Journal of Current Pharma Research
(An Official Publication of Human Journals)
An International Peer Reviewed Journal For Pharmacy, Medical & Biological Science
DOI: 10.25166 CODEN: JCPRD6 NLM ID: 101744065
ISSN: Print: 2230-7834 Online: 2230-7842
SJIF Impact Factor: 6.913

Human Journals
Research Article
January 2024 Vol.:20, Issue:1

Hesperidin and Silymarin Helpful in Preventing and Slowing the Progress of Gout-Related Disease

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Submitted: 05 January 2024

Accepted: 20 January 2024

Published: 25 January 2024

Keywords: Hesperidin, Silymarin and gout related disease

ABSTRACT

The study suggested that hesperidin and silymarin possessed xanthine oxidase inhibitory and antioxidant activities, which might help prevent or slow the progress of gout-related disease. The results obtained from the present study indicate that the hesperidin at the dose of 100mg/kg exhibited higher activity. In the present study, in social interaction test as well as in the elevated plus maze both the test drugs i.e., hesperidin, silymarin and allopurinol showed significant antianxiety effects. All the compounds showed significant antianxiety effects in both the models consistently. The results were comparable to the control animals. The significant antianxiety effect shown by the xanthine oxidase inhibitors can be attributed to the increased level of tryptophan by the inhibition of xanthine oxidase.

INTRODUCTION:

Gout[1-4] is an inherited metabolic disease and results from hypouricemia, an elevation in level in serum uric acid level. Control of hypouricemia is most often achieved by the use of anti-inflammatory agents for symptomatic relief and of reducing uric acid production from purines by xanthine oxidase inhibitor. The enzyme XO catalyse the conversion of hypoxanthine to xanthine and to uric acid, which are the terminal biochemical reaction in purine metabolism resulting in an increase in the level of uric acid. This eventually leads to the deposition of monosodium urate crystals in the joints. Moreover, an increase in the level of XO also cause renal stone formation, ischemic myocardium and free radical-induced diseases. Recent finding shoe that the occurrence of gout is increasing worldwide, possibly due to the changes in dietary habits like intake of foods rich in nucleic acid, such as meats, some type of sea food. Hypouricemic agents are commonly employed for the treatment of chronic gouty arthritis which includes xanthine oxidase inhibitors and uricosuric agents. Allopurinol a xanthine oxidase inhibitor interferes with the conversion of hypoxanthine to xanthine and xanthine to uric acid. In general, allopurinol is the drug of choice for gout. However, it has been observed that allopurinol include adverse effects such as hepatitis, nephropathy and allergic reactions. So there is a need to development of new compound with high therapeutic activity and less side effects.

Tryptophan is a serotonin precursor and tryptophan pyrrolase is the enzyme that metabolises tryptophan. Xanthine oxidase[5-10] endogenously stimulates tryptophan pyrrolase thus resulting in increased metabolism of tryptophan. Tryptophan level is controlled or inhibited by the inhibition of xanthine oxidase.

In the present study, in social interaction test as well as in the elevated plus maze both the test drugs i.e., hesperidin, silymarin[11-15] and allopurinol showed significant antianxiety effects. All the compounds showed significant antianxiety effects in both the models consistently. The results were comparable to the control animals. The significant antianxiety effect shown by the xanthine oxidase inhibitors can be attributed to the increased level of tryptophan by the inhibition of xanthine oxidase. Many studies have demonstrated that Tryptophan has been used in the treatment of depression in the past. Researchers have found that the breakdown of L-tryptophan into kynurenine production instead of serotonin production by the enzyme tryptophan pyrrolase is linked to anxiety. Some studies showed that L-tryptophan ingestion caused a reduction in social anxiety disorder and at the same time depletion induced a significant increase of anxiety in treated Seasonal Affective disorder (SAD) patients.

Tryptophan is the only aromatic acid precursor of serotonin, a chief neurotransmitter in the brain which has a key role responsible for various brain physiologies such as alterations in appetite, energy, sleep, mood, libido, and cognitive functioning seen in affective disorders. It is produced in the brain by a short two-step process. In the first step, L- Tryptophan is first converted into 5-Hydroxytryptophan by the enzyme tryptophan hydrolase. 5Hydroxy-tryptophan is then decarboxylated by another enzyme, aromatic enzyme decarboxylase in the second step. For about 80% of our body's total serotonin content, the source is the gut, i.e., the enterochromaffin cells - where it regulates intestinal movements. The rest is synthesized in the serotonergic neurons in the central nervous system as serotonin cannot cross the blood-brain barrier Therefore serotonin that is used inside the brain must be produced within it. Serotonin facilitates defensive responses to potential threats like inhibitory avoidance related to anxiety. The role of serotonin in anxiety is supported by its modulatory effects on the locus coeruleus and its dense projections to the amygdale. Allopurinol has been on the market from many decades now & is tolerated well by most of the patients with few drug interactions unlike TCAs and SSRIs. It is known to cause an increase in the volume of distribution of tryptophan. Fever, myalgia, malaise hepatomegaly are some of the rare side effects caused by allopurinol. Hesperidin is more potent compared to allopurinol and silymarin. It is the non purine xanthine oxidase inhibitor. The activity of these xanthine oxidase inhibitors were studied on depression models and a significant antidepressant activity was seen. The putative role of xanthine oxidase on levels of tryptophan and serotonin has to be studied.

