Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 2, 1799 – 1810

Ameliorative Effect of Some Medicinal Plants Against Experimentally Hyperuricemic Rats

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ABSTRACT

The present study was conducted to investigate changes in expression of xanthine oxidase (XO) enzyme during hyperuricemia and the ameliorating effect of natural safe source as medicinal plants on the hyperuricemia in rats. For this purpose, 50 albino rats were categorized into 5 equal groups as follows: (GI) Control group, (GII) Potassium Oxonate (PO) Hyperuricemic group, (GIII) Febuxostat Hyperuricemic group,(GIV) Gingko biloba hyperuricemic group and (GV) Onion juice hyperuricemic group . At the end of 4 weeks, blood samples were collected and sera were separated and analyzed for determination of xanthine oxidase, ALT, AST, ALP activities and uric acid and total protein concentrations, liver and kidney specimens divided into 2 parts. Part for estimation of MDA, GSH, uric acid contents and SOD, GSH-Px activity and part (liver only) for studying xanthine oxidase gene expression. PO increased XO gene expression and enzyme activity it , uric acid, MDA, ALT,AST,and ALP., while it decreased GSH, SOD,GSH-Px and total proteins. Febuxostat decreased XO gene expression and enzyme activity, uric acid, MDA, GSH, GSH-Px and total proteins but it increased SOD, ALT, AST, and ALP. activities. Both Gingko biloba and onion juice showed a similar influence as Febuxostat but with lower degree in concerning XO gene expression and enzyme activity and uric acid, but they have more potent antioxidative effect than Febuxostat.

Key words: Hyperuricemia, potassium oxonate, Febuxostat, Ginkgo biloba, Onion juice, Xanthine oxidase gene, Xanthine oxidase enzyme and Uric acid.

INTRODUCTION

In hyperuricemia, which persists at a serum saturation of 6.8 mg/dl, urates are deposited on the articular cartilage, causing gout. Even with high levels of serum uric acid, very few people get gout. More than 90 percent of instances of hyperuricemia are due to poor renal excretion of urate, (**Conway and Schwartez, 2009).** Because of its severe and debilitating nature, gout is an old kind of arthritis that has been around for ages. Most commonly, the feet, and notably the big toe, are affected by severe bouts of excruciating swelling. Unswollen areas might be hot and swollen. Food and drug triggers, as well as medications that might aid, can be avoided in order to decrease the severity of gout episodes **(Schumacher, 2015).**

Gout is an uncommon disease caused by enzyme abnormalities in purine metabolism. Gout in males is typically caused by a high hereditary predisposition, even though the genetic basis is unclear. Recent research has focused on genes that regulate the transfer of the salty substance, known as urate. Urate transporter 1 (URAT1), encoded by SLC22A12, is a member of the organic anion transporter family and plays a crucial role in regulating uric acid reabsorption from the renal tubules. Hyperuricemia has been linked to a polymorphism of this gene**, (Graessler, et al , 2006).** The mouse XO gene has 36 exons and spans roughly 70 kb, according to **Cazzaniga et al. (1994)**, whereas the human XO gene has 36 exons and spans at least 60 kb, according to **Xu et al. (1996)**. Exon lengths range from 53 to 279 bp, whereas intron lengths

range from 0.2 to over 8 kb. Xanthine Oxidoreductase (XOR) is a rate-limiting enzyme in production of uric acid where it catalyzes hypoxanthine and xanthine oxidation to produce uric acid. It has two forms: xanthine dehydrogenase (XDH) and xanthine oxidase (XO) **(Furuhashi, 2020)**. XO inhibitors can be powerful therapeutic medicines for the prevention of hyperuricemia. Many XO inhibitors have been identified and described from plants, and it is expected that they can be utilized as alternatives to allopurinol since they have less side effects **(Zanabaatar, et al , 2010).** XO through its catalysis in coversion of hypoxanthine into xanthine and then uric acid, it produces superoxide (O2•-) and hydrogen peroxide (H_2O_2), **(Schmidt, et al, 2019)**. Potassium oxonate increased XO activity in rats,**(Haidari et al. , 2008)**, while **Chau , et al, (2019)** stated that hyperuricemia was induced through inhibition of uricase enzyme by using potassium oxonate (PO). Febuxostat suppressed both the oxidized and reduced forms of XO **(Takano, et al, 2005)**. Febuxostat drug is a nonpurine inhibitor used to treat hyperuricemia and persistent gout, **(Khosravan, et al, 2006)**. **Jansvinder et al. (2015)** found that Febuxostat was more successful than allopurinol in treating gout and decreasing blood UA levels.

