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ORIGINAL ARTICLE

Evaluation of Anti-Hyperuricemic Potential of Fumaria Indica Extract on Xanthine Oxidase Activity in Vitro

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ABSTRACT

Introduction: A metabolic condition called hyperuricemia is caused by increased uric acid levels, which can range from 6.6 to 7.6.Treatment for hyperuricemia is, low protein diet and drugs like allopurinol and Febuxostat. Medicinal plants and herbs are safer, almost completely nontoxic, and less likely to cause adverse effects than medications. Fumaria Indica (indica) a Herb belongs to family (Fumariaceae), was chosen to evaluate its Invitro uric acid lowering activity.

Objective: To evaluate the effect of Fumaria indica extract on xanthine oxidase activity in vitro.

Method: The study was designed to evaluate the xanthine oxidase inhibitory activity of Fumaria indica extract might inhibit xanthine oxidase in vitro. The inhibitory activity of xanthine oxidase in vitro was evaluated spectrophotometrically at 295nm using a UV/VIS spectrophotometer. The inhibitory concentration was estimated using the EZ fit enzyme kinetics tool.

Results: Fumaria indica dramatically reduced uric acid generation. FIE inhibited xanthine oxidase by 80% at 100 g/ml, but febuxostat inhibited it by 97.50% at the same concentration. The inhibitory concentration was determined using the EZ Fit enzyme kinetics test. FIE and febuxostat had IC50s of 12.59g/ml and 9.30g/ml, respectively.

Conclusion:The observed results indicate that fumaria indica extract could be a viable source for the development of novel XOI. FIE, based on its toxicity profile and uric acid reducing action, can be used to treat hyperuricemia and other hyperuricemia-related disorders, particularly gout.

Keywords: febuxostat, Fumaria indica ,Invitro, uric acid **.**

INTRODUCTION

A metabolic condition called hyperuricemia is brought on by increased uric acid levels, which can range from 6.6 to 7.6[1]. Both in industrialized and developing nations, the prevalence of hyperuricemia has increased recently on a global scale [2].Its prevalence in pakistan is 39% [3].Uric acid is oxidized by uricase in mammals and other animals to produce allantoin, a hydrophilic filtrate found in the kidney [4]. There is a uricase enzyme deficit in humans, This explains why hyperuricemia is more common in humans[5]. Hyperuricemia has become recognized as a metabolic disorder that poses a hazard to human health[6] . Its risk factors include cardiovascular disease, kidney disease, and stroke[7] One of the main risk factors for gout is hyperuricemia, an inflammatory condition brought on by the deposition of urate crystals. Urate crystal deposition in the joint and surrounding tissues causes hyperuricemia, an inflammatory state that is one of the main risk factors for gout[8,9].Fumaria Indica (F. indica) consists of 46 species in the world, distributed in Asia, Europe and Africa belongs to family (Fumariaceae)[10]. It is known as ''Shahtrah'' in Pakistan[11]. The plant has been used in traditional and folk medicine . The purpose of this study was to assess the antihyperuricemic activity of F. indica . Based on the phytochemical study of F. indica, it was decided to evaluate the plant's inhibitory effect on xanthine oxidase activity in vitro.

Hypothesis: F. indica extract has activity of xanthine oxidase in vitro

Objectives: To evaluate the impact of F. indica extract on the Invitro enzymic activity of xanthine oxidase

MATERIAL AND METHODS

Study Design and Settings: The experimental study, approved by the Ethical Review Committee and Advance Studies Research Board at the University of Health Sciences in Lahore, Ltr No.UHS/EDUCATION/126-17/2192,was conducted in March 2017 and completed in October 2017.

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Drugs and Reagents: Sigma Company provided xanthine (X7375-26G), xanthine oxidase (X1876-25U). Febuxostat purchased from Pharmaceutical company **.** Reagents and kits compatible with the Chemistry Analyzer (UA Kit, catalogue no., UA 230, Randox Laboratories Limited, United Kingdom) were purchased From the local market. Other chemicals and reagents used in our study were of pharmaceutical quality.

