

**EFFECT OF HYDROALCOHOLIC EXTRACT OF *PSIDIUM GUYAVA LINN.*
ON COMPLETE FREUND'S ADJUVANT INDUCED ARTHRITIS IN
LABORATORY ANIMALS**

Kirti Jahagirdar, Pinaki Ghosh, Shaikh .M. Adil, Ziyaurrahman A.R.*

Department of Pharmacology, M.C.E. Society's, Allana College of Pharmacy,
Azam Campus, Camp, Pune-01, India.

Summary

The objective of the study was to investigate the anti arthritic activity of the hydroalcoholic extract of the leaf of *Psidium guyava linn* against complete Freund's adjuvant induced arthritis in laboratory rats.

Arthritis was induced in male wistar rats by administration of complete Freund's adjuvant in the sub plantar region of the hind paw. Diclofenac sodium (4 mg/kg/day p.o) was used as the standard drug. The hydroalcoholic extract of *Psidium guyava* (HEPG) was administered at the following doses 50, 100, 200mg/kg/day p.o. The following parameters were measured: change in paw volume, body weight, diameter of the tibiotarsal joint and total leukocyte count in the blood.

The results demonstrate that hydroalcoholic extract of *Psidium guyava linn.* at a dose of 200 mg/kg/day p.o showed significant anti arthritic activity.

Keywords: *Psidium guyava linn*; HEPG; Complete Freund's adjuvant induced arthritis; Immunomodulatory activity.

Abbreviations: HEPG hydroalcoholic extract of *Psidium guyava*, CFA complete Freund's adjuvant

*Author for correspondence:

Ziyaurrahman A.R.,
H.O.D. Pharmacology,
Allana College of Pharmacy,
Azam Campus, Camp, Pune - 400 001, India.
E-mail: rahman92@reddifmail.com
Tel. No. : +91-20-26442074,
Fax No.: +91-20-26442074

Introduction

Rheumatoid arthritis is a chronic, progressive, systemic autoimmune disease causing inflammatory erosion of synovial joint ultimately culminating to joint destruction, deformity, and disability. It affects nearly 1% of the population of the world (1). The pathogenesis in this disease has been attributed to the development of the autoantibodies which infiltrate the synovial joint leading to degradation of structural macromolecules in connective tissue and proteoglycans present in the cartilage of the joint(2).

Psidium guyava leaf linn belongs to family *myrtacea*. the plant is commonly known as amrud ki patti in hindi. It is a low evergreen tree or shrub 6 to 25 feet high, with wide-spreading branches and square, downy twigs, is a native of tropical America. It is a common vegetation cover by roads and in waste places in Hawaii. This tree is also cultivated nearly all over India and in common in Bengal (3).

All the parts of plant have medicinal uses. In folk medicine, the plant is reportedly used in treating epileptic convulsions, mental illnesses and hemicranias (4). The whole herb is used in ayurvedic preparation to treat epilepsy and mental disorders (5). It is used to treat vitiated conditions of hemicranias, jaundice, hepatopathy, Antibacterial activity, Anti-inflammatory effect, Antispasmodic, CNS activit, Conjunctivitis y, Coughs, Diabetes, Kidney Problems, Malaria, Rheumatism, Vaginal disorder. It is also used as a nerving tonic. The oil prepared using the plant root and bark is reportedly useful in astringent, febrifuge, antiseptic. The external application of a paste of this herb is beneficial in treating pruritus and edema, arthritis, filariasis, gout, and cervical adenopathy. It also treats piles and hepatitis (6).

The hydroalcoholic extract showed potent anxiolytic activity against various animal models of anxiety (16). It also showed immunomodulatory activity in vitro (17, 18).

Hence, we designed the present study to unravel the immunomodulatory and anti arthritic activity of the hydroalcoholic extract. Complete Freund's adjuvant induced arthritis which mimics the human pathophysiological state was used as the animal model to investigate the activity of hydroalcoholic extract of *Psidium guajava* (HEPG) in laboratory rats.

Materials and methods:

Collection of plant material

The leaves of *P.guajava* were collected at Loni village of Poona, Maharashtra. They were authenticated by Dr. J. Jayanthi Scientist.'C' (Head Dept.of botany), NO.BSI/WRC/Tech/2010/. (V.NO=PSIGADS1). Botanical Survey of India, Pune. A voucher specimen was deposited at the herbarium in the Botanical survey of India.