The mechanism that might give us a possible explanation about the effect of Ginkgo biloba on uric acid, was xanthine oxidase inhibition effect by flavonoids which was the main active ingredient in Ginkgo biloba**,** these flavonoids rich compounds are structurally similar to xanthine oxidase substrate and so can inhibit the enzyme activity **(Owen and Johns, 1999).** Flavonoids are the main active constituents in Ginkgo biloba L., which have been suggested to have broad-spectrum free-radical scavenging activities. More than 70 kinds of flavonoids have been identified in this plant, **(Liu, et al, 2015).**

Onion is a flavonoid-rich staple food and it has been shown that it ranked highest in quercetin content among 28 vegetables and 9 fruits assayed by **Finnegan, et al., (1992).** The inhibitory effects of both onion juice and allopurinol on XO activities in the potassium oxonate-induced hyperuricemia are more dominant than their effects on the normal activities of the either two forms of the enzyme, **(Yoshisue, et al., 2000).** Onion elicited significant inhibitory actions on the xanthine oxidase (XO) activities in rat liver homogeneities but it might be acting via other mechanisms apart from simple inhibition of enzyme activities, **(Haidari, et al., 2008).**

This study has been planned to investigate the changes which may be occur in the expression of xanthine oxidase gene during hyperuricemia. Also, to investigate the hyperuricemic-ameliorating effect of some medicinal plants (Ginkgo biloba and onion) in comparison with an anti-hyperuricemic drug (febuxostat,).

MATERIALS AND METHODS

Animals: 50 male albino rats, Sprague Dawley strain, of body weight range 80-110 g were used, they were obtained from faculty of veterinary medicine, Cairo university, Egypt. Rats were kept in a room maintained at 25-30 °C with about 25% relative humidity. The room was lighted on a daily photoperiod of 12 hr. light and dark. Rats were fed on a balanced basal ration formulated according to national institute of health **(NRC., 1978)**.

Chemicals and solutions of treatment: Potassium oxonate (Sigma Chemicals Company) was used to induce experimental hyperuricemia. Febuxostat (Feburic) tablets (Hikma pharma Company), extracted Ginkgo biloba tablets (EMA Pharm pharmaceuticals Company) and onion juice were used for treatment of experimentally hyperuricemic rats.

Potassium oxonate was freshly prepared daily by dissolving in saline and injected i.p in dose of 250 mg /Kg. b.wt.**(Kwon, et al, 2011)**. Febuxostat solution was freshly prepared daily by grinding the tablets to getting it in powder form then 35 mg of febuxostat powder were dissolved in 10 ml of dimethyl sulfoxide (DMS) **(Sánchez-Lozada, et al, 2008)**. Ginkgo biloba solution was prepared by dissolving of 80 mg of Ginkgo biloba extraction powder (from inside capsule tablets) in 10 ml of distilled water.**(Mostafa ,et al, 2016).**

Preparation of Onion Juice: Onion was obtained from Agriculture College, Cairo University, Egypt. The outer dry skins and any inedible outer portions of onion were removed and the remaining edible portion was weighed and completely blended in distilled water (1:1 w/v). The freshly prepared juicy sample was

administrated to each animal by gastric gavage, **(Haidari et al., 2008)**.

Experimental design:

The animals were categorized into five equal groups (10 rats each):

Group I : Control group which was unsupplemented, only given basal diet. Other groups (40 rats) were injected i.p with 1ml of potassium oxonate sol. by dose of 250 mg/kg/ b wt. daily. Group II : Hyperuricemic group did not received treatment and used as positive control. After one hour the remaining 3 groups were treated as follows: Group III : Febuxostat hyperuricemic was injected i.p with 1 ml of Febuxostat sol. (35 mg/kg/ b wt.) daily. Group IV : Gingko biloba extraction hyperuricemic was injected i.p with 1 ml of gingko biloba sol (80 mg/kg/ b wt.) daily. Group V : Onion juice hyperuricemic was administered 1.5 ml of onion juice (50 %) by gastric gavage daily.The experiment continued for four weeks.

