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TO EVALUATE THE ANTI-ARTHRITIC AND IMMUNOMODULATORY ACTIVITY OF HYDROALCOHOLIC AND METHANOLIC EXTRACT OF QUISQUALIS INDICA LINN IN RATS

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ABSTRACT

Rheumatoid arthritis (RA) is characterized by joint inflammation and swelling, rheumatoid factor (RF) and anticitrullinated protein antibody (ACPA) production, and bone and cartilage destruction. In the present study the hydroalcoholic and methanolic extract of Quisqualis indica was administered orally at the dosage levels of 100 mg/kg and 200 mg/kg. The anti-inflammatory activity was test using acute inflammatory models like Formaldehyde induced arthritis and chronic models like Freund's complete adjuvant arthritis. Oral administration of the extract at the doses 100 and 200 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in acute (formaldehyde induced arthritis, $p< 0.0001$) and chronic (freund's adjuvant induced arthritis, $p<0.0001$). The pharmacological screening included evaluation of anti-arthritic and immunomodulatory activity of hydroalcoholic and methanolic extract of Quisqualis indica at the dose of 100 mg/kg, 200mg/kg in rats with CFA-induced arthritis and formaldehyde induced arthritis. In injected (right) paw, low dose and high dose hydroalcoholic extract exhibited 36.29 %, 44.28 % inhibition and low dose, high dose methanolic extract exhibited 37.38 %, 46.09 % inhibition respectively against CFA induced paw edema on 21st day as compared to indomethacin (49.01 %). Administration of Quisqualis Indica was found to increase phagocytic activity by stimulation of macrophages, total WBC and differential leukocytes count. So anti inflammatory activity of Hydroalcoholic and methanolic extract of Quisqualis indica can be attributed to bradykinin and PG synthesis inhibition property of polyphenols. Hence, present investigation established some pharmacological evidences to support the folklore claim that *Quisqualis indica* L. is use as anti-inflammatory, anti-arthritic agent and immunomodulatory activity.

KEYWORDS: Arthritis, Quisqualis indica, immunomodulation, indomethacin.

INTRODUCTION

Rheumatoidarthritis

Arthritis or Joint inflammation is an auto invulnerable problem described by torment, solidness, synovial expansion, irritation, ensuing obliteration like distortion of joints or annihilation of ligament and bone.[1] Its incidence varies with age. It affects women more often than men. It is a synovial joint inflammation caused by an immune-mediated response. Since anti-inflammatory drugs do not inhibit T-cell and B-cell-mediated responses, they are not anti-arthritic. Rheumatoid arthritis is not a hereditary condition. Some individuals, according to researchers, have genes that make them more vulnerable to the disease. Rheumatoid arthritis does not always occur in people who have these genes. People with these genes will not automatically develop rheumatoid arthritis.[2] The genes are normally activated by a "trigger," such as an infection or an environmental influence. The immune system reacts inappropriately when the body is exposed to this cause. The immune system starts to develop substances that threaten the joint instead of defending it. This is what may lead to the development of rheumatoid arthritis.[3] It is an autoimmune disease, which means that the immune system of the body attacks healthy tissues by accident. These cytokines cause adjacent articular cartilage synovial fibroblasts and chondrocytes to secrete enzymes that destroy proteoglycan and collagen, causing tissue destruction.[4] The release of numerous cytokines and inflammatory mediators causes the synovial to proliferate and spread, which is known as pannus. The next step is fibrosis, which results in a loss of joint mobility. This stage is known as asankylosis.[4]

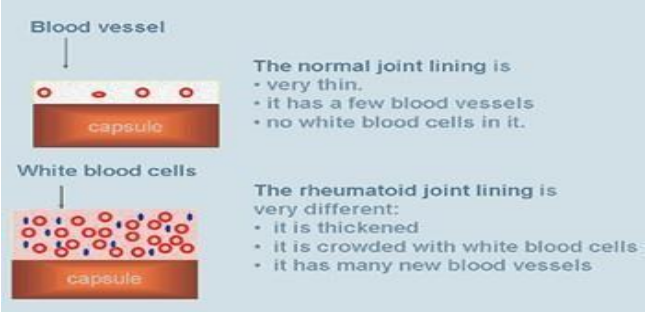

Fig. 1: Difference between normal joint lining and rheumatoid joint lining[4]

Diagnosis of Rheumatoid arthritis

The diagnosis of rheumatoid arthritis is primarily clinical. The typical presentation is polyarticular, with pain, stiffness, and swelling of multiple joints in a bilateral, symmetric pattern. A minority of patients present with oligoarticular asymmetric involvement.[5] The onset is usually insidious, with joint symptoms emerging over weeks-months and often accompanie by anorexia, weakness, or fatigue. Patients usually note morning stiffness lasting more than an hour. Commonly involved joints are the wrists, proximal interphalangeal, metatarsophalangeal, and metatarsophalangeal joints; with distal interphalangeal joints and spinal joints usually spared. Typical examination findings include swelling, bogginess, tenderness and warmth of, with atrophy of muscles near, theinvolved joints. Weakness is out of proportion to tenderness.[6]

Epidemiology

- Prevalence varies from 0.5% to 1.5% of the population.
- RA affects more women than men (ratio 3:1).
- The age of onset is between 30 and 55 years.
- RA results in progressive disability, with nearly half of all patients experiencing significant functional impairment within 10 years.
- RA shortens life expectancy by a number of years in both men andwomen.[7]