Preparation of extract

The powdered plant material (500 g) was soaked in 10 % alcohol (2000 ml) and allowed to stand for 48 h, with occasional shaking. The macerate was decanted and filtered, through cloth, and then, through Whatman filter paper (No.1). This process of extraction was repeated with the same volume of hydroalcohol. The macerates was pooled and evaporated. The residue, called 'marc', (after the extraction with hydroalcohol) was dried and extracted with alcohol (10%) by the same procedure to yield a reddish brown semisolid. The extract of hydroalcoholic was stored in air-tight glass bottle, at room temperature.

Preparation of dosage form

The emulsion of hydroalcoholic extract (HEPG) and diclofenac sodium suspension was prepared with 0.25% polysorbate 80 (Tween 80) in a glass mortar, with the gradual addition of water for injection (WFI), to make up the required volume.

Animals Used

Male albino rats of Wistar strain weighing 230-250 gms was procured from National Toxicological centre, Pune for the present study. The animals were housed in groups of 4 in solid bottom polypropylene cages. They were maintained at $24\pm 1^{\circ}\text{C}$, with relative humidity of 45- 55% and 12:12 h dark/light cycle. Acclimatization period was two weeks. The animals have free access to food (Standard chow pellets, Chakan Oil Mills, Sangli) and water, *ad libitum*. The acute toxicity protocol was approved by Institutional Animal Ethics Committee (IAEC) of Allana College of Pharmacy, Pune. Approved the pharmacological and acute toxicity protocol.

Acute Toxicity Testing

The acute oral toxicity study was carried out as per the guideline set by the Organization for Economic Co-operation and Development (OECD) received from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Acute toxicity study was performed in female Swiss albino mice. The extracts were administered orally (i.p.) at doses of 500, 1000, 2000, 5000 mg/kg. They were then observed for signs of toxicity, continuously for 2 h, and for mortality up to 24 h, after injection.

Induction of arthritis

Arthritis was induced by a single intra dermal injection of Freund's complete adjuvant (CFA) containing 1.0 mg dry heat killed *Mycobacterium tuberculosis* per milliliter sterile paraffin oil into the sub plantar region of the foot pad of the left hind paw of male rats (19).

1 ml tuberculin syringe with 26 gauge needle was used to administer the Freund's complete adjuvant. The rats were anesthetized with light ether inhalation prior to and during adjuvant injection.

Experimental setup

Animals were divided into five groups of 6 animals in each group as follows:

Group 1: Control group (1ml of 0.25% tween 80/day).

Group 2: Diclofenac Sodium (4 mg/kg/day in 0.25% Tween 80)

Group3: HEPG 50 (50 mg/kg/day in 0.25% tween 80)

Group4: HEPG 100 (100 mg/kg/day in 0.25% tween 80)

Group5: HEPG 200 (200mg/kg/day in 0.25% tween 80)

AIEC Approval

The proposed work has been approved by IAEC.

Approval No: CPCSEA 13/9/2009.

Assessment of arthritis

The progression of Complete Freund's adjuvant induced arthritis was evaluated by measuring the following parameters on 0, 4, 7, 10, 13,14,17,19 and 21st day after adjuvant injection.

Paw volume

The swelling in the hind paw from the ankle was measured periodically on the days mentioned above using Plethysmometer (Ugo Basile, Italy) (19).

Arthritis score

Rats were scored for arthritis (arthritis index) daily by a set visual criterion (20).

The following scoring system was used:

Normal paw	= 0
Swelling and erythema of the digits	= 2
Mild swelling and erythema of the digits	= 3
Gross deformity and inability to use the limb	= 4

Body weight

The body weight of all the animals was recorded using electronic balance (21).

WBC Count

The total WBC count was measured using Neubauer's chamber as an indication of the inflammatory response (22, 23).

Joint Diameter:

The joint diameter was measured in millimeters with the help of vernier calipers and change in joint diameter was calculated (24).

Statistical Analysis:

All data are presented as Mean±SEM and analyzed by two-way ANOVA followed by bonferroni test. The groups treated with extracts were compared with the respective vehicle group. The diclofenac sodium treated group was compared with vehicle 0.25% tween 80 solution in sterile water for injection. *P* values <0.05 was considered statistically significant.