Sampling:

At the end of trial blood samples were collected from retinobullbar venous plexus by using fine heparinized capillary glass tubes according to **Schemer, (1967)** . The blood samples were left at room temperature for 30 minutes and then serum was separated by centrifugation at 3500 r.p.m for 15 minutes and frozen at -20°C in plastic vials for subsequent biochemical analysis. After decapitation of rats, liver and kidney were rapidly excised and washed with ice cold saline (0.9% NaCl) to get rid of tissue debris. Liver and kidney specimens were used for investigation of oxidative stress markers, but RNA extraction for Xanthine Oxidase gene expression was performed in hepatic tissue only.

Methods:

Analysis of Xanthine Oxidase Gene Expression:

RNA isolation: Total RNA was isolated from liver of six rats in each group. The RNA isolation was performed using miRNeasy Mini kit (Qiagen, Germany), according to manufacturer's instructions, the tissue was homogenized by potter-elvehjem homogenizer (Sartorius, Germany). Total RNA was reverse transcribed using High-Capacity cDNA Reverse Transcription Kits without RNase inhibitor (Applied Biosystems, CA, USA) according to the manufacturer instructions. cDNA was synthesized using random primers supplied by the kit. RNA and cDNA quantity and purity was measured using UV/Vis Spectrophotometer (Unico, USA). PCR was performed on all the strains to detect the Eimeria steadi specific gene. The PCR amplification was performed on a Biometra T Professional_thermocycler.

Amplification of all the genes has been carried out with the following reaction mixture composition.

DNA template	1µl
Forward primer (2.5µM)	1µl
Reverse primer (2.5µM)	1µl
GoTaq PCR Master Mix	12.5 µl
PCR grade water (Nuclease free)	ml

All PCR amplifications were performed using thermal cycler (Biometra TProfessional) using the following conditions;

Initial denaturation for 5 mins.	Denaturation for 30 seconds	Primer annealing for 30 seconds	Extension for 45 seconds	Final extension 7 mins.	Number of cycles
94 °C	94 °C	55 °C	72 °C	72 °C	35

The PCR products were analysed on 1% agarose gel, stained with ethidium bromide and the bands were visualized under UV illumination.

Real-time PCR: The PCR products are labeled and detected by using a fluorescently tagged substrate during the amplification process. This method is more time efficient and allows a precise quantification of the PCR products due to the high sensitivity of the fluorescent dye used for the detection of the amplification products. It also requires less RNA than end point assays, and is more resistant to nonspecific amplification **(Fraga, et al., 2011** and **VanGuilder, et al., 2008)**.

Chemical Analysis:

The collected sera of different groups were analyzed for estimation of activities of xanthine oxidase **(Gerd, et al, 1989)**, alanine aminotransferase (ALT), aspartate aminotransferase (AST) **(Wilkinson, et al, 1972)**, alkaline phosphatase (ALP) **(Belfield and Goldberg,1971)**. Also determination of serum uric acid (UA) **(Yunsheng et al, 2008)** and total protein **(Kevser, et al ., 2006)** concentrations was performed.

Oxidative stress markers in both hepatic and renal tissue homogenates were investigated through determination of malondialdehyde (MDA) **(Albro et al.,1986)** and reduced glutathione (GSH) **(Anderson., 1985)** contents as well as activities of superoxide dismutase (SOD) **(Marklund 1974; Nandi and Chtterjee, 1988)**, Glutathione peroxidase (GSH-Px) **(Rotruck et al.,1973)**. Also contents of uric acid **(Yunsheng et al., 2008)** and total proteins (**(Bradford, 1976).**

The obtained data were analyzed statistically by using SPSS for Windows version 16.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results and Discussion:

Purine metabolism produces uric acid, which is mostly generated in the liver by xanthine oxidase (XO) and expelled via the kidney. A high amount of uric acid in the blood is a symptom of hyperuricemia. Gout is a metabolic disease in which extracellular urate supersaturation causes the deposition of monosodium urate crystals in the joints and other organs **(Chau , et al, 2019)**

The obtained data in table (1) showed that potassium oxonate in group II enhanced expression of xanthine oxidase gene and increased significantly serum xanthine oxidase activity as well as concentration of uric acid in liver, kidney and serum than control and other treated groups. Only treatment with Febuxostat in group III inhibited xanthine oxidase gene expression significantly lower than group II but non-significantly lower than control and other treated groups. On the other hand, treatment with either Gingko biloba extraction (group IV) or onion juice (group V) inhibited xanthine oxidase gene expression slightly lower than group II. All administered treatments in groups III, IV and V decreased significantly xanthine oxidase activity and uric acid concentrations in serum, liver and kidney lower than group II and returned it into control range.

