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Research Article

INVITRO ANTI-INFLAMMATORY ACTIVITY OF *MOMORDICA CHARANTIA* BY INHIBITION OF LIPOXYGENASE ENZYME

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ABSTRACT

The invitro anti-inflammatory activity of *Momordica charantia* was studied by inhibiting the action of lipoxygenase (LOX) enzymes. The anti-inflammatory activity of *Momordica charantia* extract (MCE) was assessed by assay of lipoxygenase activity at different time intervals, pH, temperature and different extract concentration. A protein free *Momordica charantia* extract (PFMCE) was also used to characterise the chemical component which is responsible for inhibition of lipoxygenase activity. In this study we observed that the plant extract had anti inflammatory activity as it inhibited the activity of lipoxygenase. The enzyme activity of 5-LOX, 12-LOX and 15-LOX was found to be 199.38, 289.82 and 198.36 U/ml respectively. The activity of LOX in the presence of plant extract was less when compare to control. The study also gave a clear picture that a protein is not responsible for the activity as removal of protein from the extract also gave the same result.

Keywords: Lipoxygenase, Anti-inflammation, *Momordica charantia,* Phytochemicals

INTRODUCTION

Inflammation is a normal protective response to tissue injury that is caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is the result of concerted participation of a large number of vasoactive, chemotactic and proliferative factors at different stages and there are many targets for anti inflammatory action [1]. The mechanisms of inflammation involve a serious of events in which the metabolism of arachidonic acid plays an important role. Prostaglandins are involved in the complex process of inflammation and are responsible for the pain[2]. It can be metabolised by the cyclooxygenase (COX) pathway to prostaglandins and thromboxane A_2, or by the lipoxygenase (LOX) pathway to hydroperoxy-eicosatertraenoic acids (HPETE'S) and leukotrienes (LT's), which are important biologically active mediators in a variety of inflammatory events [3, 4].

Upon appropriate stimulation of neutrophils, arachidonic acid is cleaved from membrane phospholipids and can be converted to leukotrienes and prostaglandins through 5-lipoxygenase (5-Lox) or cyclooxygenase (Cox) pathways respectively [5]. Many anti-inflammatory drugs (NSAIDs and corticosteroids) have been developed but their safety profile studies have shown that none of them is clearly safe [6]. They show wide ranges of adverse effects. Due to adverse reactions of synthetic and chemical medicines being observed round the globe, herbal medicines have made a comeback to improve our basic health needs. Many plants and herbs such as ginger, turmeric, olive oil, have been shown to exhibit potent anti-inflammatory effect.

The establishment of new invitro test systems has stimulated the screening of plants aiming to find leads for the development of new drugs. The plant lipoxygenase pathway is in many aspects the equivalent of the 'arachidonic acid cascades' in animals [7]. For this reason, the invitro inhibition of soybean lipoxygenase constitutes a good model for the screening of plants with anti-inflammatory potential [8].

Momordica charantia is a plant which belongs to Family Cucurbitaceae, it is a well known plant and widely distributed and cultivated in many parts of India. It is known as bitter gourds in English, pavakai in Tamil, karela in Hindi and Bengali, Karke in Marathi, and Kaippa or kaippa-valli in Malayalam [9]. All parts of the plant have a bitter taste including the fruits.

The fruits of the plant is widely used as vegetable as well as it is used in ayurvedic and unani system of medicines for the treatment of many diseases. The fruits and leaves of *M. Charantia* are useful in piles, leprosy, jaundice, diabetes, snake-bite and it is found to have vermifuge and antioxidant properties [10]. The earlier reports showed that the plant also has anti- malarial, anti-plasmodial properties [11, 12] and insecticidal activity against mustered saw fly [13]. The present study was carried out to investigate the anti-inflammatory potential of MEC by inhibition of lipoxygenase activity.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used were of analytical grade and obtained from sigma chemical company and used without further purification.

Plant material

Momordica charantia were collected from hosur, Tamilnadu, India. 10gms of plant was extracted with 0.2M sodium phosphate buffer (pH 6.5). The extract was filtered with cheese cloth and the filtrate was centrifuge for 10min at 16300g (4^o C). The precipitate was discarded and the supernatant was used for the study. The protein free extract of *Momordica charantia* was prepared by salting out method and used for study.