Results

Acute toxicity:

Extract was found to be safe in the dose used and there was no mortality up to dose of 2000 mg/kg, i.p.

Paw volume:

The change in paw volume elicited a biphasic response. It was maximum on 4th day showing an early inflammatory response and on the 14th day post inoculation exhibiting a late inflammatory response in all the groups of animals. A chronic phase of inflammation reached a plateau on the nineteenth and twenty first days. Administration of HEPG at a dose of 200 mg/kg/day for a period of 21 days to arthritic animals suppressed the chronic phase of inflammation significantly ($p < 0.001$) when compared with the control group of animals. A similar pattern was observed in the animals treated with diclofenac sodium at a dose of 4 mg/kg/day. However, at lower doses of 50 and 100 mg/kg/. HEPG did not inhibit the acute and chronic phases of inflammation and calculated percentage inhibition showed a dose dependent effect of HEPG. When

compared to control, HEPG 200 mg/kg showed significant reduction of paw volume. Diclofenac sodium was prove effective than HEPG 200 mg/kg. Figure 1 portrays the change in the paw volume during the entire treatment schedule of 21 days. (Figure.1).

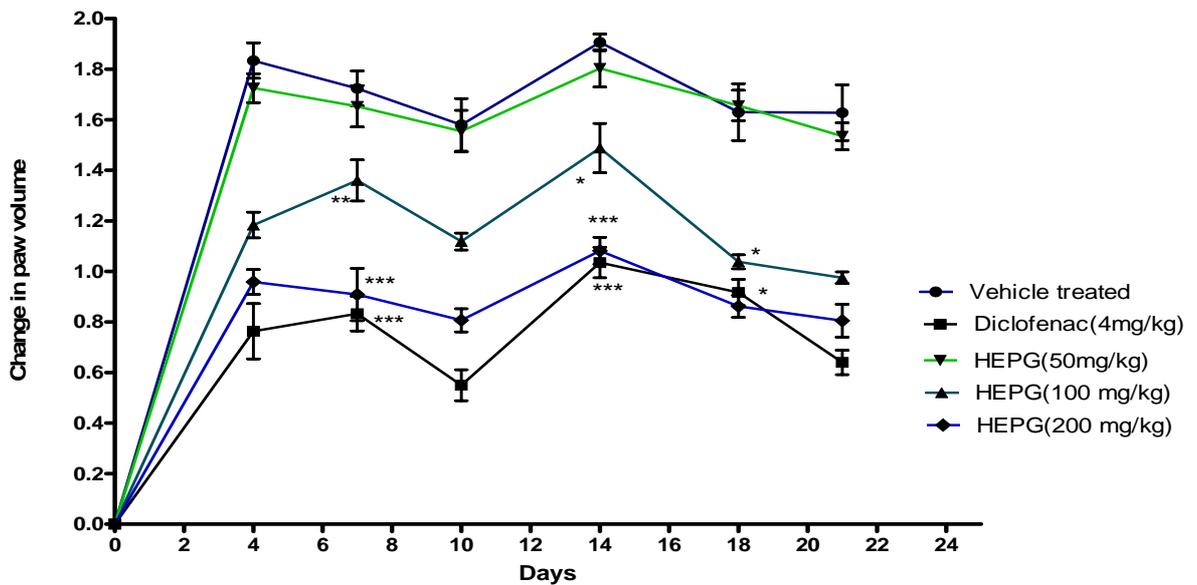


Figure No. 1: Effect of Hydroalcoholic Extract of *Psidium guyava Linn.* On change in rat paw volume are expressed as mean SEM, n = 6 in each group. *p<0.05, **p<0.01, ***p<0.001 when compared to vehicle. (Two-way ANOVA followed by Bonferroni test.)

