ORIGINAL ARTICLE

Effect of *Mimosa Himalayana* Extract on Xanthine Oxidase Activity in Vitro

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ABSTRACT

Hyperuricemia is a disorder of purine metabolism. Xanthine oxidase inhibitors has central role in the treatment of chronic gout and hyperuricemia. Current xanthine oxidase inhibitors have multiple adverse effects which limit their prolonged use. Natural products are considered as an important source for the search of new drugs. *Mimosa himalayana* is a shrub that has been used in traditional medicine for the treatment of rheumatism, leucoderma, leprosy and fungal infections. In this study, in vitro xanthine oxidase inhibitory activity of *Mimosa himalayana* extract (MHE) was measured with the help of spectrophotometer. Inhibitory concentration 50% (IC_{50}) was calculated with EZ-Fit Enzymes Kinetics programme. *Mimosa himalayana* extract showed xanthine oxidase inhibitory activity with IC_{50} of 83.5 µg/ml while 9.21 µg/ml was IC_{50} observed with allopurinol.
Keywords: Hyperuricemia, xanthine oxidase inhibitory activity, spectrophotometer.

INTRODUCTION

Gout is painful inflammatory arthritis which can ultimately lead to poor quality of life. Hyperuricemia (HU) is a key factor in the development of gout (Hendriani et al., 2016). The prevalence of HU and gout has been increased in the past 4 decades (Ali et al., 2018). Uric acid is the final end product of purine catabolism. Hypoxanthine and xanthine are the intermediate products (Lima et al., 2015). Xanthine oxidase (XO) catalyses these reactions. Unlike lower primates, humans cannot oxidize uric acid, resulting in higher uric acid levels in them (Szczurek et al., 2017).

Uric acid levels in the human serum are maintained within normal range by the beauty of balance between production from the liver and excretion through kidneys and gut(Wang et al., 2016). Xanthine oxidase inhibitors and Uricosuric drugs are the mainstay of treatment for chronic hyperuricemia but adverse effects and drug interactions limit their use (Grosser and Fitzgerald, 2011).

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Natural products of plant origin have long been used in traditional medicine because of their potential beneficial effects. *Mimosa himalayana (M. himalayana)* is a large straggling deciduous shrub belonging to Leguminosae family (Flora of Pakistan, 2016). Traditionally, it has been used in the treatment of leucoderma, rheumatism, leprosy, fungal infections and for cuts & wounds (Nandipati et al., 2014). Its phytochemical analysis showed that it is rich in flavonoids like quercetin (65.38 %) and luteolin (2.5%) (Khan et al., 2012). Quercetin and luteolin possess hypouricemic effect and they have ability to inhibit XO (Huang et al., 2011).

In the light of composition of *M. himalayana* and limited data available on its xanthine oxidase inhibitory activity, it was planned to see the effect of MHE on XO activity in vitro.

MATERIALS AND METHODS

Preparation of Plant Extract: Fresh flowering shoots were collected, washed and shade dried. The crushed dried plant material was macerated in 95% ethanol for 48 hrs, then filtered and concentrated it by rotary evaporator under reduced pressure at 34˚C temperature. The concentrated *Mimosa himalayana* extract (MHE) was freeze dried at - 44ºC using

lyophilizer and stored at 4˚C temperature till further utilization (Valle et al., 2015).

In Vitro Xanthine Oxidase Inhibitory Activity Assay: The inhibitory effect on XO was measured spectrophotometrically at 295 nanometer (nm) with the help of double beam Ultravoilet / Visible (UV/VIS) spectrophotometer. Allopurinol, a well known XOI was used as a positive control for the inhibition test. Various concentrations of allopurinol and *M. himalayana* extract were made starting from 100 microgram per ml (µg/ml) in distilled water and serially diluted upto 5 µg/ml. The reaction mixture consisted of 1.8 ml of 50 milliMoles (mM) potassium phosphate buffer (pH 7.5), 0.1 ml of test sample solution (allopurinol or *M. himalayana* extract) and 0.1 ml of freshly prepared enzyme solution (0.2 units/ml of xanthine oxidase in phosphate buffer). The assay mixture was pre-incubated at 37°C for 15 minutes. Then, 1 ml of substrate solution (0.15 mM of xanthine) was added into the mixture. The mixture was incubated again at 37°C for 30 min. Next, the reaction was stopped with the addition of 0.1 ml of 0.5 M HCl. The absorbance was measured using UV/VIS spectrophotometer against a blank prepared in the same way but the enzyme solution was replaced with the phosphate buffer. Another reaction mixture was prepared (control) having 0.1 ml of distilled water instead of test compounds. The inhibition percentage of xanthine oxidase activity was calculated according to the formula

Percent xanthine oxidase inhibition =

$$\frac{\text{(Control absorbance – Sample absorbance)}}{\text{Control absorbance}} \times 100$$

(Bergmeyer et al.. 1974; Alsultanee et al., 2014)

Statistical analysis:

Mean ± Standard deviation (SD) was used to present the data. Inhibitory concentration 50 % (IC_{50}) for allopurinol and MHE was calculated with EZ-Fit Enzymes Kinetics programme.

