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Comparison of Xanthine Oxidase Inhibitory Effect of *Swertia chirayita* and Febuxostat in Vitro

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ABSTRACT

Introduction: Plasma uric acid ≥ 6.5 to 7.0 mg/dl increases the incidence of gout, hypertension, diabetes mellitus type 2, stroke and heart failure. Low purine diet, use of xanthine oxidase inhibitors allopurinol and febuxostat; or uricosuric agent's probenecid, sulfinpyrazone and Benzbromarone are being used for treatment. *Swertia chirayita* extract (SCE) being practiced as anti-inflammatory and anti-oxidant was evaluated for inhibition of xanthine oxidase. **Aims & Objectives:** To observe the in vitro effect of *Swertia chirayita* ethanolic extract on xanthine oxidase inhibition and its comparison with medicine febuxostat. **Place and duration of study:** The study was conducted in Pharmacology Department, University of Health Sciences Lahore during June-August 2017. **Material & Methods:** Xanthine oxidase (XO) inhibition by ethanolic extract of the herb was compared with febuxostat. Serial dilutions of test compounds ranging from 1.25µg/ml to 100 µg/ml were prepared in absolute ethanol. Fixed volumes of xanthine, xanthine oxidase, phosphate buffer and test compounds were incubated. Uric acid concentration in the reaction mixture was calculated by measuring absorbance at 295nm by using Ultraviolet- Visible Spectrophotometer. Mean values and percent inhibition (± SD) was calculated. EZ Fit Enzyme Kinetic program was also used to calculate inhibitory concentration 50 percent (IC_{50}), Michaelis constant (Km) and maximum rate of reaction (Vmax). **Results:** At maximum dose, (100 µg/ml) xanthine oxidase inhibition by febuxostat and *Swertia chirayita* was 98% and 80% percent respectively (p=0.01). *Swertia chirayita* extract showed IC_{50} at 9.15± 1.2 µg/ml whereas IC_{50} of febuxostat was 7.91± 0.9 µg/ml. **Conclusion:** In the light of XO inhibition, *Swertia chirayita* has potential to be explored as antihyperuricemic agent.

Key words: Uric acid, Febuxostat, *Swertia Chirayita*

INTRODUCTION

Hyperuricemia is a metabolic disease characterized by increase in serum uric acid (SUA) levels≥ 7.0 mg/dl in males and ≥ 6.0 mg/dl in females. Prevalence of hyperuricemia has increased globally in the last 40 years and it is on a substantial rise.[1]Humans lack uricase enzyme predisposing them to hyperuricemia.[2]

Hyperuricemia is considered an important risk factor for gout. In addition, hyperuricemia promotes the incidence of metabolic syndrome (hypertension, diabetes mellitus type 2, kidney disease, stroke, peripheral artery disease and even heart failure).[3]

Hyperuricemia can be managed by low purine diet, inhibition of uric acid formation by allopurinol and febuxostat or increased excretion by probenecid, sulfinpyrazone and benzbromarone. However, the incidence of adverse effects to these drugs is a major clinical problem.[4]

Medicinal plants have vital role in the health evolution of humankind. In modern era, researchers are working on herbal medicine, their chemical constituents and their extracts in management of various diseases. For hyperuricemia, theleading cause ofvarious diseases allopathic treatment is available but numerous side effects have restricted their prolonged use. Hence, it is necessary to explorenew alternatives, which decrease serum uric

acid level with fewer side effects and are cheap and easily available.[5]

Swertia chirayita (SC), commonly known as chiraita, a popular herb from Gentianaceace family is used in numerous home remedies, due to established, hepatoprotective, anti-carcinogenic, antimalarial and antioxidant effects. Moreover, it has anti-inflammatory and antimicrobial effects.[6] SC has been used in unani system of medicine for treatment of gout and its associated arthritis.[7] Therefore, a plant that targets both hyperuricemia and its related inflammatory arthritis gout will serve as a good alternative with minimum side effects. In the light of anti-inflammatory and anti-gout effect reported in literature xanthine, oxidase inhibitory activity was evaluated. This work was designed to observe in vitro effect of this medicinal herb and compare it with newly inducted medicine febuxostat.

MATERIAL AND METHODS

Study Design and Setting:

It was a comparative in vitro study, carried out in Pharmacology Department and Experimental Research Laboratory, University of Health Sciences Lahore during June- August 2017. The work was approved by Institutional Review Board # UHS/REG-17/ERC/4388.

Chemicals & Drugs:

Xanthine (X7375-25G), xanthine-oxidase (X1875-25U), potassium oxonate (156124-5G) and absolute ethanol (32205) were purchased from Sigma Aldrich Company. Hydrochloric acid and ethanol (Merck Darmstadt, FR, Germany) were procured from local vendor. Febuxostat was provided by Sharooq Pharma Limited. All the reagents and chemicals used were of research grade.

