Research Article

In-vitro Anti-inflammatory Activity of *Mimosa pudica* Against Inhibition of Protein Denaturation and Heat induced Haemolysis Methods

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Received: 14-06-2023; **Revised:** 22-08-2023; **Accepted:** 30-08-2023; **Published on:** 15-09-2023.

ABSTRACT

The present study was executed to evaluate the In-vitro anti-inflammatory activity of aqueous extract of whole part of *Mimosa pudica* against inhibition of protein denaturation & heat induced haemolysis methods. Six different concentrations of *Mimosa pudica* aqueous extract and Paracetamol solution (31.25 µg/ml, 62.5 µg/ml, 125.5 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml) were made and incubated with egg albumin and 10% RBC solution in standard experimental conditions and it is taken to determine the absorbance to obtain the anti-inflammatory activity. Paracetamol was used as a standard drug. In both protein denaturation & heat induced haemolysis methods, the effect of paracetamol was found to be less effective when compared with the plant extract. Hence from the results, protein denaturation method at concentration of 1000 µg/ml extract (81.12%) showed maximum anti-inflammatory activity compared to the Heat Induced Haemolysis methods at concentration of 1000 µg/ml extract (67.51%). From the current study it can reveals that the presence of different phytochemicals such as alkaloids, flavonoids, tannins, phenols and glycosides are responsible for the maximum anti-inflammatory activity against protein denaturation &heat induced haemolysis methods.

Keywords: *Mimosa pudica*, anti-inflammatory, protein denaturation, Paracetamol, Heat-induced haemolysis.

INTRODUCTION

Inflammation is an immune response to tissue injury, infection or other cell destruction by different factors like physical trauma, noxious substances or microbial agents and hampered the biological functions[1]. Inflammation is characterized by swelling, pain, heat, redness on the skin etc. It is the body's normal immune response to immobilize or suppress the invading microorganisms to remove and starts the tissue repairing process. During inflammation the body's immune system is triggered to release the various allergic mediators from the damaged cells. It is also reported that there are lyses of lysosomes are produced which will release their enzymes to produce various disorders. Protein denaturation and RBC membrane lysis are well established causes of inflammatory disease. The commonly used drug for the treatment of inflammatory conditions are NSAID, which is having a various adverse drug reactions like irritation of GIT, which causes gastric ulcer[2]. The *in-vitro* anti-inflammatory activity of plant extracts was determined by protein denaturation of egg albumin and heat-induced haemolysis method. Now a days Natural products have shown a significant result towards the invention of new drugs. The rich natural sources can illustrate a pioneer source of newer compounds with notable anti-inflammatory activity. *Mimosa pudica* also known as Lojjabati (Fabaceae) is used in this research work to evaluate the anti-inflammatory activity. The plant is native of tropical America and naturalised nearly all through the tropical and subtropical parts of India[3]. It is also having several important medicinal uses throughout the world. The previous researchers have found some pharmacological properties on *Mimosa pudica* but the current research was conducted to evaluate the possible *in-vitro* anti-inflammatory activity against protein denaturation of egg albumin and heat-induced haemolysis method.

MATERIALS AND METHODS

Plant material

The whole plant of *Mimosa pudica* (Family: Fabaceae) were collected from the surrounding of Gandhinagar, Haldia, District Purba Medinipur, West Bengal. The authentication of the plant was done by Scientist-in-charge, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal. The Whole plant of *Mimosa pudica* was collected and powdered by grinder after shade drying process and kept it in a air-closed container for further studies[4].

Drugs and chemicals

Paracetamol was collected from the chemistry lab of Haldia Institute of Pharmacy, Hatiberia, Haldia, East Medinipur, WB, 721657. All the other chemicals were obtained from the college laboratory with laboratory grade. Double distilled water was used throughout the whole study.

Preparation of extract

The powder plant material (100 g) was dissolved in 800 ml distilled water and kept for boiling for 45 minutes as per decoction process. After boiling solution kept for 2-3 hours for cooling it. After cooling filter the whole solution and kept the filtrate in glass bottle. Then the solvent was evaporated to dryness by using a water bath at 56°C temp to get the yield of the dry extract (Yield: 3.8%). The dry

extract was kept in a well closed container for further use[5-8].