Arthritis score :

The pattern of amelioration in the arthritis was evaluated according to the above mentioned scoring system. Diclofenac Sodium 4 mg/kg and HEPG 200 mg/kg significantly reduced (p<0.001) arthritis score compared to control group. (Figure 2)

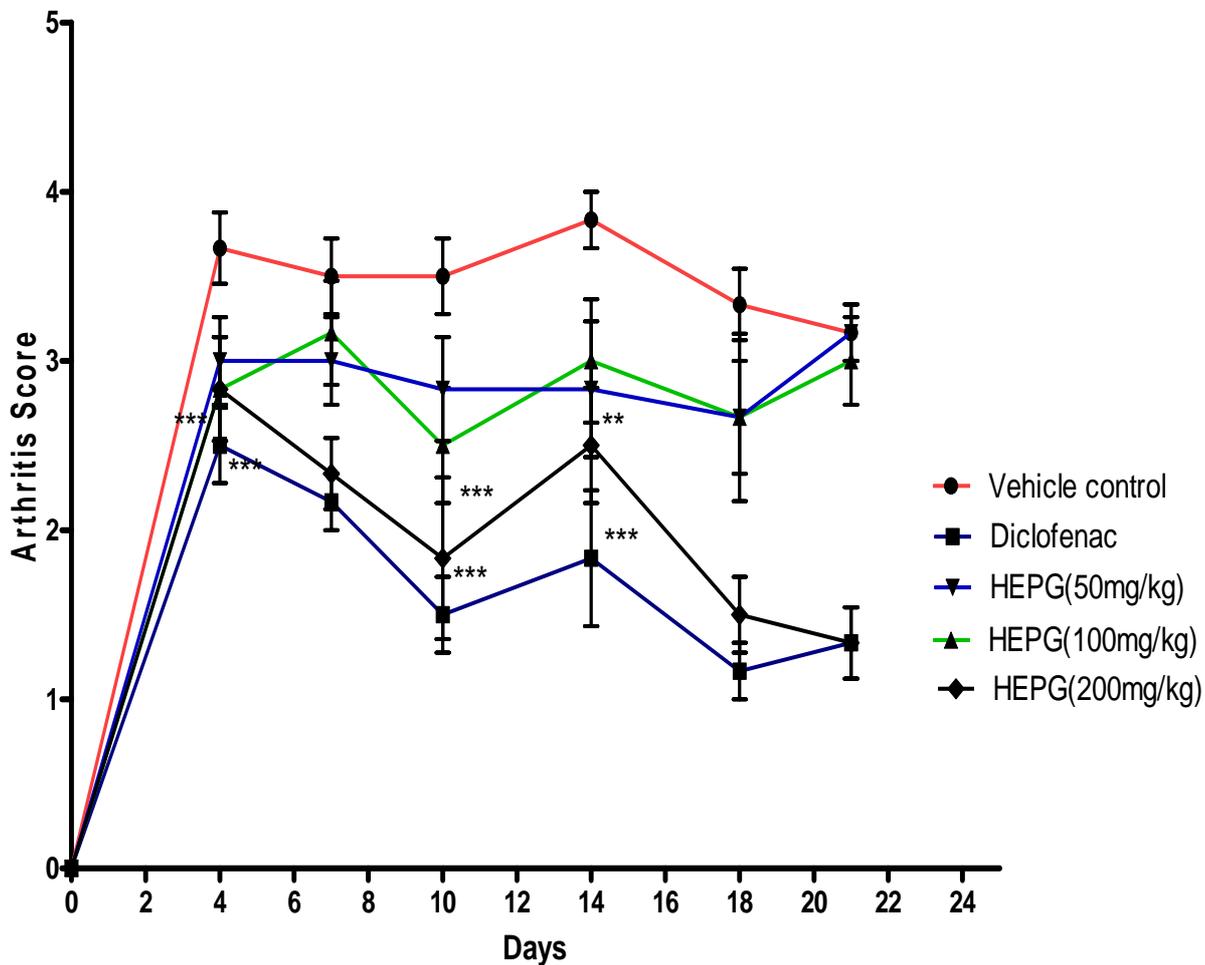


Figure No.2: Effect of Hydroalcoholic Extract of *Psidium guyava Linn.* On arthritis score. Result are expressed as mean SEM, n=6 in each group. *p<0.05, **p<0.01, ***p<0.001 when compared to vehicle. (Two-way ANOVA followed by Bonferroni test.)

Joint diameter:

In control group the tibiotarsal joint was increased while it was significantly decreased (p<0.001) diclofenac Sodium 4 mg/kg and HEPG 200 mg/kg treated groups as illustrated in the (Figure 3).