This result came in parallel with that of **Haidari, et al, (2008)** who found that the oxonate administration lead to an increase in XO activity in rat liver when compared with the control group. **Xiaoying, et al, (2016)** recorded increasing activity of XOD in potassium oxonate-induced hyperuricemia, they illustrated

that PO is a well-documented effective uricase inhibitor, to induce a hyperuricemic condition in rats.

Also **Oh, et al, (2019)** recorded significant increase in activity of xanthine dehydrogenase in serum and liver of mice injected i.p with potassium oxonate. **Chen, et al, (2019)** found that PO increased significantly XOD level in serum and liver higher those levels in the control group.

Table: (1): Relative expression of hepatic xanthine oxidase gene, Xanthine oxidase Activity, Uric Acid concentration in different groups of rats (Mean ± SD)

Group	Hepatic Gene Expression. Fold change ($2^{-\Delta\Delta CT}$)	Serum Xanthine Oxidase Activity (U/mg protein)	Hepatic Uric Acid (ng/mg protein)	Renal Uric Acid (ng/mg protein)	Serum Uric Acid (mg /dl)
G I	1.05 ± 0.12^{ab}	2.58 ± 0.39^{b}	9.83±1.65^{b}	8.16±0.99^{b}	2.2 ± 0.2^{c}
G II	1.25 ± 0.25^{a}	3.27 ± 0.43^{a}	15.73±2.30^{a}	10.95±1.32^{a}	2.85 ± 0.27^{a}
G III	0.98 ±0.16^{b}	2.59 ±0.35^{b}	9.95±1.28^{b}	8.22±0.84^{b}	2.31± 0.36$^{b c}$
G IV	1.10 ± 0.19^{ab}	2.59 ± 0.45^{b}	10.92±2.12^{b}	8.65±0.85^{b}	2.39 ± 0.15$^{b c}$
G V	1.19 ± 0.21^{ab}	2.91 ± 0.33^{b}	11.17±1.70^{b}	8.91±0.78^{b}	2.51 ± 0.2^{b}
p-value	>0.05	<0.001	<0.001	<0.001	<0.001

- Different letter in the same column means presence of significant variation

Hassoun et al. (1994) found that the activities of xanthine dehydrogenase/xanthine oxidase (XD/XO) in bovine endothelial cells (EC) are inversely controlled by the oxygen tensions to which the cells are exposed, with the highest activity observed at the lowest O2 level. The impact of O_2 concentration on XD/XO mRNA expression in rat epididymal fat pad EC was investigated, and it was discovered that XD/XO mRNA concentration was higher in hypoxia and lower in hyperoxia compared to normoxic cells. The transcriptional control of XD/XO by oxygen tension is most likely the case..

Anjana et al. (2011) reported that Febuxostat, a xanthine oxidase inhibitor, fully inhibits the enzyme's activity by preventing substrate binding and inhibits both the oxidized and reduced forms of xanthine oxidase. Also, **Johji, et al, (2014)** has proved that febuxostat significantly reduced XO activity.

G.biloba leaf acetone extract showed strong inhibition for XO activity by 100 % when compared with the control group **(Bong, et al, 2002)**. **Hui et al, (2018)** revealed that total onion polyphenols inhibited XO activity well in experimental and screening experiments utilizing gradient multiplexing at various doses. These findings revealed a dose-effect connection between XO concentration and inhibition of XO activity.