Evaluation of *in vitro* anti-inflammatory activity

Inhibition of protein denaturation

The 5 ml of reaction mixture was comprised of 0.2 ml of eggs albumin, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying conc. of extracts so that the final concentration becomes 31.25 µg/ml, 62.5 µg/ml, 125.5 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml. Similar volume of double distilled water served as a control. Then the mixture was incubated at 37 °C in incubator for about 15 mins and then heated at 70 °C for 5 mins. After cooling, their absorbance was measured at 660 nm by using pure blank. Paracetamol (standard drug) at the final conc. of (31.25 µg/ml, 62.5 µg/ml, 125.5 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml) and was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula mentioned below:

% Inhibition = $[(V_c – V_t)/V_c] \times 100$,

Where, V_c = Absorbance of Control

V_t = Absorbance of Test/sample

Heat Induced haemolysis

The 2 ml of reaction mixture is consisted of 1 ml of test extract at various concentrations (31.25 µg/ml, 62.5 µg/ml, 125.5 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml) and 1 ml of 10% RBCs suspension, instead of drug only saline was added to the control test tube. Paracetamol was taken as a standard drug. All the centrifuged tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. % of membrane stabilization activity was calculated by the formula mentioned below:

% Inhibition = $[(V_c – V_t)/V_c] \times 100$,

Where, V_c = Absorbance of Control

V_t = Absorbance of Test/sample.

IC_{50} value was calculated by plotting the % inhibition with respect to the control against treatment concentration.

RESULTS AND DISCUSSION

In the current study the in vitro anti-inflammatory activity of *Mimosa pudica* was studied against inhibition of protein denaturation & heat induced haemolysis methods. The results are illustrated in table 1 & 2. This present study shows a concentration dependent inhibition of protein (Albumin) denaturation and heat induced haemolysis method by using *Mimosa pudica* extract throughout the concentration range of 31.25 to 1000 µg/ml. Paracetamol was used as a standard drug throughout the concentration range of 31.25 to 1000 µg/ml which also exhibit a concentration dependent inhibition. Although, the effect of paracetamol was found to be less by both methods as compared to *Mimosa pudica* plant extract which is illustrated in figure 1 & 2. In case of inhibition of protein denaturation & heat induced haemolysis methods the aqueous extract of *Mimosa pudica* shows IC_{50} value 192.12 µg/ml & 382.79 µg/ml whereas the IC_{50} value of Paracetamol shows 435.42 µg/ml & 520.32 µg/ml respectively. A statistical comparative study was also done for two methods and illustrated in fig 3 & 4. There are several problems in using experimental animals in pharmacological research, such as ethical permission and the lack of resoning for their use where other methods are also available to execute the study[9-10]. Here, the current studies inhibition of protein denaturation & heat induced haemolysis methods were selected to evaluate in vitro anti-inflammatory activity of aqueous extract of *Mimosa pudica.* Protein denaturation is a common cause of inflammation & arthritis. Destruction of RBC by heat is also a sign of inflammation. Researchers should focus on the anti-inflammatory drug discovery process to prevent the protein denaturation[11-12].

