 50% inhibition was found to be 111.3 µg/ml (Table 3).

Discussion
Karapxa decoction had dose-dependent effects on uric acid concentration and xanthine oxidase activity in two different hyperuricemic mice models. In addition, KD had free radical scavenging activities in vitro.

Gout and hyperuricemia are increasingly common disorders, reportedly afflicting more than 2 million men and women in the United States alone [21], and is progressing rapidly in China due probably to recent changes in dietary habits [22]. Gout and hyperuricemia are metabolic disorders associated with abnormal uric acid concentrations in the body, resulting in the deposition of urate crystals in the joints and kidneys that lead to inflammation, as well as gouty arthritis and uric acid nephrolithiasis. In addition to an increased risk of hyperuricemia and gout, excess uric acid is also related to cardiovascular disorders, nephrolithiasis, diabetes [7,23-32].

Two major mechanisms have been proposed for hyperuricemia in man, excess production and insufficient metabolism of uric acid. Yeast extract paste and potassium oxonate were used to mimic both mechanisms; yeast represents excess production of UA, probably the main mechanism in man, and oxonate impairs metabolism [15,23]. Yeast disturbs normal purine metabolism by increasing xanthine oxidase (XO) activity and generating large quantities of uric acid. This model is similar to human hyperuricemia, which is induced by high-protein diets. Another mouse hyperuricemia model was generated by a single intraperitoneal injection of potassium oxonate 250 mg/kg. Potassium oxonate, a urate oxidase inhibitor, can raise the serum uric acid concentration by inhibiting the decomposition of uric acid by uricase, an enzyme that does not exist in man. Karapxa was found approximately equally effective in both models, but less than allopurinol.

Many bioactive products have been identified from the herbs in the KD formula and pharmacological activity of those herbs has been reported. Celery seeds are used in arthritic pain relief, for treating rheumatic conditions and gout [33,34]. Essential oil, fatty acid, flavonoids isolated from Apium graveolens L., possesses antibacterial, antioxidi-

tive, hepatoprotective, anti-tumor, and anti-cardiovascular disease abilities [35,36]. Certain indole-like compounds and indole alkaloids isolated from A. graveolens seeds were found to have antioxidant, cyclooxygenase and topoisomerase inhibitory activity [37]. The seeds of Cuscuta chinensis Lam. (Convolvulaceae), is a commonly used traditional Chinese herbal medicine used in improving and conditioning the liver and the kidney and also possesses anticancer effects, immunostimulant, antioxidant, and hepatoprotective activities [13,38-41].

The active constituents of C. chinensis include flavonol, flavonoids, lignans, quinic acid, and polysaccharide [42-45], which have been suggested to be responsible for the pharmacological activities observed from C. chinensis [46,47]. Cichorium glandulosum Boiss. et Huet is well known in Uighur medicine use for curing liver diseases, etc. In the Chinese Pharmacopoeia of the People's Republic of China, “Juju” (Herba Cichorii and Radix Cichorii) refers to the aerial parts and roots of Cichorium intybus L. and Cichorium glandulosum Boiss. et Huet. Different parts of these plants have been analysed, mostly for the presence of phenolics and sesquiterpene lactones. The major phenolics include flavonoids, coumarins and caffeic acid derivatives [48].

Many pharmacological studies on the same compounds have been performed earlier, finding anti-diabetic [49], antibacterial [50], hepatoprotective [12] and antioxidant effects. These constituents may be present at different amounts in the KD extracts and it is far from established which constituent(s) are responsible for the effects of the extracts. Further investigations are warranted to identify
the active principle(s) of the extracts from KD, responsible for the observed hypouricemic effects.

**Conclusion**

The objective of the present study was to test the hypouricemic and antioxidant effects of the KD, which is used in traditional Uighur medicine to treat gout and hyperuricemia. The results suggest that KD at the dosage of 200, 400 and 800 mg/kg has hypouricemic effects in both hyperuricemic mouse models. KD was also found to have inhibitory effects on mouse liver XO activity. Antioxidant activity was evident, through the ability to scavenge several free radicals (DPPH, NO and \( \text{O}_2^\cdot \)) and the effect on lipid peroxidation.

These results, though there is still a need for clinical validation, support the traditional use of KD to prevent or treat hyperuricemia and gout. It was less powerful than allopurinol, but may have the advantages of low cost and high acceptability in countries with limited resources or with a tradition of herbal medicinal treatment of diseases.

**Abbreviations**

KD: Karapxa Decoction; XO: Xanthine oxidase; DPPH: 1, 1-diphenyl-2-picrylhydrazyl radicals; PMS: Phenazine methosulfate; NBT: Nitroblue tetrazolium; NADH: Nicotinamide adenine dinucleotide; AA: Ascorbic acid; TBA: Thiobarbituric acid; UA: Uric Acid; NO: Nitric oxide; \( \text{O}_2^\cdot \): Superoxide; BHT: Butylated hydroxytoluene.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

NA, MA, Gi carried out most of the pharmacology studies, participated in the data analysis, and participated in its design and coordination and drafted the manuscript. MH helped with the preparation of samples. HU participated in the design of the study. RA, AK and NM participated in its design and coordination and drafted the manuscript. All authors read and approved the final manuscript.

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