All the compounds such as hesperidin, silymarin and allopurinol have got significant antianxiety effects as demonstrated in both social interaction test and the elevated plus maze test when compared to control.

Flavonoids are secondary plant metabolites containing a high group of polyphenolic compounds. They are involved in the formation of pigmentation in flowers). To-date more than 8000 structure varieties of flavonoids are identified which possess low toxicity. Flavonoids are distributed in various food materials and medicinal plants such as fruits, vegetables, herbs and beverages such as tea and red wine. All flavonoids share a basic C6-C3-C6 phenyl-benzopyran backbone which is responsible for their pharmacological actions. Flavonoids have also been found to possess xanthine oxidase inhibitory activity.

In silico docking study, is carried out to identify the inhibiting potential of selected 50 flavonoids against xanthine oxidase enzyme from bovine milk source.

The hesperidin was isolated from orange peel and purified, commercially available flavonoids such as silymarin, rutin and diosmin were collected from Sigma-Aldrich.

Hesperidin, silymarin, rutin and diosmin exhibited *In vitro* xanthine oxidase inhibitory activity. Among the four compounds, hesperidin possesses highest XO inhibitory activity (IC_{50} 13.16±0.20).

MATERIALS AND METHODS:

IN SILICO BINDING INTERACTION STUDIES

Software's used

- MGL tools-Auto Dock 4.2 was downloaded from www.scripps.edu
- Python 2.7.8 was downloaded from www.python.com
- Cygwin 64 was downloaded from www.cygwin.com
- Accelrys Discovery Studio 4.0.1 was downloaded from www.accelerys.com
- Online SMILES translation using cactus.nci.nih.gov/translate
- ChemSketch was downloaded from www.acdlabs.com
- Graphpad 3.10

In silico docking study on xanthine oxidase enzyme using AutoDock 4.2

In vivo experiments demonstrate that the flavonoids possess significant hypouricemic activity in oxonate-induced hyperuricaemic mice. Potassium oxonate, an uricase inhibitors partially blocks the conversion of uric acid to allantoin and the serum uric acid level was significantly elevated to approximately two-fold for the hyperuricemic mice. The hesperidin and silymarin at the dose of 50mg/kg, and 100mg/kg restored the plasma uric acid level of the hyperuricaemc animals similar to the control group. These results indicate that the hypouricemic activity may be due to the xanthine oxidase inhibitory action.

The XO and XDH enzyme activities were also evaluated in the liver homogenate of the treated animals. In the present study, we observed that hesperidin and silymarin exhibited inhibitory action on XO and XDH enzymes in mouse liver. Among the two compounds, hesperidin possessed higher activity, at a dose of 100mg/kg compared to the standard allopurinol.

The XO and XDH enzyme activities were also evaluated in the liver homogenate of the treated animals. In the present study, we observed that the hesperidin and silymarin exhibited inhibitory action on XO and XDH enzymes in mouse liver. Among the two compounds in various dose hesperidin possessed higher activity at the dose of 100mg/kg compared to the standard allopurinol.

Lipid peroxidation is an autocatalytic process, which is a common cause of cell death. Reactive oxygen species and particularly free radical including lipid peroxidative tissue damage has been implicated in the pathogenesis of various diseases. The determination of MDA and LH level, the end product of lipid peroxidastion is one of the most commonly used methods for monitoring LPO. Our results suggests that there was a dramatic increase in lipid peroxidation after oxonate treatment and it was inhibited by the treatment with hesperidin and silymarin at various dose which proves its potent activity towards inhibition of lipid peroxidation.

Measurement of protein concentration is mainly used to calculate the level of purity of a specific protein. In Lowry method, the Cu^{2+} ions combine with the peptide bond and produce Cu^{+} peptide bond complex which is purple blue in colour solution. Potassium oxonate treated mice causes a depletion in total protein. Treatment with hesperidin and silymarin significantly increases the level of total protein.

XO/XDH is an important source of oxygen-free radicals. The enzyme catalyzes the reduction of oxygen leading to the formation of superoxide and H_2O_2 as well as hydroxyl radicals. It has been proposed as a central mechanism of oxidative injury in some situations. Thus, the determination of the *in vivo* antioxidant enzymes like SOD, CAT,GPx, GSSH, peroxidase and non-enzymatic antioxidant like GSSH were carried out.