RESULTS

In Vitro Xanthine Oxidase Inhibitory Activity: The results obtained showed that XO inhibition was in dose dependent manner. The results of MHE on XO inhibition activity are summarized in table no. 1. At 100 µg/ml concentration of MHE, XO inhibition was 66.1 % while allopurinol showed 88.8 % inhibition at the same concentration. This indicates that allopurinol is more potent as compared to MHE. The IC_{50} values for test samples were calculated by EZ Fit Enzyme Kinetic programme which were 9.21 µg/ml and 83.5 µg/ml for allopurinol and MHE respectively.

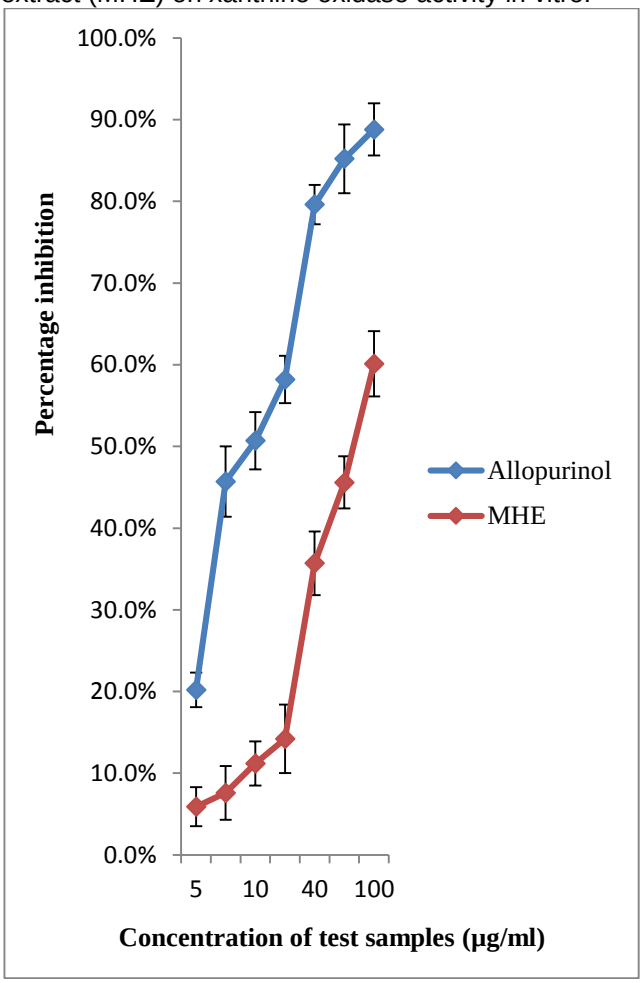
Table 1: Effect of *Mimosa himalayana* extract (MHE) on xanthine oxidase activity in vitro

Concentrations	Percentage of XO inhibition

	Allopurinol %	MHE %
5 µg/ml	20.20 ± 2.1	5.90 ± 2.4
7.5µg/ml	45.70 ± 4.3	7.60 ± 3.3
10 µg/ml	50.70 ± 3.5	11.20 ± 2.7
20µg/ml	58.20 ± 2.9	14.20 ± 4.2
40µg/ml	79.60 ± 2.4	35.70 ± 3.9
80 µg/ml	85.20 ± 4.2	45.60 ± 3.2
100 µg/ml	88.80 ± 3.2	83.5µg/ml
IC_{50}	9.21 µg/ml	**83.5µg/ml**

Results are expressed as Mean ± S.D (n=3).
Where IC_{50} is 50 % inhibitory concentration.

Figure 1: Inhibitory effect of *Mimosa himalayana* extract (MHE) on xanthine oxidase activity in vitro.



Results are expressed as Mean ± S.D (n=3)

DISCUSSION

The present study was carried out to investigate the xanthine oxidase inhibitory activity of MHE in search for substances that might have potential as alternatives to treat hyperuricemia and gout. XO inhibitors are the mainstay of treatment for hyperuricemia and gout (White, 2018). Allopurinol and febuxostat are currently available XO inhibitors with multiple adverse effects which limit their use (Sarvaiya et al., 2015). Medicinal plants are potential source for such compounds. Polyphenolic compounds including falvonoids have been reported to possess xanthine oxidase inhibitory activity (Al-Azzawie and Abd, 2015).

Xanthine oxidase inhibitory effect of MHE was observed with IC_{50} as 83.5 µg/ml. The inhibitory activity of MHE is attributed to its phenolic constituents (flavonoids). Many flavonoids are recognized as potent inhibitors of several enzymes including XO, cyclooxygenase, lipoxygenase and phosphoinositide 3-kinase (Panche et al., 2016). This explains the abovementioned XO inhibitory effect.

CONCLUSION

MHE possesses xanthine oxidase inhibitory activity in vitro with IC_{50} of 83.5 µg/ml. This result suggests the potential of *Mimosa himalayana* as xanthine oxidase inhibitor. Further researches should be carried out to find out the particular active constituents of the plant responsible for XO inhibitory activity. This will help to make an alternative drug to combat hyperuricemia and gout.

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Conflict of Interest: The authors declare no conflict of interest.

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