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Research Article

IN-VITRO ANTI-ARTHRITIC ACTIVITY OF METHANOLIC EXTRACT OF MOMORDICA CHARANTIA FRUIT

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ABSTRACT

The present study was aimed at the investigation of anti-arthritic activity in methanolic extract of M. charantia. The anti-arthritic activity of M. charantia Methanolic Fruit Extract was done by inhibition of protein denaturation and human red blood cell membrane stabilization (HRBC) in-vitro methods. The methanolic extract of M. charantia was subjected to in-vitro inhibition of protein denaturation in various concentrations i.e. 10, 20, 40, 80, 100 and160µg/ml. HRBC method was also used for the estimation of anti-arthritic activity from in various concentrations 10, 20, 40, 80, 100 and 160 µg/ml. In the results M. charantia Methanolic Fruit Extract exhibited a concentration dependent both in-vitro models (inhibition of protein denaturation& stabilization of HRBC membrane) showed a concentration dependent anti-arthritic activity, and the protection percent increased with increase in the concentration of the M. charantia Methanolic Fruit Extract. So, in the conclusion the present study is support to the isolation and use of phytoconstituents from fruit M. charantia in the treatment of arthritis.

Keywords:*Anti-arthritic, Human red blood cell membrane stabilization, Inhibition of protein denaturation, M. charantia, Phytoconstituents.*

INTRODUCTION

Momordica charantia or bitter melon, also known as balsam pear or karela, is a tropical vegetable, is a common food in Indian cuisine and has been used extensively in folk medicine as a remedy for diabetes. It is a very common herb having various medicinal properties for the treatment of different kind of disease, viz. antifungal, wound healing and antidiabetic agents [1]. Bitter melon has been used in various Asian traditional medicine systems for a long time [2].

Momordica charantia is an edible plant, which widely cultivated all around the world especially in tropical areas including part of Amazon, East Africa, Asia, and Caribbean [3]. For a long time, several workers have studied the effects of this plant in DM. Treatment with *M. charantia* fruit juice reduced blood glucose levels, improved body weight and glucose tolerance. *M.charantia* fruit juice can also inhibit glucose uptake by the gut and stimulate glucose uptake by skeletal muscle cells. Moreover, the juice of this plant preserves islet β cells and β cell functions, normalizes the systolic blood pressure, and modulates xenobiotic metabolism and oxidative stress [4].

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In the present work was undertaken to check the potential of *M.charantia* fruit in the treatment of arthritis.

MATERIALS AND METHODS

Collection and authentication of plant

The fruit of *M. charantia* were collected from the local market of Jaipur in April 2015. A voucher specimen (Voucher no. RUBL211526) was kept at the Department of Botany, University of Rajasthan, Jaipur after identified and authenticated of the plant.

Preparation of m. charantia methanolic fruit extract

The fruits were dried in room temperature without exposure of sun light and were crushed with the help of mortar-pestle then powdered mechanically through mesh sieve number 20. Powdered fruits were soxhleted with methanol (95%) as solvent for 9-10 hours at the pharmacognosy department. The methanolic extract was concentrated to dryness in an evaporator under reduced pressure and controlled temperature (50– 60°C) to obtain the dry crude extract [5].

Chemicals

Diclofenac sodium was used. Other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

EXPERIMENTAL PROCEDURE

Inhibition of protein denaturation method: The following procedure was followed for evaluating the percentage of inhibition of protein denaturation:-

- **Preparation of solutions**
- **Bovine albumin solution:** was prepared by dissolving 5gm of bovine albumin in 100ml of distilled water.
- **Phosphate buffer (ph 6.4):** 1.79gm Disodium hydrogen phosphate, 1.36gm Potassium hydrogen phosphate and 7.02gm of Sodium chloride were dissolved in 1000ml of distilled water.
- **Stock solution of mcmfe:** was prepared by dissolving 0.3gm of extract in 100ml of methanol.
- **Stock solution of reference / standard drug:** was prepared by dissolving 0.3gm of Diclofenac sodium in 100ml of methanol.
- **Preparation of control solution (5ml):** consists of 0.2 ml of egg albumin (from fresh hen's egg) and 2.8 ml of phosphate buffered saline (PBS, pH 6.4) in 5 each test tubes.
- **Preparation of standard solution:** consists of 0.2 ml of egg albumin (from fresh hen's egg) and 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentrations of standard drug (Diclofenac sodium) so that final concentrations become 10, 20, 40, 80 ,100 & 160µg/ml.
- **Preparation of test solution:** consists of 0.2 ml of egg albumin (from fresh hen's egg) and 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentrations of plant extract (Momordica charantia Methanolic Fruit Extract) concentrations of 10, 20, 40, 80, 100 & 160µg/ml.