Catalase plays a major role in the cellular antioxidant defense system by decomposing H_2O_2 therapy preventing the generation of the hydroxyl radical through the Fenton reaction. In hyperuricaemic mice, significant diminution was observed in liver catalase activity.

Therefore it is likely that catalase inhibition and resulting evaluation and resulting elevation of extracellular H_2O_2 level by increased blood uric acid causes oxidative stress. Flavonoids significantly the catalase level in hyper uricemic mice liver which may be a compensatory mechanism for the decline of blood uric acid level as well as H_2O_2.

The first enzyme involved in the antioxidant defense is superoxide dismutase. It is a metalloproteinase found in both prokaryotic and eukaryotic cells. The oxygen radical, generated by the interaction of Fe2+ and H2O2 are the species responsible for the oxidation of epinephrine and was strongly inhibited by superoxide dismutase.

GPx has a major role in degrading the levels of H_2O_2 in cells. Since GPx acts on hydroperoxides of unsaturated fatty acids, the enzyme plays an important role in protecting membrane lipids, and thus cell membranes from oxidative disintegration.

The levels GPx, GSSH, SOD, and peroxidase in oxonate-treated groups were decreased when compared to normal control group. The hesperidin significantly increases the level of antioxidant enzymes such as GPx, GSSH, peroxidase and SOD.

GSH is widely distributed in cells. GSH is an intracellular reductant and plays a major role in catalysis, metabolism and transport. It orotect cell against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and several metabolic effects. The various dose of hesperidin and silymarin significantly increase the level of non-enzymatic antioxidant GSH when compared to oxonate-treated mice.

RESULTS AND DISCUSSION:

To conclude, the study suggested that hesperidin and silymarin possessed xanthine oxidase inhibitory and antioxidant activities, which might be helpful preventing or slowing the progress of gout related disease. The results obtained from the present study indicate that the hesperidin at the dose of 100mg/kg exhibited higher activity.

Effect of the flavonoids on serum urate level in control and experimental animals

Administration of the uricase inhibitors, potassium oxonate resulted in significant hypouricaemic in mice, as indicated by an increase in the serum uric acid levels when compared to the control group. Pre-treatment with hesperidin lown (50mg/kg) and high (100mg/kg), silymarin low (50mg/kg) and high (100mg/kg) dose for seven days significantly reduced the serum urate levels, when compared with the hypouricemic control group. The reduction in urate level produced by hesperidin was more potent than that of other compound. The standard drug allopurinol at a dose of 10mg/kg elicited a significant reduction of serum urate level compared to hypouricaemic mice.

Table 1. Effect of the flavonoids on serum urate level in control and experimental animals

Treatment	Dose (mg/kg)	Serum urate level (mg/dl)
Control CMC 0.5% w/v	10ml/kg, p.o	3.72±0.17
Negative control	Potassium oxonate (280mg/kg i.p.)	7.39±0.04
Hesperidin Low dose	50mg/kg, p.o	4.9±0.13
Hesperine High dose	100mg/kg, p.o	4.2±0.01
Silymarin Low dose	50mg/kg, p.o	6.1±0.01
Silymarin High dose	100mg/kg, p.o	5.3±0.21
Allopurinoll	10mg/kg	4.3±0.01

All drugs were given orally and potassium oxonate injected intraperitoneally.

Values are mean± SEM; n=6 in each group.

Effect of the flavonoids on XO/XDH activities in mouse liver

Animals treated with potassium oxonate produced a significant increase in XO/XDH enzyme activities in mouse liver compared to the control group. Pre-treatment with hesperidin lown (50mg/kg) and high (100mg/kg), silymarin low (50mg/kg) and high (100mg/kg) dose for seven days produce significant ($p<0.01$) inhibition towards XO () and XDH () respectively, when compared with the hypouricaemic control. The inhibition of the XO/XDH activities of the hesperidin 100mg/kg was found to be the highest among the silymarin 50mg/kg and 100mg/kg. Allopurinol inhibited both the XO and XDH activities at a dose of 10mg/kg exhibiting more potent activity than the test compounds.