The observed increase in uric acid concentration in serum or hepatic and renal tissues may be attributed mainly to the attained activation of xanthine oxidase enzyme. On the other hand, **Su, et al, (2018),** considered potassium oxonate as a hyperuricemic agent by inhibition of uricase enzyme. **Bisht and Bist, (2011),** stated that febuxostat is a new orally given anti-hyperuricemic medication that inhibits XOD and therefore decreases the synthesis of UA in the body. Also,**Takashi, et al, (2016)**, found that febuxostat therapy reduced plasma uric acid levels in rats substantially. **Luma, & Imad , (2019)** recorded a significant effect of decreasing the uric acid level by using of G. biloba. The hypouricemic property of onion juice could be explained by the inhibitory effects of its flavonoids on XDH and XO activities, (**Zhu et al., 2004).** Oral administration of onion

as a flavonoid-rich food can reduce the elevated uric acid levels in hyperuricemic rats in a dose- and time-dependent manner, (**Haidari, et al., 2008). Shivrajm and Se (2014),** found that onion juice had a substantial impact on lowering uric acid levels in hyperuricemic rats.

Changes in oxidative stress markers (MDA, GSH, SOD, and GSH-Px) in liver and kidney of different groups of rats were illustrated in tables (2 and 3). These changes happened by same pattern in both hepatic and renal tissues but in varying degrees. So, the attained data showed that injection of PO in group II increased significantly MDA content and decreased significantly GSH content and inhibited SOD and GSH- Px activities significantly in comparison with control group in both liver and kidney. Treatment with febuxostat in group III caused significant decrease in MDA content and increase in SOD activity in comparison with both groups I and II. Unfortunately, febuxostat cannot increase GSH content and GSH-Px activity over group II and stilled them significantly lower than control group. Administration of both Gingko biloba extraction (group IV) or onion juice (group V) caused significant decrease in MDA content and increase in GSH concentration ,SOD and GSH-Px activities in comparison with both groups I and II. The obtained data showed that antioxidative effect of Gingko biloba extraction is more potent than that of onion juice.

Table (2):Changes in hepatic oxidative stress markers (MDA, GSH, SOD & GSH-Px) in different groups of rats

Group	MDA (µM/mg protein)	GSH (µM/mg protein)	SOD (u/g protein)	GSH-Px (µM/mg protein)
GI	2.13±0.16 [b]	5.35± 1.50 [a]	43.74±4.72 [b]	2.00±0.31 [b]
GII	2.44±0.26 [a]	4.14±0.81 [b c]	38.70±2.98 [c]	1.72±0.18 [bc]
GIII	1.73± 0.42 [c]	3.82±0.40 [c]	48.16±2.27 [a]	1.58±0.37 [c]
GIV	1.81±0.46 [c]	4.88±0.87 [ab]	50.27±4.52 [a]	2.73±0.41 [a]
GV	1.74± 0.29 [c]	4.73±0.85 [ab]	48.35±2.4 [a]	1.79±0.29 [bc]
p-value	<0.001	<0.01	<0.001	<0.001

- Different letter in the same column means presence of significant variations

Table (3) : Changes in renal oxidative stress markers (MDA, GSH, SOD & GSH-Px) in different groups of rats

Group	MDA (µM/mg protein)	GSH (µM/mg protein)	SOD (u/g protein)	GSH-Px (µM/mg protein)
GI	1.83±0.11 [b]	4.90±0.88 [a]	49.54±4.35 [b]	1.81± 0.28 [b]
GII	2.22± 0.35 [a]	3.79±1.15 [bc]	44.50±2.87 [c]	1.55±0.25 [c]
GIII	1.55± 0.34 [b c]	3.58±0.31 [c]	53.61± 2.24 [ad]	1.42± 0.25 [c]
GIV	1.50±0.30 [c]	4.50±0.95 [ab]	54.62± 3.27 [a]	2.18± 0.39 [a]
GV	1.44± 0.56 [c]	4.39± 0.52 [ab]	51.61±2.39 [bd]	1.61± 0.16 [b c]

p-value	**<0.05**	**=0.005**	**<0.001**	**<0.001**

- **Different letter in the same column means presence of significant variations**

McCord, (1985) reported that oxidative stress marker (MDA) arise from activation of xanthine oxidase enzyme which produces reactive oxygen species (ROS; superoxide anion and H_2O_2). Xanthine oxidase generates ROS superoxide anion (O°-) and hydrogen peroxide (H_20_2) through oxidation of hypoxanthine and xanthine into uric acid. **Satoshi, et al, (2006)** recorded a significant inhibition in glutathione peroxidase activity in liver of PO-induced hyperuricemic rats when compared with the control group. **Xiaoying, et al, (2016),** found high accumulation of MDA and a significant inhibition in SOD activity in hepatic tissues of rats injected with potassium oxonate and induced hyperuricemia. Injection of PO caused significant depletion of GSH content in comparison with the control group, **(Al-Seeni, et al, 2018)**

Mahmoud, et al ,(2019), reported that febuxostat have a significant decline in GSH in rat liver when compared with both of PO injected group and control group.