Phytochemical screening

The preliminary phytochemical screening of *Momordica charantia* was performed by the standard methods [14, 15&16].

Assessment of invitro anti-inflammatory activity

Preparation of crude Lipoxygenase

Commercially purchased soybean flakes were defatted and decolorized with cold acetone (-20^0C) and dried in a hood overnight. The enzyme was extracted from the dried powder by stirring it for 5 hr in 10 volumes of 50mM sodium phosphate buffer, pH 6.8, at 4^0C. The slurry was forced through two layers of cheese cloth and centrifuge at 16,000 rpm for 15min in a refrigerated centrifuge. The precipitate was discarded and the supernatant was used as an enzyme source.

Effect of MCE and PFMCE on LOX activity at different incubation times

Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxygenase as enzyme. MCE samples were dissolved in 0.25ml of 2M borate buffer pH 9.0 and added 0.25ml of lipoxygenase enzymes (5-LOX, 12-LOX and 15-LOX) solution and incubated for 5 min at 25^0C. After that, 1.0ml of linoleic acid solution (0.6mM) was added, mixed well and kept for various time intervals

at 25ºC; the product HPETE formed was measured at 234nm(Model 371 ,EI make). The specific activity was calculated by estimating the amount of protein by Lowry's method[17]. The same experiment was done by using PFMCE sample.

Effect of different concentration of MCE on LOX activity

Different volume of aliquots at the concentration range of 5, 10, 20, 50,75mg/ml was taken. 0.25ml of lipoxygenase enzyme solution was added and incubated for 5 min at 25^{0}C. After that, 1.0ml of buffered linoleic acid solution (0.6mM) was added, mixed well and kept for incubation for 15mins at 25ºC and absorbance was measured at 234nm and specific activity was calculated.

Effect of MCE on LOX Activity at different pH

Test sample was dissolved in 0.25ml of borate buffer at various pH ranges (pH 4-8) and added 0.25ml of lipoxygenase enzyme solution and incubated for 5 min at 25^{0}C. After that, 1.0ml of linoleic acid solution (0.6mM) was added, mixed well and incubated for 15mins at 25ºC and the absorbance was measured at 234nm and specific activity was calculated.

Effect of MCE on LOX Activity at different Temperature

Test sample was dissolved in 0.25ml of borate buffer and added 0.25ml of lipoxygenase enzyme solution and incubated for 5 min at 25^{0}C. After that, 1.0ml of linoleic acid solution (0.6mM) was added, mixed well and incubated for 15mins at various temperatures (5, 10, 20, 40, 60&80ºC) and the absorbance was measured at 234nm and specific activity was calculated.

RESULTS

Phytochemical analysis

The phytochemical analysis of MCE showed the presence of Alkaloids, Tannins, Steroids, and Glycosides (Table 1)

Table 1: Phytochemical screening of *Momordica charantia*

S. no	Constituents	Inference
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Phenols	-
5	Steroids	+
6	Glycosides	+
7	Tannins	+

Key: + = Present, - = Absent

Invitro anti-inflammatory activity

Invitro anti-inflammatory activity of *Momordica charantia* was studied by LOX assay. Enzyme activity of 5-LOX for control is 646.75, MCE is 199.38, and PFMCE is 196.40 U/ml (figure 1) MCE and PFMCE also showed inhibitory activity against 12 LOX and 15 –LOX and the enzyme activity was less when compared to the control and the values are presented in figure 2&3. The inhibitory activity was also studied at different concentration of plant extract, pH, and temperature. The enzyme activity was calculated for each parameter and is given in figure 4, 5, 6, 7 & 8. All the studies show that the plant extract has high inhibition on all LOX enzyme activity.