Table 2. Effect of the flavonoids on XO/XDH activities in mouse liver

Treatment	Dose (mg/kg)	(nM Uric acid formed/min/mg protein)		% Inhibition	
		XO	XDH	XO	XDH
Control CMC 0.5% w/v	10ml/kg, p.o	1.33±0.07	1.51±0.04	-	-
Negative control	Potassiumoxonate (280mg/kg i.p.)	3.38±0.11	3.20±0.12	-	-
Hesperidin Low dose	50mg/kg, p.o	2.47±0.20	2.60±0.22	30.60	32.21
Hesperine High dose	100mg/kg, p.o	2.17±0.16	2.10±0.34	38.97	40.11
Silymarin Low dose	50mg/kg, p.o	2.90±0.26	2.80±0.06	26.7	34.20
Silymarin High dose	100mg/kg, p.o	2.60±0.70	2.30±0.70	28.86	37.81
Allopurinoll	10mg/kg	1.96±0.05	1.80±0.55	54.76	58.39

All drugs were given orally and potassium oxonate injected intraperitoneally.

Values are mean± SEM; n=6 in each group.

Effect of the flavonoids on tissue protein, MDA, and LH in mice

There was a significant decrease in the total protein content and an increase in the concentrations of the end product of lipid peroxidation namely malondialdehyde (MDA) and lipid hydroperoxidase (LH) in the liver tissue of mice treated with the potassium oxonate when compared to the normal control. Mice pre-treated with hesperidin, silymarin and allopurinol orally for seven days produced a significant decrease in the levels of malondialdehyde and lipid hydroperoxidase and a significant increase in the total protein when compared to oxonate control. Treatment with the hesperidin significantly increased the total protein and decreased the malondialdehyde level but a decrease in lipid hydroperoxiase level was significant when compared to oxonate control. The activity produced by the hesperidin was found to be highest compared to the standard allopurinol.

Table 3. Effect of the flavonoids on tissue protein, MDA, and LH in control and experimental animals

Treatment	Dose (mg/kg)	Protein	MDA	LH
Control CMC 0.5%w/v	10ml/kg, p.o	94.51±0.56	0.150±0.005	0.233±0.008
Negative control	Potassium oxonate (280mg/kg i.p.)	42.95±1.07	0.629±0.007	0.5081±0.0127
Hesperidin Low dose	50mg/kg, p.o	71.45±1.39	0.311±0.005	0.333±0.012
Hesperine High dose	100mg/kg, p.o	89.02±0.71	0.245±0.012	0.2654±0.003
Silymarin Low dose	50mg/kg, p.o	42.44±1.05	0.475±0.005	0.4516±0.007
Silymarin High dose	100mg/kg, p.o	63.23±1.57	0.411±0.005	0.3628±0.005
Allopurinoll	10mg/kg	82.17±1.07	0.195±0.012	0.3531±0.012

All drugs were given orally and potassium oxonate injected intraperitoneally.

Values are mean± SEM; n=6 in each group.

Protein = moles/min/mg wet tissue, MDA = µmoles/min/mg protein, LH = µmoles/min/mg protein

SUMMARY:

The present study was undertaken to develop compounds for xanthine oxidase inhibitory activity. The issue is the desirability of developing novel xanthine oxidase inhibitors for clinical use in gout and related inflammatory diseases. All the compounds such as hesperidin, silymarin and allopurinol have got significant antianxiety effect as demonstrated in both social interaction test and elevated plus maze test when compared to control.

Flavonoids are secondary plant metabolites containing high group of polyphenolic compounds. They are involved in the formation of pigmentation in flowers To-date more than 8000 structure varieties of flavonoids are identified which possess low toxicity. Flavonoids are distributed in various food materials and medicinal plants such as fruits, vegetables, herbs, and beverages such as tea and red wine (Jeevan *et al* 2004; Tapas *et al*, 2008). All flavonoids share a basic C6-C3-C6 phenyl-benzopyran backbone which is responsible for their pharmacological actions. Flavonoids have also been found to possess xanthine oxidase inhibitory activity.

In silico docking study, is carried out to identify the inhibiting potential of selected 50 flavonoids against xanthine oxidase enzyme from bovine milk source. The hesperidin was isolated from orange peel and purified, commercially available flavonoids such as silymarin, rutin and diosmin are collected from Sigma-Aldrich. Hesperidin, silymarin, rutin and diosmin exhibited *In vitro* xanthine oxidase inhibitory activity. Among the four compounds hesperidin possesses highest XO inhibitory activity (IC_{50} 13.16±0.20).

In vivo experiment demonstrate that the flavonoids possess significant hypouricemic activity in oxonate-induced hyperuricaemia mice. Potassium oxonate, an uricase inhibitors partially blocks the conversion of uric acid to allantoin and the serum uric acid level was significantly elevated to approximately two-fold for the hyperuricemia mice. The hesperidin and silymarin at the dose of 50mg/kg, 100mg/kg restored the plasma uric acid level of the hyperuricaemc animals similar to the control group. These results indicate that the hypouricemic activity may be due to the xanthine oxidase inhibitory action.

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The study suggested that the hesperidin and silymarin possessed xanthine oxidase inhibitory and antioxidant activities, which might be helpful preventing or slowing the progress of gout

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