Total ROS levels were decreased and cells were protected from apoptosis when XDH and XO enzymes were inhibited by modest pharmacological inhibitors or gene silencing **(Haixia, et al, 2019)** . **Alshahawey, et al, (2019)** found that febuxostat caused a significant decrease in MDA in renal tissue in PO injected group. Also **Ibrahim,et al , (2020)** , mentioned that febuxostat decreased serum UA elevation which accompanied with significant elevation in SOD activity in liver and kidney in comparison with Po-induced hyperuricemia. **Hidetoshi, et al,** (**2012)** recorded that treatment of hyperuricemic rats with febuxostat exhausted GSH-Px activity in hepatic and renal tissues lower than both control and hyperuricemic groups.

Both **Mansour , et al, (2013)** and **Niu,et al, (2019)** reported that the supplementation of G. biloba linearly increased hepatic GSH-Px activity when compared with PO hyperuricemic rats. Also renal content of MDA was significantly decreased after administration of either G. biloba extract (**Juan, et al, (2019)**, or onion juice (**Stephen, 2008)** into hyperuricemic rats. **Fahim, (2008)**, found that treatment of PO hyperuricemic rats with onion juice showed a non-significant change of renal GSH-px activity when compared with PO group and control group.

Concerning liver functions, Table (4) illustrates changes in some liver functions (ALT, AST and ALP activities) and total protein concentration in serum of different groups of rats. The obtained data revealed that PO, febuxostat and onion juice in groups II, III and V caused significant increase in ALT, AST and ALP activities and decrease in total protein concentration as compared with control and G. biloba groups (I & IV).

Table (4) : Changes in ALT, AST, ALP activities and Total proteins level in serum of different groups of rats

Group	**ALT(U/L)**	**AST (U/L)**	**ALP (U/L)**	**Total protein (g/dl)**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
GI	32.54 ±2.80^{b}	49.19±4.40^{c}	37.66± 4.62^{b}	6.45±0.44^{a}
GII	53.52±8.66^{a}	79.22± 13.03^{a}	42.07±2.32^{a}	5.37±0.24^{b}
GIII	54.11±7.2^{a}	80.03±10.30^{a}	41.8± 3.37^{a}	5.35± 0.23^{b}

GIV	33.71± 3.51 [b]	60.69±19.58 [b]	38.24±3.17 [b]	6.39± 0.35 [a]
GV	53.85± 6.60 [a]	79.97± 10.72 [a]	41.33± 1.90 [a]	5.38± 0.25 [b]
p-value	<0.001	<0.001	<0.05	<0.001

- Different letter in the same column means presence of significant variations

Dool, et al, (2019), recorded that PO caused liver damage and increase ALT & AST activity. Also **Hariprasad , et al ,(2020)** found a significant decrement of TP after injection of PO when compared with control group**.**

Khattab, (2012), mentioned that pretreatment with G. biloba caused significant restorations of ALT, and AST enzymes level in induced liver damage in rats.

Yuanyuan, et al, (2015), reported that G. biloba application noticeably mitigated fibrosis and improved the functions of the liver. **Chávez, et al , (2020)** reported that the G. biloba reduced the amount of necrotic areas in the central lobe area and decrease ALT & AST activities.

Conclusion:

Potassium oxonate induced hyperuricemic state through slight upregulation of xanthine oxidase gene and produced oxidative stress through marked increase in xanthine oxidase activity. Febuxostat relieved the hyperuricemia through marked downregulation of xanthine oxidase gene expression and inhibition of xanthine oxidase activity. Although febuxostat decreased MDA and increased SOD activity it did not increase GSH content and GSH-Px activity. Ginkgo biloba leaf extract and onion juice ameliorated the hyperuricemia through its inhibitory effect upon xanthine oxidase enzyme and its potent antioxidant role due to its polyphenol and flavonoid contents.

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