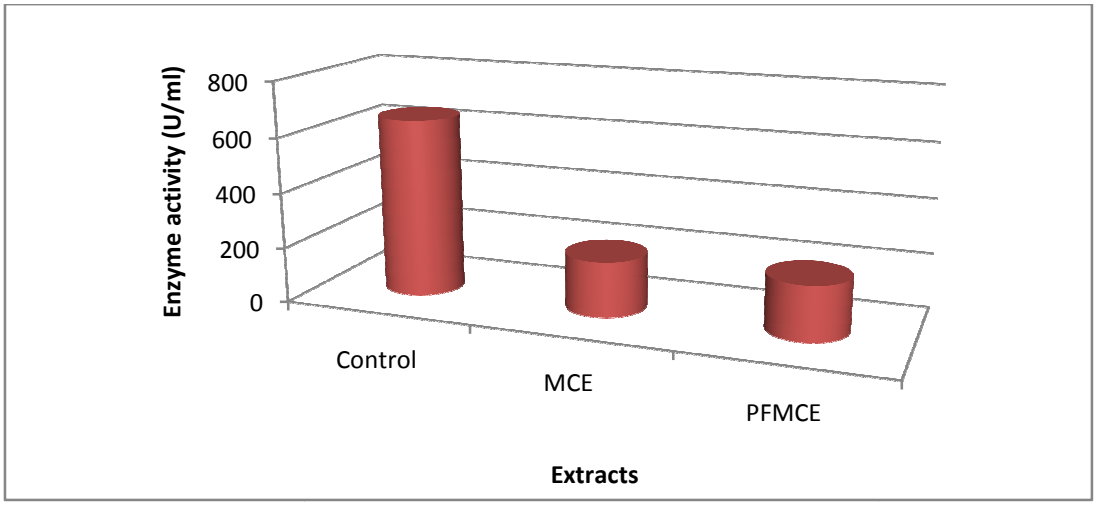

Fig. 1: Effect of MCE and PFMCE on the activity of 5-LOX

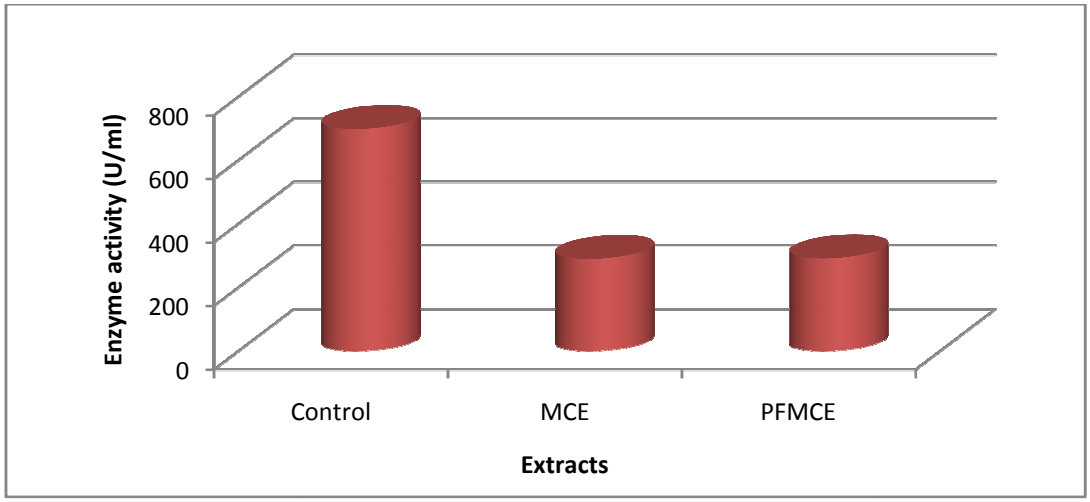

Fig. 2: Effect of MCE and PFMCE on the activity of 12-LOX

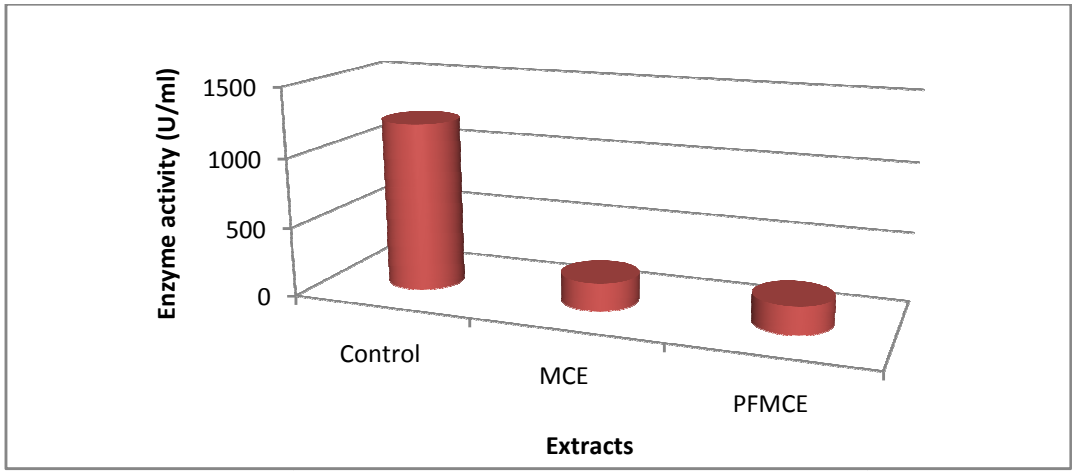

Fig. 3: Effect of MCE and PFMCE on the activity of 15-LOX

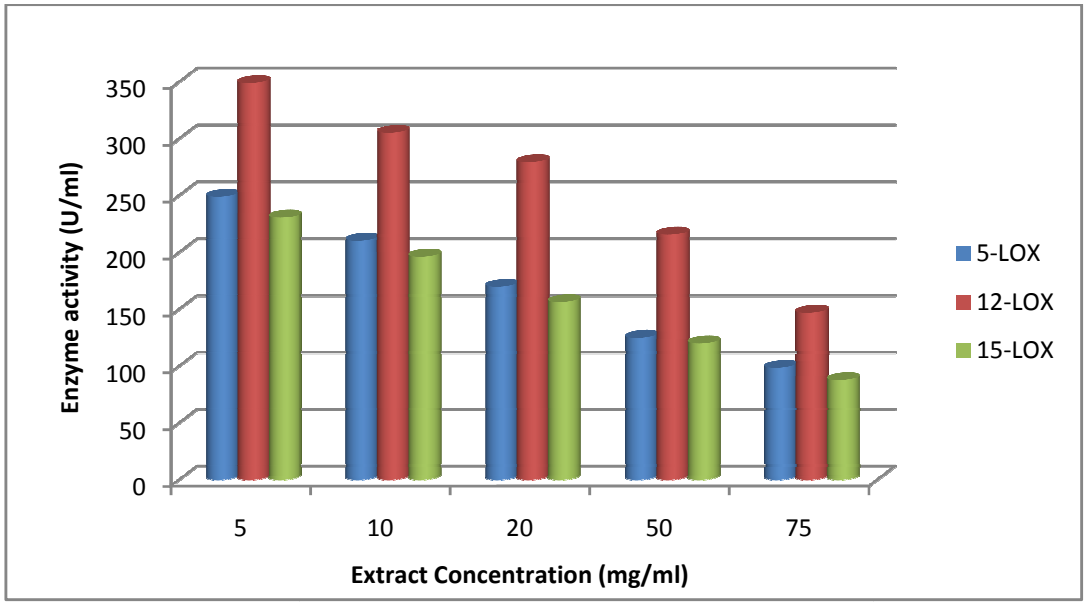

Fig. 4: Effect of different concentration of MCE on LOX activity

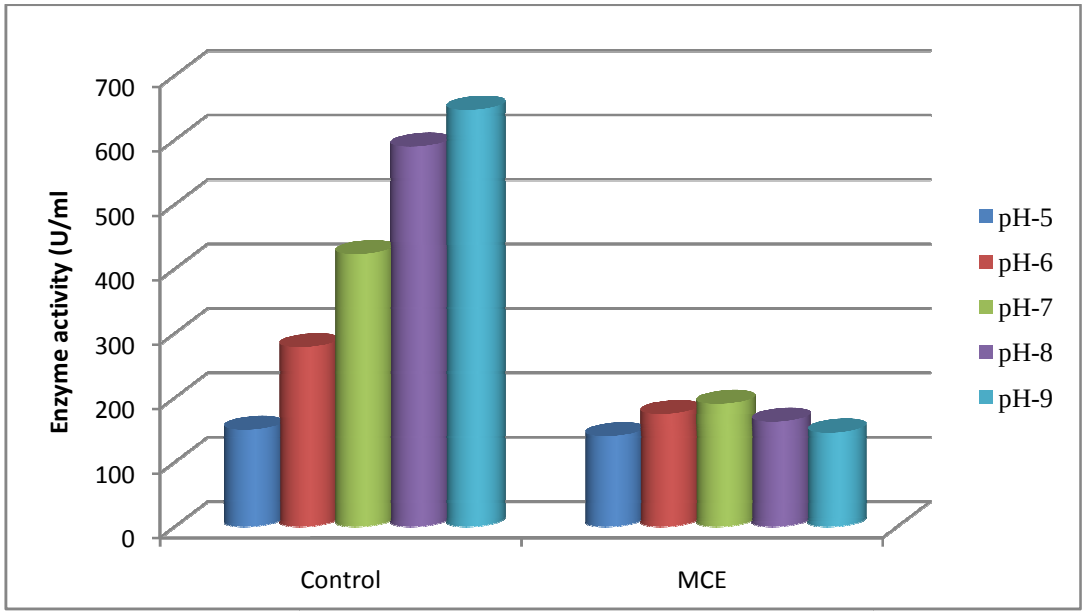

Fig. 5: Effect of MCE on 5-LOX Activity at different pH ranges

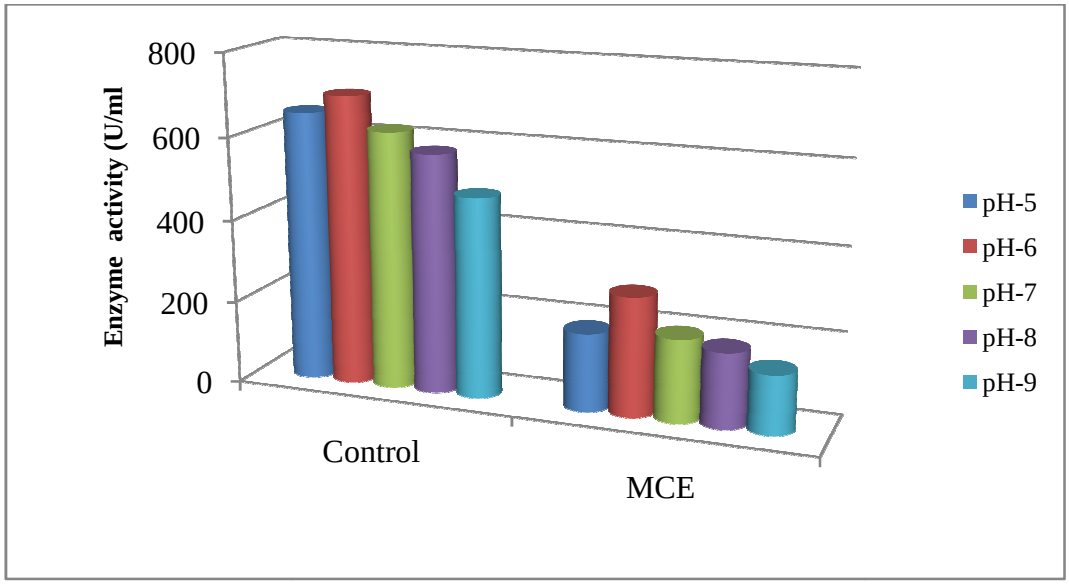

Fig. 6: Effect of MCE on 12-LOX Activity at different pH ranges

