*The Pharmaceutical and Chemical Journal*, 2016, 3(2):173-177

*Available online www.tpcj.org*

**Research Article**

**ISSN: 2349-7092**
**CODEN(USA): PCJHBA**

# Evaluation of anti-arthritic activity of *Momordica charantia* root by *in-vitro* models

**Lekhni Soni[1], Rupesh K Gautam[1*], Prateek K Jain[2]**

[1]Department of Pharmacology, [2]Department of Pharmaceutical Chemistry, ADINA Institute of Pharmaceutical Sciences, Bhopal Road, Sagar (MP), India-470002
*Corresponding author –Email: drrupeshgautam@gmail.com

**Abstract** The present study is aimed to evaluate the anti-arthritic activity of ethanolic extract of *Momordica charantia* root (EEMR) by two *in-vitro* models *i. e.* human red blood cell (HRBC) membrane stabilization and inhibition of protein denaturation. The standard drug was diclofenac sodium. The results of both models showed concentration dependent inhibition of protein (egg albumin) denaturation as well as stabilization towards HRBC membrane. On the basis of present findings, it can be concluded that EEMR showed anti-arthritic activity due to presence of phytocompounds such as flavonoids, alkaloids, tannins etc.

**Keywords** Anti-arthritic activity, *Momordica charantia*, Karela, HRBC.

## Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease with chronic inflammation characterized by hyperplasia of synovial cells and angiogenesis in affected joints, which ultimately leads to the destruction of cartilage and bone. RA is a long lasting disease that can affect joints in any part of the body, most commonly the hands, wrists, and knees. RA affects three times more women than men [1-2]. RA is believed to be a T lymphocyte driven disease in which a sudden influx of T cells into the affected joint(s) is followed by an increased number of fibroblasts and macrophages, drawn by the release of cytokines, particularly interleukin-1(IL-1) and tumor necrosis factor alpha (TNF-α). This cytokine release and subsequent migration of cells is thought to be responsible for the chronic inflammation and characteristic destructive alteration in rheumatoid joints [3].

*Momordica charantia* (Family: Cucurbitaceae), is commonly known as bitter gourd or bitter melon in English and karela in Hindi [4]. It is a climber, widely cultivated as food in Asia, Africa and South America. It is also found all over India and cultivated up to an altitude of 1500 m. The word *Momordica* is derived from the Latin word *Mordeo* which means to bite and the species name is derived from Greek word and it means beautiful flower [5]. *M. charantia* is very useful as antidiabetic, anti-inflammatory, antioxidant, antitumor, antiulcer, hypoglycemic, immunostimulant etc [6]. Our research group has already reported the anti-arthritic activity of fruit of *M. charantia* [7]. The root of *M. charantia* is useful in arthritis [8]. On the basis of traditionally use; we have selected root part of *M. charantia* for the present study.

## Materials and Methods

### Plant Materials

*Momordica charantia* roots were collected from Sanjay Nursery, Mohara, Sagar (M.P.). The root was identified and authenticated by Dr. Archana Verma, Head, Department of Botany, Govt. Girl's Degree College, Sagar (M.P.).

### Preparation of Extract:

The roots of *M. charantia* were powdered mechanically through mesh sieve. The powdered plant parts were extracted with solvent ethanol by continuous hot percolation method using soxhlet apparatus. The filtrate of the extracts was concentrated to dryness [9].

**Phytochemical screening**
Preliminary photochemical screening of EEMR was carried out by previous established procedures [10].

***In-vitro* anti-arthritic activity**
The following models have been performed—
**Human red blood cell (HRBC) membrane stabilization method** [11]
**Preparation of reagents**
2 gm dextrose, 0.8 gm sodium citrate, 0.05 gm citric acid and 0.42 gm sodium chloride were dissolved in distilled water. The final volume was made up to 100 ml with distilled water. This mixture was used as Alsevers solution. Hypotonic saline was prepared by dissolving 0.36 gm of sodium chloride in 100 ml of distilled water. Isotonic saline was prepared by dissolving 0.85 gm of sodium chloride in 100 ml of distilled water. 2.38 gm disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8 gm of sodium chloride were dissolved in 100 ml of distilled water. This was served as phosphate buffer (pH 7.4, 0.15 M).
**Preparation of suspension (10% v/v) of human red blood cell (HRBC)**
The blood was collected from healthy human volunteer who had not taken any NSAID'S for 2 weeks prior to the experiment and was mixed with equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the study.