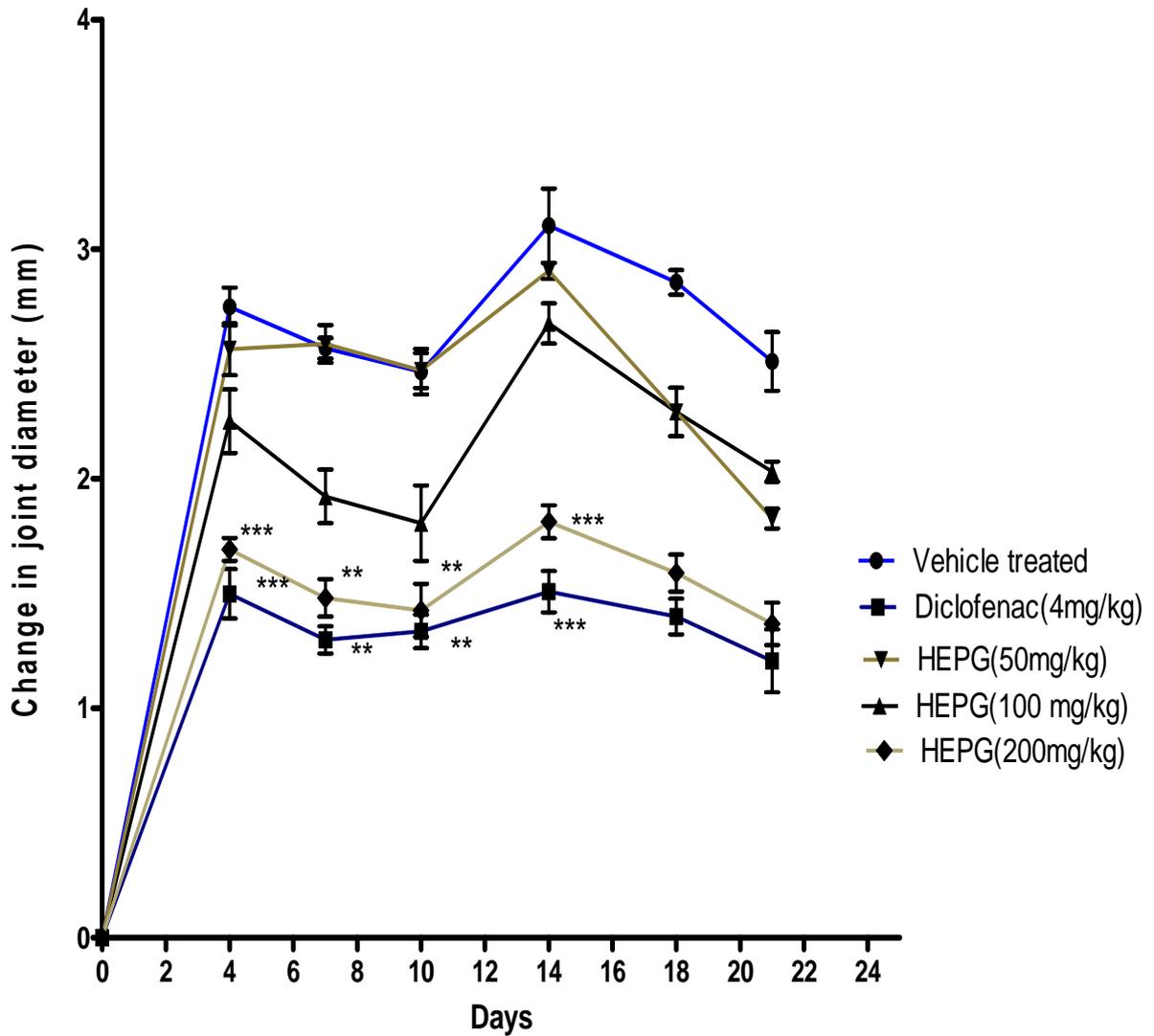


Figure 3: Effect of Hydroalcoholic Extract of *Psidium guyava* Linn. On rat joint diameter. Results are expressed as MeanSEM, n=6 in each group.*p<0.05, **p<0.01, ***p<0.001 when compared to vehicle. (Two-way ANOVA followed by Bonferroni test.)

Total leukocyte (WBC) count:

The total leukocyte count showed a steep rise during both the acute and delayed inflammatory response in the control group and the animals treated with HEPG at doses of 50 and 100 mg/kg of animals. Whereas in the Diclofenac Sodium and HEPG treated group the elevation of the WBC count was inhibited significantly ($p < 0.001$) in the chronic phase of inflammation (Figure 4).