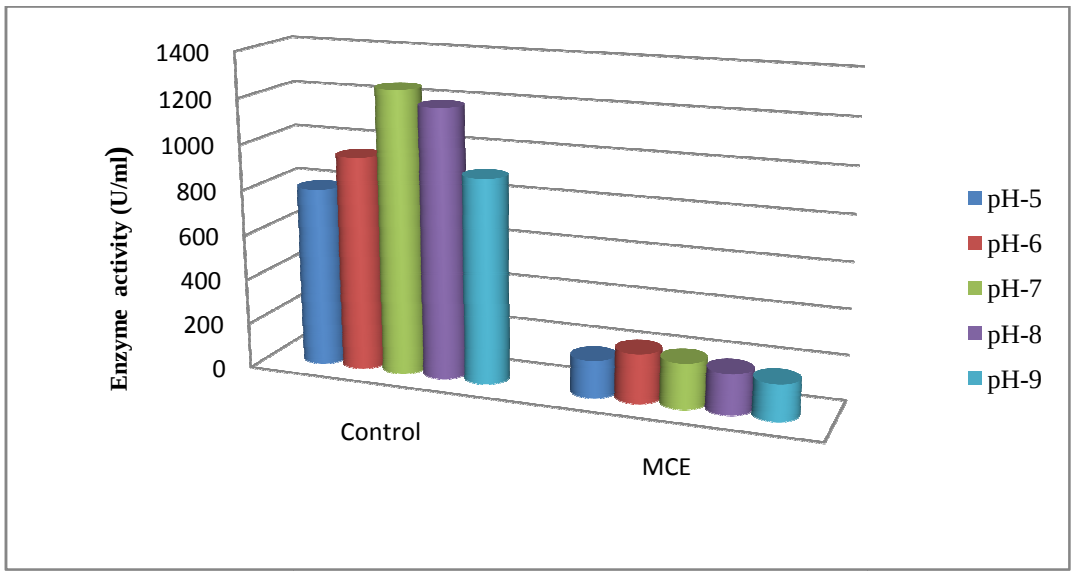

Fig. 7: Effect of MCE on 15-LOX Activity at different pH ranges

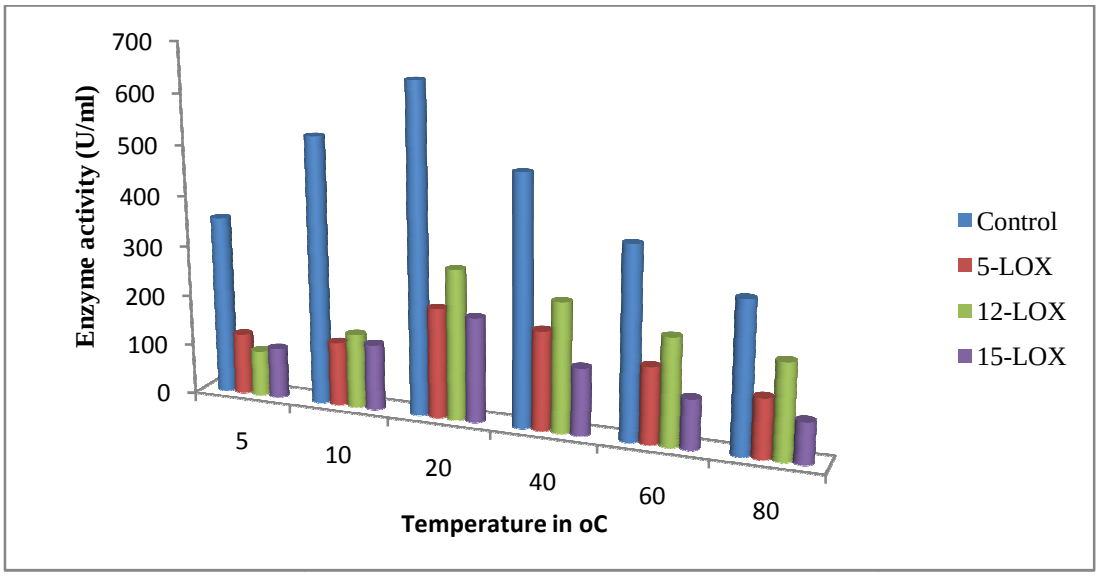

Fig. 8: Effect of MCE on LOX Activity at different Temperature

DISCUSSION

The present study was done by using the lipoygenase enzyme extracted from soybean seed. The inhibitory effect of *Momordica charantia* on the activity of the enzyme was studied as the enzyme has a same mechanisms of inflammation involve a series of events in which the metabolism of arachidonic acid. The enzyme in the presence of the plant extract showed a good inhibitory action at the parameters such as pH and temperature. The inhibition of the enzyme was directly proportional to the extract concentration. This shows that *Momordica charantia* has a good anti-inflammatory effect as reported in the previous study [18]. Previous studies have reported that *Momordica charantia* has good antioxidant activity. Antioxidants are known to inhibit plant lipoxygenases [19] and so we conclude presence of antioxidant in the study plant is responsible for the anti inflammatory activity. In our study we observed that the primary metabolite protein in the extract is not having much influence on lipoxygenase inhibitory activity and thus it was understood that some phytochemicals in *Momordica charantia* may have antioxidant nature which intern is responsible for anti-inflammatory activity.

CONCLUSION

The present study showed less LOX activity in the presence of the study plant extract than the control. Thus we identified that the plant extract was responsible for the inhibition of LOX activity and we concluded that the non protein components of *Momordica charantia* has anti inflammatory activity.

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