**Assay of membrane stabilizing activity**
The assay mixtures contains 1ml of phosphate buffer, 2 ml of hypo saline and 0.5 ml of HRBC suspension and 0.5 ml different concentrations of extract, reference sample and control were separately mixed.
1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of plant extract of various concentration (100, 200, 400, 800 and 1600 µg/ml) and 0.5ml of 10% w/v human red blood cells were used as test solution. 1ml of phosphate buffer and 2ml of water and 0.5ml of 10%w/v human red blood cells in isotonic saline were served as test control.1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of standard drug (Diclofenac sodium) of various concentration (100, 200, 400, 800 and 1600 µg/ml) and 0.5ml of 10% w/v human red blood cells were taken as standard solution. All the assay mixtures were incubated at 37ºc for 30 min. and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the hemolysis produced in control as 100%. [15,16] The percentage of HRBC membrane stabilization or protection was calculated by using the following formula-
**Percentage protection**: - 100- [(optical density sample/optical density control) × 100]

**Inhibition of protein denaturation method:** [12]
2ml of egg albumin (from fresh hen's egg), 28 ml of phosphate buffer (PBS, pH 6.4) and 20ml distilled water were used as control solution (50 ml). 2ml of egg albumin, 28 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) (10, 50, 100, 200, 400, 800, 1000 and 2000µg/ml) were served as standard drug solution (50 ml). 2ml of egg albumin, 28 ml of phosphate buffer and various concentrations of plant extract (10, 50, 100, 200, 400, 800, 1000 and 2000 µg/ml) were taken as test solution (50 ml).
All of the above solutions were adjusted to pH, 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using UV-Visible spectrophotometer at 660nm and their viscosity was determined by using Ostwald viscometer. The percentage inhibition of protein denaturation was calculated using the following formula-
**Percentage inhibition** = (Vt/Vc-1) x 100
Where, Vt = absorbance of test sample, Vc = absorbance of control.

**Results**
**Phytochemical screening**
Preliminary photochemical screening of EEMR showed the presence of flavonoids, alkaloids, carbohydrates, protein and tannin.
**Human red blood cell (HRBC) membrane stabilization method**
Both diclofenac sodium and EEMR at different concentrations (100 to 1600 µg/ml) showed stabilization towards HRBC membrane. The effect of EEMR was found to be less than standard drug (diclofenac sodium). The results are summarized in Table 1 and 2.

**Table 1:** Effect of diclofenac sodium on HRBC membrane stabilization

| S. No. | Concentration µg/ml | %Protection |
|---|---|---|
| 1 | 100 | 21.76 |
| 2 | 200 | 43.60 |
| 3 | 400 | 59.59 |
| 4 | 800 | 71.48 |
| 5 | 1600 | 86.85 |

**Table 2:** Effect of EEMR on HRBC membrane stabilization

| S. No. | Concentration µg/ml | % Protection |
|---|---|---|
| 1 | 100 | 11.64 |
| 2 | 200 | 18.56 |
| 3 | 400 | 26.61 |
| 4 | 800 | 38.95 |
| 5 | 1600 | 52.29 |

**Inhibition of protein denaturation method**

Both diclofenac sodium and EEMR at different concentrations (10 to 2000 µg/ml) showed inhibition of protein denaturation. The effect of EEMR was found to be less than diclofenac sodium. The results are summarized in Table 3 and 4.

**Table 3:** Effect of diclofenac sodium against protein denaturation

| S No. | Concentration (µg/ml) | %Inhibition | Viscosity (Cps) |
|---|---|---|---|
| 1 | Control | - | 1.41 |
| 2 | 10 | 18.08 | 0.85 |
| 3 | 50 | 58.66 | 0.86 |
| 4 | 100 | 172.99 | 0.94 |
| 5 | 200 | 247.07 | 0.97 |
| 6 | 400 | 302.44 | 0.99 |
| 7 | 800 | 454.63 | 1.02 |
| 8 | 1000 | 624.92 | 1.06 |
| 9 | 2000 | 813.25 | 1.14 |

**Table 4:** Effect of EEMR against protein denaturation

| S. No | Concentration (µg/ml) | %Inhibition | Viscosity (Cps) |
|---|---|---|---|
| 1 | Control | - | 1.41 |
| 2 | 10 | 14.50 | 0.81 |
| 3 | 50 | 39.59 | 0.83 |
| 4 | 100 | 112.55 | 0.87 |
| 5 | 200 | 221.67 | 0.91 |
| 6 | 400 | 285.72 | 0.96 |
| 7 | 800 | 398.71 | 0.99 |
| 8 | 1000 | 518.24 | 1.01 |
| 9 | 2000 | 626.18 | 1.04 |

**Discussion**

Rheumatoid arthritis is a chronic, inflammatory, autoimmune disorder affecting about 1% of adults worldwide. In recent years, natural products are becoming an important area of interest for the development of new therapeutic entities due to their lower cost and higher safety [13]. For the present study two *in-vitro* models *i. e.* human red blood cell (HRBC) membrane stabilization and inhibition of protein denaturation were used and both models are well established model for screening of anti-inflammatory and anti-arthritic activity.

HRBC method was selected for the *in-vitro* evaluation because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [14].

Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo*. The increments in absorbance of test sample with respect to control indicate stabilization of protein *i.e*. inhibition of protein (albumin) denaturation by plant extract (EEMR) and standard drug diclofenac sodium [15]. This anti-denaturation effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on denaturation. However, the viscosities were found to decrease with simultaneous decrease in concentration of test extract (EEMR) and standard drug as well. Although, the viscosities of the test samples of all concentrations were always less than that of control. Even so, the viscosity data indicated inhibition of protein (albumin) denaturation [16-17].