Preparation of Plant extract:

Fresh shoot of *Swertia chirayita* was identified by Botany Department, University of the Punjab Lahore. Half gram of air dried plant material was soaked in 5 liter (95%) ethanol for 48 hrs. The Plant material was filtered using Whattman filter paper no 1. Filtrate was concentrated by Rotary evaporator (Hei-Vap HL, Heidolph, Germany) and freeze-dried by lyophilizer (Alpha 1-2 LD plus Germany)[8]. Extract was light brown and shiny. Percentage yield was 5% w/w.

Preparation of Test Compounds:

Method described by Abu-Gharbiehet al.[9] was adopted. Febuxostat and plant extract were dissolved in 100µl` of dimethyl sulfoxide to ensure complete solubility. Serial dilutions were made in distilled water ranging from 100µg/ml to 1.25µg/ml. Final volume of the reaction mixture was 3ml, which includes 1.6 ml potassium phosphate buffer, 0.1ml xanthine oxidase, 0.3 ml test compounds. The contents were vortexed and pre-incubated at 37°Cfor 15 minutes. Then 1ml of xanthine was added into the solution. Reaction mixture was incubated again for 30 minutes at 37°C**.** Reaction was stopped by adding 0.1 ml of HCl. Absorbance was measured on double beam UV/VIS spectrophotometer (model UV-1602, Biotechnology Medical services, Canada) at 295nm. Control solution was prepared to observe maximum uric acid formation. Value of absorbance at spectrophotometer is inversely proportional to the potency of test compounds. All samples were run in triplicate. The reactions were repeated for outliers. Mean was taken and compared with control. The following formula was used to calculate the percentage inhibition of xanthine oxidase.[10]

Percentage inhibition = (Sample absorbance/Control absorbance x 100) -100

Statistical analysis:

Statistical analysis was performed using Statistical Package of Social Sciences (SPSS) version 20. Data was expressed as Mean ± S.D. One-way analysis of variance (ANOVA) was used to detect significant differences between *Swertia chirayita* extract and febuxostat. Probability value ≤ 0.05 was considered statistically significant. EZ-Fit Enzymes Kinetics program using Michaelis Menten equation was applied to calculate inhibitory concentration 50 % (IC_{50}), Km and Vmax for in-vitro study. Line weaver Burk plot was used for graphical representation of in vitro results.

RESULTS

Xanthine oxidase inhibition:

Febuxostat and *Swertia chirayita* have graded dose response relationship in xanthine oxidase inhibition. Maximum response was 80% with *SCE* at 100 µg/ml, whereas it was 98% with febuxostat at the same concentration; and the difference was significant (p value 0.001)(Fig-2). Line weaver Burk plot was plotted using Microsoft Excel sheet (2010**)**. There was dose dependent inhibition of XO (Fig-1). Inhibitory concentration 50 percent (IC50) was calculated by EZ fit Enzyme Kinetic program. For febuxostat IC50, Km and Vmax were 7.91 ± 0.9, 6.1± 1.14 and 98.24± 1.9 µg/ml respectively; while with *SCE* values were 9.15 ± 1.2 , 2.31 ± 0.73 and 71.9 ± 1.4 µg/ml respectively (Table-1).

In vitro results thus showed that XO inhibition of *SCE* was lesser but comparable to febuxostat.

Parameter	Febuxostat	*Swertia chirayita*
IC50	7.91 ± 0.9 µg/ml	9.15± 1.2 µg/ml
Km	6.1± 1.14 µg/ml	2.31 ± 0.73 µg/ml
Vmax	98.24± 1.9 µg/min	71.9 ± 1.4µg/min

Table-1: Comparison between kinetic parameters of Febuxostat and *Swertia chirayita.*

IC50 is 50% inhibitory concentration.
Km is concentration of substrate at which half Vmax is achieved.
Vmax= Reaction velocity.
Kinetic model= MICHAELIS-MENTEN

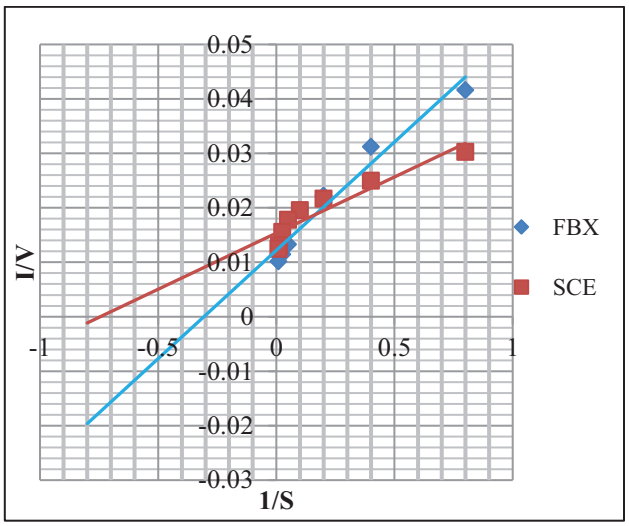
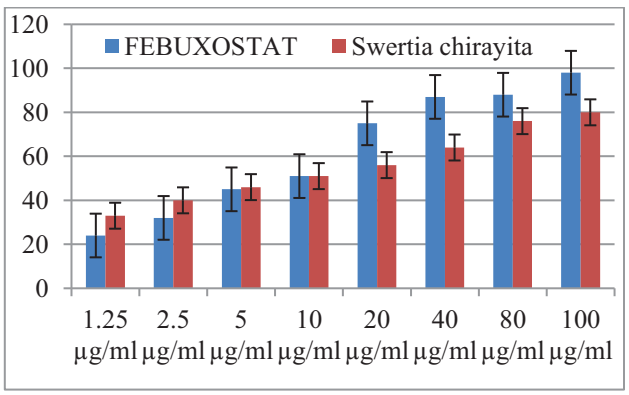