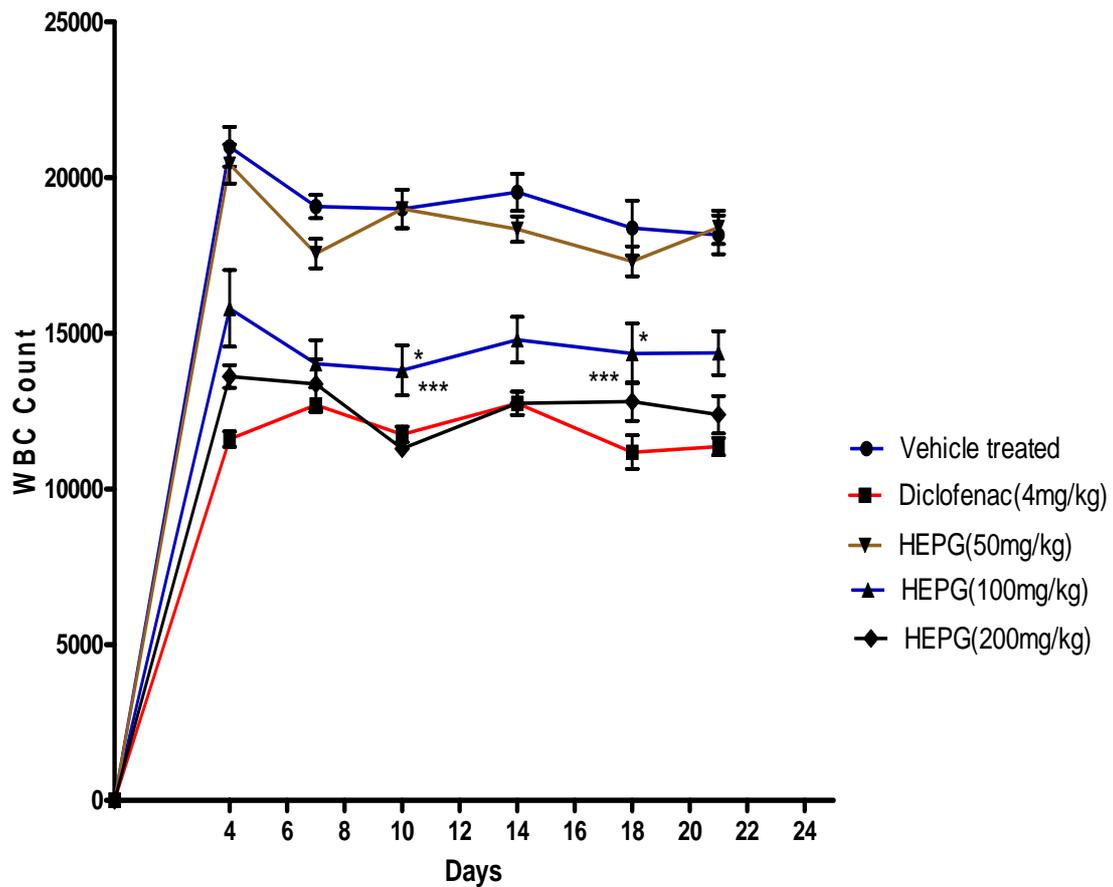


Figure 4: Effect of Hydroalcoholic Extract of *Psidium guyava Linn.* On rat leukocyte count. Results are expressed as SEM, n=6 in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to vehicle. (Two-way ANOVA followed by Bonferroni test.)

Body weight:

The animal in the control group suffered continuous loss in body weight throughout the period of 21 days whereas the animals in the Diclofenac Sodium and HEPG treated group did not lose weight significantly when compared to control. (Figure 5)

