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RESEARCH ARTICLE

In-vitro Anti-arthritic Activity of *Manilkara zapota* Linn

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ABSTRACT:
The present study is aimed to evaluate the leaf extract of *Manilkara zapota* for acclaimed anti-arthritic activity using *in-vitro* inhibition of protein denaturation model. Acetyl salicylic acid was used as a standard drug. Results revealed that the ethanolic extract of *Manilkara zapota* at two different concentrations (100mcg/ml and 250mcg/ml) possessed significant anti-arthritic activity as compared to standard used drug acetyl salicylic acid. The plant extract showed dose dependent activity.

KEYWORDS: *Manilkara zapota*, Anti-arthritic activity

INTRODUCTION:
Rheumatoid arthritis is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage.[1] It is a common disease having peak incidence in 3rd to 4th decades of life with 3-5 times higher preponderance in female.[2] Its prevalence depends upon age.[3] Herbal drugs constitute a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity.[4] The factors responsible for the continued and extensive use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and shortage of practitioners of modern medicine in rural areas.[5] Number of synthetic medicines has been derived from medicinal herbs.[6] The sapodilla is large, evergreen, forest tree more than 30 m in height and with a diameter up to 1.5 m, under cultivation it varies between 9 and 15 m, depending on location, and generally does not exceed 50 cm in diameter. The gummy latex of sapodilla, called chicle, is used to make chewing gums, and the fruit is used to treat diarrhea and pulmonary diseases. [7] The crushed seeds are claimed to expel bladder and kidney stones and effective in rheumatism.[8] Leaf decoction used for fever, hemorrhage, wounds and ulcers. [9] Hence, the present study was undertaken to evaluate *in vitro* antiarthritic activity of plant extract.

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MATERIALS AND METHODS:
Plant material:
The leaves of plant *Manilkara zapota* were collected from local area of Bhopal, M.P., India, and authenticated by Mr Anil Prakash, department of Biotechnology, Barkatullah university, Bhopal, M.P. where a voucher specimen No. has been submitted. (Voucher specimen No. 28 SAP)

Preparation of plant extract:
Collected leaves of *Manilkara zapota* were converted into moderately coarse powder and extracted with solvent ethyl alcohol for 27 hours by soxhlet. The solvent was removed under reduced pressure and the yield of extract was calculated.

ASSESSMENT OF *IN VITRO* ANTI-ARTHRITIC ACTIVITY:
Inhibition of protein denaturation method:[10]
The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Manilkara zapota* extract (100 and 250 mcg/ml of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCl. The samples were incubated at 37˚C for 20 min and then heated at 57˚C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm for control test 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

Percent inhibition=
100- (O.D. of test – O.D. of product control) X 100

$$\frac{}{\text{O D.. of control}}$$

The control represents 100% protein denaturation. The results were compared with acetyl salicylic acid (250 mcg/ml) treated samples.

Statistical Analysis:
Data are presented as the mean ± SEM of each triplicate test. The analysis was performed by using Dunnett vs. Control test and by ANOVA. P‘0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION:

Anti-arthritic effect of ethanolic extract of *Manilkara zapota* was studied significantly by using *in-vitro* inhibition of protein denaturation model. The effect of ethanolic extract of *Manilkara zapota* on inhibition of protein denaturation is shown in table 1. Extract of *Manilkara zapota* at two different concentrations (dose levels) provided significant protection against denaturation of proteins. Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. Obtained data stated that *Manilkara* could be used as potent anti-arthritic agent.

Table 1 Effect of ethanolic extract of *Manilkara zapota* on inhibition of protein denaturation

S. N.	Groups	Protein denaturation (%)
1	*Manilkara zapota* (100 mcg/ml)	58.89 ± 6.29
2	*Manilkara zapota* (250 mcg/ml)	75.84 ± 2.31
3	Acetyl salicylic acid	51.25 ± 7.14

Values (Mean ± SD) are presented in comparison to control and standard.

CONCLUSION:

From the results obtained in the present study, it may be concluded that *Manilkara zapota* possess significant anti-arthritic activity. Hence it could be beneficial for further work as active anti-arthritic agent.

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