Then the mixtures were incubated at 37 ± 2^0C in an incubator for 15 minutes and then heated at 70^0C for 5 minutes. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank. The percentage inhibition of protein denaturation was calculated by using the following formula:

Percentage of inhibition = 100X [Vt / Vc – 1]

Where, Vt= absorbance of test sample, Vc = absorbance of control [6].

- **Human red blood cell membrane stabilization method**
- **Preparation of solutions**
- ***Preparation of Normal Saline:*** was prepared by dissolving 0.9gm sodium chloride in 100ml of distilled water.
- **Preparation of 10%v/v red blood cells (rbcs) suspension*:*** Fresh whole human blood (10ml) was collected and transferred to the heparin zed centrifuged tubes. The tubes were centrifuged at 3000rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

- **Stock solution of mcmfe*:*** was prepared by dissolving 0.1gm of extract in 100ml of methanol.
- **Stock solution of reference / standard drug:** was prepared by dissolving 0.1gm of Diclofenac sodium in 100ml of methanol.
- **Preparation of control solution (5ml):** consists of 2ml of normal saline in 5 each test tubes.
- **Preparation of test solution:** consists of 1 ml of 10% RBCs suspension and 1ml of varying concentrations of plant extract (*Momordica charantia* Methanolic Fruit Extract) concentrations of 10, 20, 40, 80, 100 & 160µg/ml.
- **Preparation of standard solution:**consists of 1 ml of 10% RBCs suspension and 1ml of varying concentrations of standard drug (Diclofenac sodium) so that final concentrations become 10, 20, 40, 80 ,100 & 160µg/ml.

All the centrifuged tubes containing reaction mixture were incubated in a water bath at 56^0C for 30 minute. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5 min and the absorbance of the supernatants was taken at 560nm [6]. The experiment was performed in triplicates. Percent membrane stabilization was calculated by the formula: [7]

% protection = [100- (optical density sample/optical density control)] × 100

RESULTS

Inhibition of protein denaturation method

The effect of *Momordica charantia* Methanolic Fruit Extract on inhibition of protein denaturation and membrane stabilization is shown in Table I. *Momordica charantia* at different dose levels (10, 20, 40, 80, 100 and 160µg/ml) provided significant inhibition against denaturation of proteins and hypotonic saline induced RBC membrane damage [6]. Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis [7]. The result is compared with Diclofenac sodium (standard) in Figure 1 [8]. Diclofenac sodium was used as standard drug which at different concentrations (10 to 160µg/ml) showed inhibition of protein denaturation. The effect of MCMFE was found to be more than Diclofenac sodium in Figure 1 [9].

Table I: In-vitro activity of MCMFE and Diclofenac sodium by inhibition of protein denaturation method.

Group	*Concentration (µg/ml)*	*% inhibition*
MCMFE	Control	0.248
	10	32.25806452
	20	110.8870968
	40	183.0645161
	80	259.6774194
	100	292.3387097
	160	565.3225806
Diclofenac Sodium	*Concentration (µg/ml)*	*% inhibition*
	Control	0.248
	10	18.14516129
	20	86.69354839
	40	137.5
	80	195.1612903
	100	259.6774194
	160	472.5806452

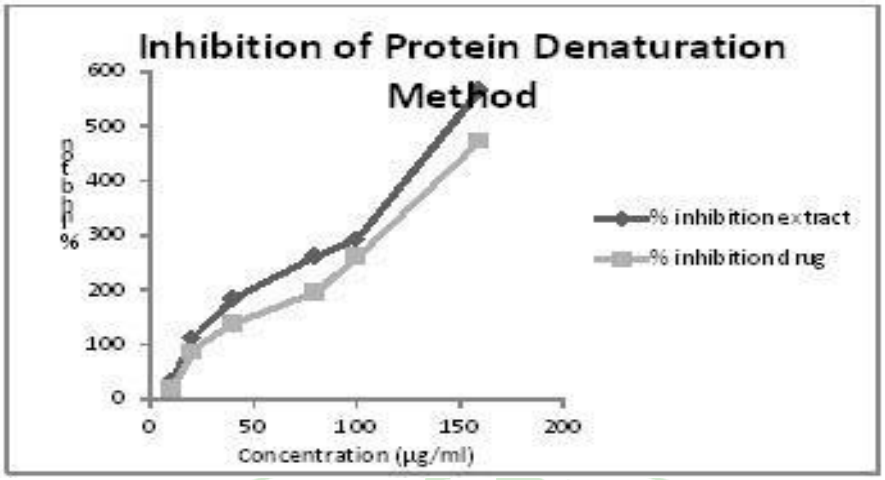

Figure 1: In-vitro anti arthritic activity by inhibition of protein denaturation method.