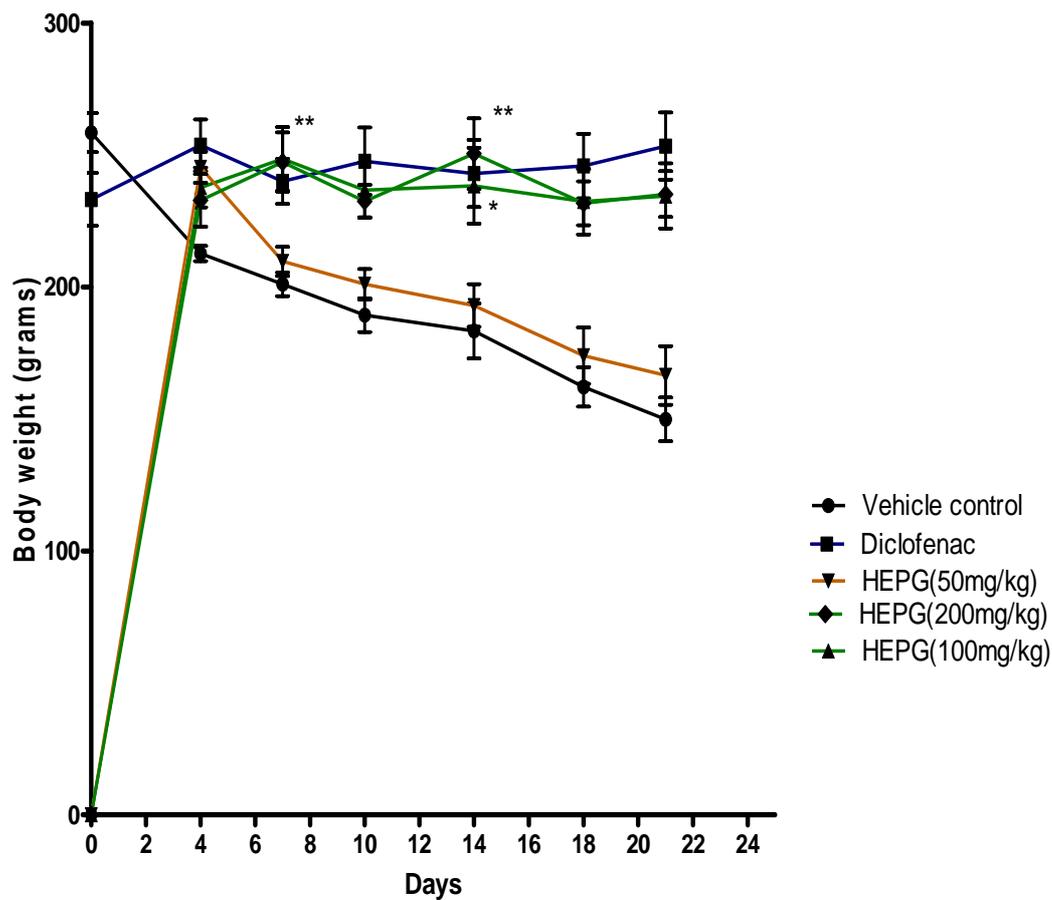


Figure 5: Effect of Hydroalcoholic Extract of *Psidium guyava* Linn. On rat body weight. Results are expressed as SEM, n=6 in each group. *p<0.05, **p<0.01, ***p<0.001 when compared to vehicle. (Two-way ANOVA followed by Bonferroni test.)

Discussion

Arthritic lesions are characterized in the rat by a biphasic response when complete Freund's adjuvant is injected in the paw of the animal. The two phases occur due to an initial inflammatory phase and secondary immunological state.

The disease peaks in on the fourth and 14th day after the injection of the CFA. The inflammation on the 4th day is due to the generation of the prostaglandin whereas the inflammatory phase on the 14th day is due to the auto antibodies generated at a later stage. In the present investigation the HEPG inhibited the initial inflammatory as well as the subsequent immunological inflammatory phase in a dose dependent manner. Hence, it could be deduced that HEPG possesses both anti inflammatory and immunomodulatory properties.

The various parameters of arthritis like paw volume, joint diameter, WBC count; body weight and arthritis score signify the activity of HEPG against experimentally induced arthritis in laboratory animals. HEPG treatment was able to significantly inhibit the increase in the paw volume, joint diameter, rise in WBC count, and arthritis score and fall in the body weight at the doses of 100 and 200 mg/kg which provide pharmacological credence to the ethno botanical claims. (Fig.no1-5.)

Further studies could be carried out using fractions of the extract to elucidate the bioactive moieties involved in the amelioration of the disease and unravel the mechanism of drug.

In conclusion, the present investigation demonstrates the HEPG possesses potent anti inflammatory and anti arthritic potential against complete Freund's adjuvant induced arthritis at a dose of 200mg/kg/day p.o. unraveling a novel facet in its pharmacological profile

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