Plant Extract Preparation: The flowering shoots (F. Indica) were taken from the wheat fields in the vicinity of Lahore, last week of March . The plant was identified by the Botany Department Punjab University. Whole plant material was washed under running tap water for removal of dust.Plant material was dried under shade in the UHS Pharmacology Department lab for 10-12 days . Then it was broken in very little pieces . Dried plant of (500g) was macerated in 95% methanol for 48 hours. Whatman filter paper No.1 was used to filter the plant. It was concentrated by using rotary evaporator (Hei-Vap HL, Heidolph, Germany) under pressure at 35°C. The concentrated sample was dried at – 44˚C using lyophilizer (Alpha 1-2 LD Plus, Germany) and stored at 4˚C till further use. 5% by weight was Percentage yield. We labeled F. indica extract as FIE.

In vitro Xanthine Oxidase Inhibitory Activity Assay: Under aerobic conditions, a double beam Ultravoilet / Visible (UV/VIS) spectrophotometer (model UV-1602, Biotechnology Medical Services, Canada) was used to spectrophotometrically assess the inhibitory effect of FIE on xanthine oxidase (XO) activity at 295 nanometers (nm). Alsultanee reported this method and we followed it with some modifications[14]. A xanthine oxidase inhibitor (XOI), Febuxostat, was employed as the positive control. Febuxostat and FIE were produced at different concentrations starting at 100 micrograms per milliliter (µg/ml) and serially diluted up to 1.25 µg/ml. Dimethyl sulfoxide (DMSO) in the volume of 100 microliters (µl) was added to the reaction mixture to guarantee the solubility of febuxostat. The assay mixture consisted of1.6 ml potassium phosphate buffer (pH 7.5), 0.3 ml test sample solution (febuxostat or F. indica extract), 0.1 ml of enzyme solution prepared. Assay mixture was pre-incubated at 37°C for 15 minutes. In the mixture 1 ml freshly prepared substrate xanthine was added. The reaction mixture was incubated at 37°C for about 30 min. The reaction was stopped by adding 0.1ml of HCL. Freshly

prepared solutions were used for the assay. The absorbance was measured by spectrophotometer against a blank prepared in the same way but the enzyme solution (xanthine oxidase)was replaced with the phosphate buffer to know the absorbance of the components and ingredients other than uric acid. To have minimum uric acid formation, an additional reaction mixture (control) was prepared having 0.1 ml of distilled water instead of having test compounds (febuxostat or F. indica extract). In triplicate, the test samples were run. The mean and S.D were taken. According to the formula, the inhibition percentage of xanthine oxidase activity was calculated

a. $$\text{Percent xanthine oxidase inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control Absorbance}} \times 100.$$

Statistical Analysis: A free trial version of the EZ-Fit Enzyme Kinetics software was used. Michaelis Menten equation was applied to calculate inhibitory concentration 50% (IC_{50}),Km and Vmax.

RESULTS

F.indica FIE was evaluated for its potential inhibition of XO enzyme activity using an in vitro Xanthine Oxidase Inhibitory Assay.Freshly produced FIE concentrations ranging from 1.25 to 100 µg/ml were tested. XO enzymatic activity was reduced in a dosage-dependent manner durinthe graded dose response of FIE at varied doses. Table 1:Figure 1 summarize the XO inhibitory assay results.As a standard, febuxostat, a novel XO inhibitor, was used. At a maximal dosage of 100 µg/ml FIE, XO inhibition was 80%, while febuxostat inhibition was 97.50% (Fig: 1; Table 1). The lowest XO inhibition was seen at 1.25 µg/ml FIE concentration, while it was 12.98% with febuxostat (Fig. 1; Table 1).

Table1: Effect of FIE on xanthine oxidase inhibitory assay in vitro.