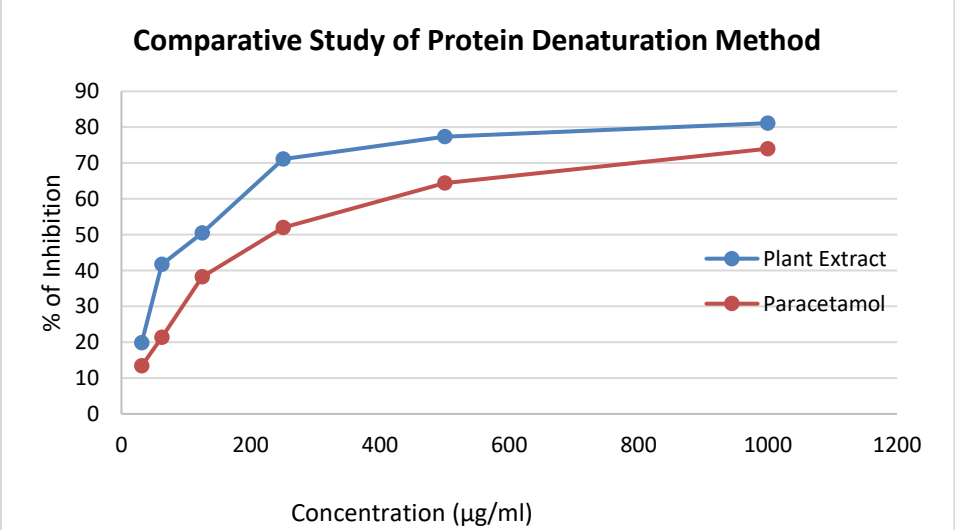

Figure 1: Effect of *Mimosa pudica* extract & PCM on protein denaturation method

Table 1: Effect of aqueous extract of *Mimosa pudica* & PCM on protein denaturation

Conc. (µg/ml)	Absorbance	% Inhibition	Conc. (µg/ml)	Absorbance	% Inhibition
Control	0.14	-	Paracetamol	0.14	-
31.25	0.15	19.83	31.25	0.15	13.38
62.5	0.21	41.7	62.5	0.17	21.32
125	0.22	50.49	125	0.22	38.21
250	0.44	71.1	250	0.28	51.95
500	0.53	77.36	500	0.36	64.42
1000	0.68	81.12	1000	0.50	73.99

Table 2: Effect of aqueous extract of *Mimosa pudica* & PCM on heat-induced haemolysis

Conc. (µg/ml)	Absorbance	% Inhibition	Conc. (µg/ml)	Absorbance	% Inhibition
Control	0.148	-	Paracetamol	0.18	-
31.25	0.20	20.75	31.25	0.21	18.88
62.5	0.27	37.15	62.5	0.24	26.43
125	0.32	46.93	125	0.29	41.96
250	0.37	54.02	250	0.35	51.11
500	0.42	61.10	500	0.39	56.65
1000	0.49	67.51	1000	0.41	61.46

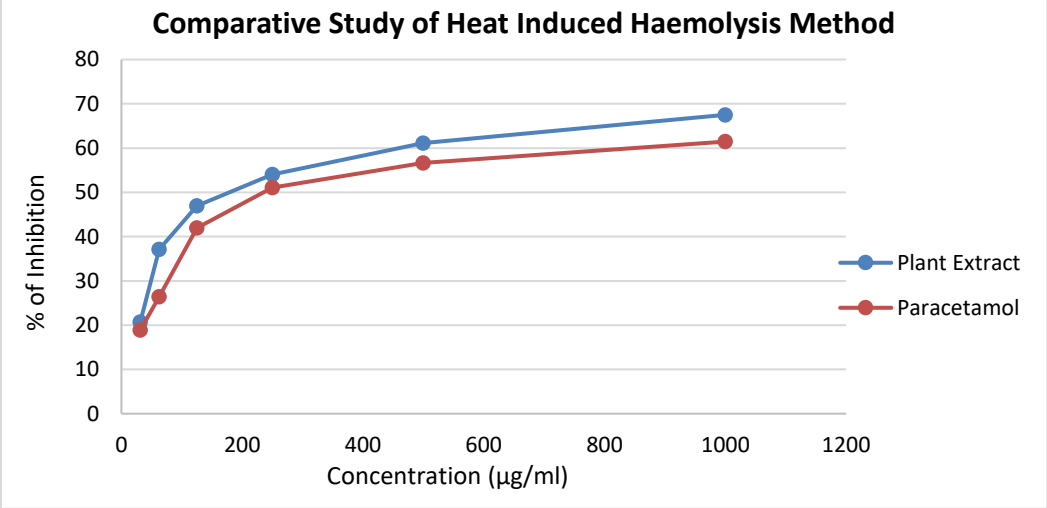

Figure 2: Effect of *Mimosa pudica* extract & PCM on Heat induced haemolysis method