Membrane stabilization method

HRBC membrane of MCMFE at different concentrations (10 to 160µg/ml) also exhibited stabilization towards HRBC membrane [7]. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula:-

Percentage of Protection = 100 X [Vt/Vc – 1]

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The effect of MCMFE was found to be more than Diclofenac sodium. The results are summarized in Table II. From the result of the present study, various fractions of the fruit of *Momordica charantia* were studied to *in-vitro* anti-inflammatory activity in various concentrations i.e. 10, 20, 40, 80, 100 and 160µg/ml and the percentage stabilization of different extract of fruit of *Momordica charantia* by HRBC membrane stabilization is depicted in Table II.

In the present investigation, extract showed positive response and dose dependent response. This effect may be due to presence of steroids, alkaloids and flavonoids present in various fractions.

From the result, it can be stated that the *Momordica charantia Methanolic Fruit Extract* are capable of controlling the production of auto antigen and thereby it inhibit the denaturation of proteins and its effect was compared with the standard drug Diclofenac sodium. The entire drug exhibited dose dependent response.

Table II: In-vitro activity of MCMFE and Diclofenac Sodium by HRBC Method

Group	*Concentration (µg/ml)*	*% protection*
MCMFE	Control	0.328
	10	28.04878049
	20	94.81707317
	40	145.1219512
	80	171.9512195
	100	200.304878
	160	275
Diclofenac Sodium	Control	0.328
	10	11.2804878
	20	64.02439024
	40	121.9512195
	80	151.2195122
	100	184.7560976
	160	229.2682927

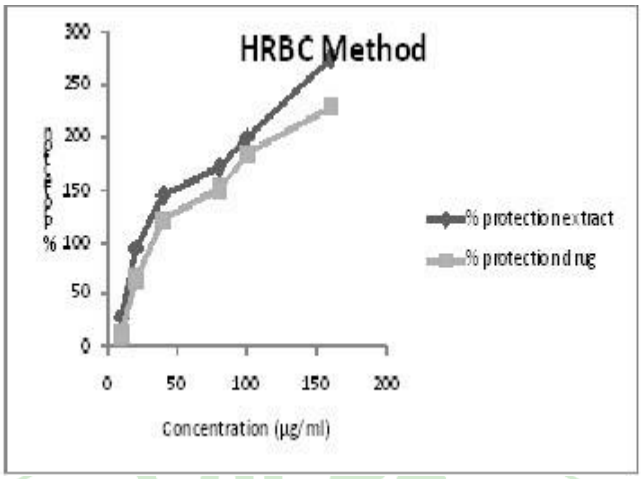

Figure 2: *In-vitro* activity by HRBC Method

DISCUSSION

The anti-arthritic effect of *Momordica charantia Methanolic Fruit Extract* (MCMFE) was evaluated against the denaturation of egg albumin *in- vitro* [8]. In the present study the protein denaturation bioassay was selected for *in-vitro* assessment of anti-arthritic property of *Momordica charantia Methanolic Fruit Extract*. The incredible development in the field of synthetic drugs during present era is accompanied by numerous undesirable side effects. Whereas plants still hold their own unique place, with lesser side effects. For the preliminary study, two *in-vitro* models i.e. inhibition of protein denaturation and HRBC membrane stabilization were selected. Both are well established model for screening of anti-inflammatory and anti-arthritic activity [9].

Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. Production of auto antigens in certain rheumatic diseases may be due to *in-vitro* denaturation of proteins [10]. HRBC method was selected for the *in-vitro* evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by MCMFE and reference drug Diclofenac sodium. Presence of MCMFE prevented this, implying inhibition of protein denaturation [10].

From the results of the present study it can be stated that all the synthesized compounds were screened for their anti-arthritis activity and the effect of all the test compounds was found to be very good. Stabilization of RBCs membrane was studied to further establish the mechanism of anti-inflammatory action of extract of *Momordica charantia*. Extract effectively inhibited the heat induced hemolysis[11]. The results provide evidences for membrane stabilization effect of the selected plant drug as an additional mechanism for their anti-inflammatory effect [11].

Further clinical studied are needed to establish its safety and usefulness in arthritic patient [12].

CONCLUSION

During the study, *in-vitro* methods (Inhibition of protein denaturation and human red blood cell stabilization) have been selected. For experimental observation it can be concluded that, the methanolic extract of *Momordica charantia* showed in the both cases (increase in inhibition of protein denaturation as well asin human red blood cell stabilization method), compared to standard (Diclofenac sodium) throughout the concentration from

low to high. From the results obtained in the study, so it may concluded that this plant have anti-arthritic activity. The *in-vitro* studies on fruit *Momordica charantia* showed the presence of significant anti-arthritic activity. The methanol extract shows more anti-inflammatory and anti-arthritic activities. The activity may be due to the presence of steroids, flavonoids and alkaloids.So that the MCMFE showed anti-arthritic activity but by comparing the results of the plant extract in both *in-vitro* models, it can be stated that MCMFE has more potent anti-arthritic activity than standard drug. Future aim is to isolate the chemical constituents responsible for the anti-arthritic activities.

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