Concentrations	Percentage of XOI	
	Febuxostat %	FIE %
100µg/ml	97.50%	80%
80µg/ml	93.65%	72%
40µg/ml	91.12%	63%
20µg/ml	74.18%	54%
10µg/ml	50.60%	48%
5 µg/ml	45.10%	44%
2.5µg/ml	22.50%	34%
1.25 µg/ml	12.98%	18%
IC_{50}	9.30 µg/ml	12.599 µg/ml
Km	9.006 ± 0.942	3.95 ± 0.965
Vmax	106.616 ± 3.01	73.883 ± 4.036

The Lineweaver-Burk plot was developed to depict data graphically.

It represents FIE's lower efficacy than febuxostat.

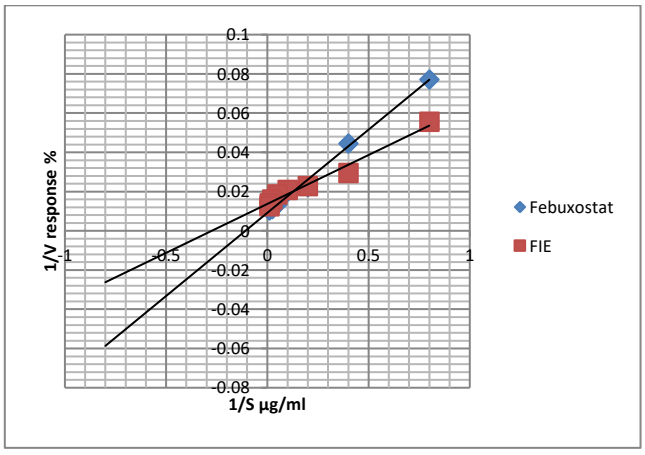

Figure 1: The Lineweaver-Burk plot was developed to depict data graphically.

DISCUSSION

The purpose of this study was to look into the anti-hyperuricemic properties of Fumaria indica (F. indica). It included invitro analysis. Because humans lack the uricase enzyme, they have greater uric acid levels than mammals. High uric acid levels can be caused by a high protein diet, alcohol consumption, or renal disease. In an animal model, fructose, a purine-rich diet, and yeast are utilized to induce hyperuricemia[15,16] .Urate-lowering medicines such as febuxostat, allopurinol, probenecid, and sulfinpyrazole are the cornerstone of hyperuricemia treatment, however they are associated with a higher risk of side effects [17,18],As a result, there is a tremendous possibility for new medicine development. .It was planned to investigate the effect of F. indica extract on xanthine oxidase activity..One of our objectives of research was to measure inhibitory activity of FIE on xanthine oxidase in vitro. Different dilutions of febuxostat were used to access xanthine oxidase inhibitions. Febuxostat were used as a standard drug for comparison in this study. The IC_{50} value of febuxostat and FIE for XO inhibitory assay was 9.30 µg/ml and 12.599µg/ml, respectively. This showed that febuxostat is more potent than FIE. Our findings are in conformity with Qurat et al. [20] . where IC_{50}values of febuxostat and allopurinol for XO inhibitory assay were8.77µg/ml and 9.07µg/ml, respectively. Febuxostat is more potent as XO inhibitor in vitro than allopurinol. But oxypurinol, a metabolite of allopurinol has non competitive inhibition, Febuxostat is a non-purine selective inhibitor of xanthine oxidase [21]. Basic alkaloids, coumarin, glycosides, steroids, quinones, flavonoids, antioxidants, and terpenoids have all been found in FIE by phytochemical study. Depending on the dose and type of substrate and inhibitor, the manner of inhibition of XO by flavonoids is both competitive and mixed.Considering that flavonoids and alkaloids are present in FIE we can speculate that the existence of these active components may be the cause of the XOI action of the FIE .

Recommendation for Further Studies: Novel drugs that target inhibition of XO in vitro and in vivo should be investigated, and their clinical safety as well as effectiveness should be evaluated in animal models to lead the way for more effective and safer herbal alternatives.

CONCLUSION

The observed results indicate that F.indica extract could be a viable source for the development of novel XOI. FIE based on its toxicity profile and uric acid reducing action, can be used to treat hyperuricemia and other hyperuricemia-related disorders, particularly gout.

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