Literature revealed that *Momordica charantia* contains a range of biologically active phytocompounds including triterpens, proteins, steroids, alkaloids, saponins, flavonoids etc. [18]. Phytochemical investigation of EEMR also showed presence of flavonoids, alkaloids, carbohydrates, protein and tannin. This is well known that flavonoids, alkaloids, tannins are responsible for significant anti-arthritic and anti-inflammatory activity. The presence of these phytocompounds in extract may be responsible for anti-arthritic activity of EEMR.

## Conclusion

On the basis of present findings, it can be concluded that ethanolic extract of *Momordica charantia* root (EEMR) showed anti-arthritic activity due to presence of phytocompounds such as flavonoids, alkaloids, tannins etc. Further *in-vivo* study is in pipe-line for supporting the present investigation.

## Acknowledgements

The authors would like to acknowledge to Dr. Sunil K. Jain, Director and Shri Rajesh Jain, Chairman, ADINA Group of Institutions, Sagar (M.P.) for their constant support and providing necessary facilities to carry out this study.

## References

1. Gautam RK, Singh D, Nainwani R. Medicinal Plants having Anti-arthritic Potential: A Review. *International Journal of Pharmaceutical Sciences Review and Research*, 2013; 19(1):96-102.
2. Soni L, Gautam RK, Jain PK. Medicinal Plants of Anti-arthritic Potential: A Review. *International Journal of Applied Pharmaceutical and Biological Research*, 2016; 1(2): 260-67.
3. Gautam RK, Nainwani R. Herbal Anti-arthritic Agents: An Overview. *Inventi Impact: Planta Activa*, 2014; 2014(3):168-73.
4. Grover JK, Yadav SP. Pharmacological Actions and Potential Uses of *Momordica charantia:* A Review. *Journal of Ethnopharmacology*, 2004, 93:123–132.
5. Desai S, Tatke P. Charantin an Important Lead Compound from *Momordica charantia* for the treatment of diabetes. *Journal of Pharmacognosy and phytochemistry* 2015, 3(6):163-166.
6. Kumar DS, Sharathnath KV, Yogeswaran P, Harani A, Sudhakar K, Sudha P, Banji D. A Medicinal Potency of *Momordica charantia. International Journal of Pharmaceutical Sciences Review and Research*, 2010, 1(2):95-100.
7. Shivali, Arora P, Gautam RK, Singh D, Chaudhary SK. *In-vitro* Anti-arthritic activity of methanolic extract of *Momordica charantia* Fruit. *Asian Journal of Pharmaceutical Research and Development*, 2016; 4(2):1-6.
8. Ahmad N, Hasan N, Ahmad Z, Zishan M, Zohrameena S. *Momordica charantia*: for Traditional Uses and Pharmacological Actions. *Journal of Drug Delivery and Therapeutics*, 2016; 6(2):40-44
9. Kokate CK, Purohit AP, Gokhle SB. *Pharmacognosy*, 46th Edition, Nirali Prakashan, Mumbai, 2010, 6.23.
10. Khandelwal KR. *Practical Pharmacognosy Technique and Experiments*, Nirali Prakashan, Pune, 2007, 149-53.
11. Gautam RK, Sharma S, Sharma K. Comparative Evaluation of Anti-arthritic Activity of *Salvadora persica* Linn. and *Asparagus racemosus* Willd: An *In-vitro* Study. *Indo American Journal of Pharmaceutical Research*, 2013; 3(10):8222-7.
12. Gautam RK, Sharma S, Sharma K. Comparative Evaluation of Anti-arthritic Activity of *Pongamia pinnata* and *Punica granatum*: An *In-vitro* Study. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2013; 5 (Supp.4):721-4.

13. Choudhary M, Kumar V, Gupta PK, Singh S. Anti-Arthritic Activity of *Barleria Prionitis* Linn. Leaves in Acute and Chronic Models in Sprague Dawley Rats. *Bulletin of Faculty of Pharmacy, Cairo University*, 2014, 52: 199–209.
14. Azeem AK, Dilip C, Prasanth SS, Shahima VJS, Kumar S, Naseera C. Anti-inflammatory Activity of the Glandular Extracts of *Thunnus alalunga*. Asian Pacific *Journal of Tropical Medicine,* 2010; 794-6.
15. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Preliminary *In-vitro* Assessment of Anti-inflammatory Property of *Mikania scandens* Flower Extract. *Journal of Advanced Pharmacy Education & Research*, 2012; 2(1): 25-31.
16. Chandra S, Dey P, Bhattacharya S. Evaluation of *In-vitro* Anti-inflammatory Activity of Coffee against the Denaturation of Protein. *Asian Pacific Journal of Tropical Biomedicine*, 2012; S178-S180.
17. Anson ML, Mirsky AE. The Effect of Denaturation on the Viscosity of Protein Systems. *Gen Physiol*, 1932; 15: 341-50.
18. Gupta M, Sharma S, Ajay K. Gautam, Bhadauria R. *Momordica Charantia linn*. (karela): Nature's Silent Healer. *International Journal of Pharmaceutical Sciences Review and Research*, 2011, 11(1), 32-37.