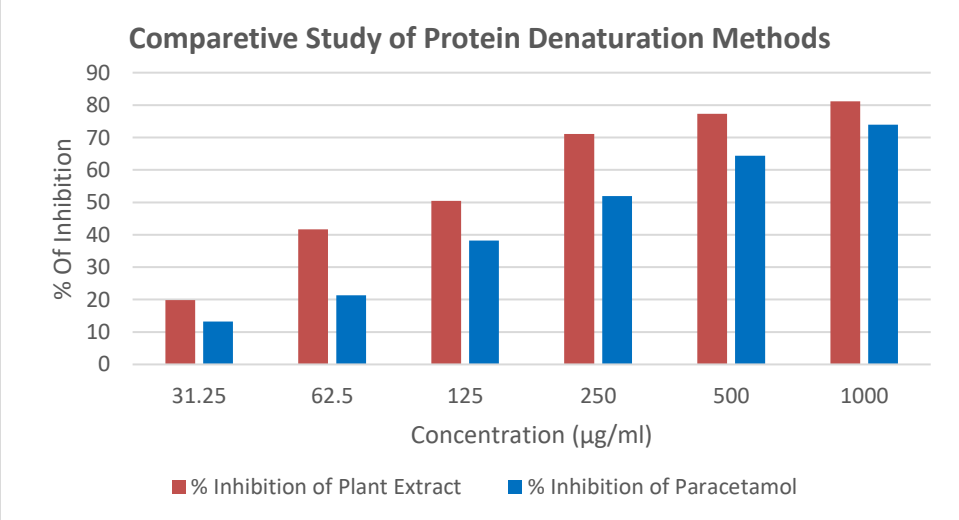

Figure 3: A comparative study of Protein denaturation method

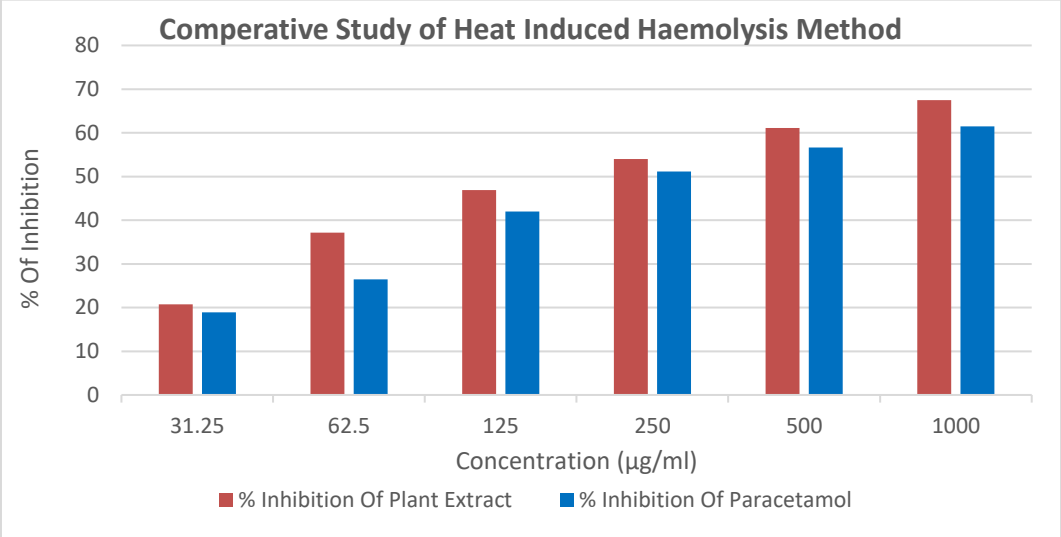

Figure 4: A comparative study of Heat induced haemolysis method

CONCLUSION

The increase of absorbances of test sample compared to reference drug paracetamol shows that it is having strong anti-inflammatory activity. From the IC_{50} values it can be said that the aqueous extract of *Mimosa pudica* is having a strong anti-inflammatory activity, more than the reference drug paracetamol. The main constituents of *Mimosa pudica* are alkaloid, Flavonoids, Tannins etc which is having several biological activity. Therefore, from the results of the current research we can draw a conclusion that the *Mimosa pudica* is having a strong anti-inflammatory activity. Further studies will be done to know the mechanism and which constituent is responsible for anti-inflammatory activity.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.