Fig-1: Lineweaver-Burk plot of inhibition of xanthine oxidase by Febuxostat (FBX) and *Swertia chirayita* extract (SCE).



*** p value < 0.001 ** p value < 0.01 * p value < 0.05

Fig-2: Bar chart showing comparison of percentage inhibition of xanthine oxidase by Febuxostat versus *Swertia chirayita*

DISCUSSION

Elevated uric acid is a primary prerequisite for gout as well as for other comorbidities like diabetes, hypertension, heart diseases and metabolic syndrome.[11] More attention has been given to natural products during the past decades in treating hyperuricemia.[12]

Currently numerous drugs are available to modulate the uric acid levels. XO is an important enzyme that catalyzes the rate-limiting step in uric acid production. Allopurinol has been available for the last four decades.[13] In 2009, FDA approved febuxostat as a non-purine, potent and selective xanthine oxidase inhibitor. Febuxostat binds with high affinity to xanthine oxidase and shows mixed type of inhibition of both oxidized and reduced forms of enzyme. Unlike allopurinol, febuxostat lacks structural resemblance to nitrogenous bases. Therefore, drug interaction with the enzymes involved in purines and pyrimidines metabolism have not been reported with febuxostat.[14]

Xanthine-XO enzymatic system was used to investigate inhibitory potential of test compounds. The potency of compound was indirectly proportional to the absorbance on UV-VIS spectrophotometer.[15] SCE has 33 % inhibition with 1.25 µg/ml whereas febuxostat showed 24 % inhibition on this concentration. At maximum concentration (100µg/ml) xanthine oxidase inhibition with febuxostat and *SCE* was 98% and 80% respectively (Table-1). Fifty percent inhibitory concentration (1C50) of febuxostat was 7.91 ± 0.9µg/ml more than *SCE* that was9.15 ± 1.2µg/ml. If IC50 of positive control is taken as 100%, the xanthine oxidase inhibition of *SCE* was 86%. Hence, we can conclude that *SCE* has significant xanthine oxidase inhibition. It is more potent but less efficacious than febuxostat

A part of this work has already been published. In an article published by Qurat et al,[16] serial dilutions of Febuxostat and allopurinol ranging from 0.75µg/ml to 100µg/ml were observed for in vitro xanthine oxidase inhibition. IC50 of febuxostat was 8.77µg/ml while Km and Vmax were 8.89 and 107.13 respectively, which are comparable to findings of this study.

In an article published by Osada et al.[17] febuxostat (TEI-6720) was investigated for xanthine oxidase inhibition in vitro. Dilutions were made in 0.1% DMSO. IC50 of febuxostat was 1.4nM and inhibition constant (Ki) was 0.7nM; whereas in our in vitro model, IC50 of febuxostat was 7.91 µg/ml and Km was 6.1± 1.14µg/ml (Mean ± S.D). We used 0.01% DMSO and distilled water was added to make final concentration. Febuxostat procured by author in his reported work was of analytical grade whereas our drug was of research grade.

Phytochemical analysis of Swertia chirayitahas revealed presence of basic alkaloids, coumarin, glycosides, steroids, quinones, flavonoid, anti-oxidants and terpenoids.[18] The mode of inhibition of XO by flavonoids is both competitive and mixed,

depending on concentration and type of substrate and inhibitor.[19] XO inhibitory effect is increased in flavonoids due to absence of C3 hydroxyl group.[20] Taking into account the presence of flavonoids and alkaloids in Swertia chirayita, we can say that this XOI effect of Swertia chirayita extract may be due to presence of these active compounds.[21, 22]

Limitation of study:

A disparity is found in the results of Km and Vmax. Values derived by Michaelis-Menten equation are much higher (Table-1) than derived from Line weaver- Burk plot (Fig-1).

CONCLUSION

Hence, we can conclude that *Swertia chirayita* extract has shown significant dose dependent xanthine oxidase inhibitory activity, which is comparable to febuxostat and the difference of inhibition is not significant particularly at low concentrations. Additionally our study has established that *Swertia chirayita* has higher affinity for the enzyme, hence higher potency (higher IC50) but less efficacy and Vmax compared to febuxostat.

Recommendation:

New drugs that target XO both invivo and invitro should be explored and their clinical safety and efficacy should be observed in experimental models, so as to pave way for more effective and safer alternatives from herbal products.

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