Pathophysiology of Rheumatoid arthritis

In the early stages of rheumatoid arthritis, the synovial membrane begins to invade the cartilage. In *established RA*, the synovial membrane becomes transformed into inflammatory tissue, the *pannus*. This tissue invades and destroys adjacent cartilage and bone. The interface between pannus and cartilage is occupied predominantly by activated macrophages and synovial fibroblasts that express matrix metalloproteinases and cathepsins.[8]

Evaluation of Anti arthritic parameter

Various experimental parameters are create to anti – arthritic activity in animal like, Paw edema, Body weight, Arthritic index, Quantitative determination of the Rheumatoid Factors (RF), Histopathology of synovial joints, Radiology (x-Ray measurements), Photographic parameter.

i- Pawedema

Paw volumes of both hind limbs are record on the day of arthritis induction, and again measured on day first; third, fifth, ninth, up to last day of experiment using mercury column Plethysmometer on last day of experiment rheumatoid arthritis becomes more evident and inflammatory changes spreads systemically and becomes observable in the limb not inducted witharthritis.[9]

ii- Arthritic Index

All the animals are closely observed for organs like ears, nose, tail, fore paws and hind paw and arthritic index (Pearson CM, 1959) was calculated.[10]

iii- Rheumatoid Factor

The latex turbidimetry method is use in the present study using RF turbilatex kit. Calibration is carried out for linear range up to 100 IU/ml. The reading of RF factor of all the groups obtained is compare with the control animals and is express as IU/mlRF.[11]

iv- Histopathology of Synovial Joints

The synovial joint is isolated for the biopsy examination of synovial proliferation, synovial lining angiogenesis, and rheumatoid inflammation. This destruction was separately graded on a scale from 0 to 3, ranging from no abnormalities to complete loss of cortical and trabecular bone of the femoral head. Cartilage and bone destruction by pannus formation was scored ranging from 0, no change; 1, mild change (pannus invasion within cartilage); 2, moderate change (pannus invasion into cartilage/subchondral bone); 3, severe change (pannus invasion into the subchondral bone); and vascularity (0, almost no blood vessels; 1, a few blood vessels; 2, some blood vessels; 3, many blood vessels.[12-15]

TREATMENT

Drug treatment generally involves a 3-pronged approach: nonsteroidal anti-inflammatory drugs and low-dose oral or intra-articular glucocorticoids; disease-modifying ant rheumatic drugs; and consideration of biologic response modifiers/biologics. Nonsteroidal anti-inflammatory drugs reduce joint pain and swelling, but do not alter the disease course and should not be used alone. Steroids (prednisone 10 mg daily or equivalent) relieve symptoms and may slow joint damage; they should be prescribed at a low dose for short duration, primarily as "bridge"

therapy, and with daily calcium (1500 mg) and vitamin D (400-800 IU) oral supplements to limit bone demineralization.[16]

MATERIALS AND METHODS

PLANTPROFILE

QUISQUALIS INDICA[17]

*Quisqualisindica*Linn. (Combretaceae) is a strong climber, ligneous vine that can reach from 2.5 meters to up to 8 meters. It is commonly known as Rangoon creeper. It is indigenous in Africa, Indo Malaysian region and cultivated all over India.

Scientific classification

Kingdom-Plantae
Division-Magnoliophyta
Class-Magnoliopsida
Order-Myrtales
Family-Combretaceae
Genus-Quisqualis
Species-*Q.indica*

Local Names

English (RangoonCreeper),
Hindi(Madhumalti),
Bengali(Modhumalati),
Telgu (RadhaManoharam),
Filipino(Niyog-niyogan),
Spanish(Quiscual),
China(Shih-chun-tzu),

It is vining and evergreen plant which is having vigorous growth needing sturdy support and can get quite out-of-hand on its favorable growing site, it doesn′trequire deep and anchoring roots. It is widely distributed all over the world especially on China, Philippines, Bangladesh, Myanmar and Malaysia and now also broadly grown in India as ornamental plant in most of the garden. Distributed over 1) Thickets and secondary forests area throughout the Philippines. 2) Ornamentally planted for its flowers. 3) Also occurs in India to Malaya. 4) Introduced in most tropical countries.

Pharmacognostic studies

Macroscopy- Morphological studies were done by using simple microscope. The shape, apex, base, margin, taste and odor of leaves and flowers were determined.

Phytoconstituents

Every plant contains several phytoconstituent in its different parts showing various pharmacological activities and / toxicities, likewise *Quisqualisindica Linn.* also showing many pharmacological activities due to the presence of medicinally active compounds. *Quisqualisindica Linn* contains phytoconstituent such as Trigonelline (alkaloid), L-proline (α- amino acid), L-asparagine (α-amino acid), quisqualic acid (agonist for both AMPA receptors), rutin (flavonoid) and two forms of the cysteine synthase, isoenzyme A and isoenzyme B (enzyme). Rutin and pelargonidin-3-glucoside have also been isolated from flowers.

Extraction procedure

About 180 gm of dry powder was taken in a closed bottle and it was defatted with Petroleum ether. The defatting was continued for 9-10 days with occasional shaking. The Petroleum ether extract was filtered. The marc left after Petroleum ether defatting was taken out and dried under shade to get a dry mass, then extracted with Methanol and water (hydroalcoholic) by using cold maceration extraction. The extraction was continued for 9-10 days with occasional shaking. The hydroalcoholic extract was filtered, concentrated under reduced pressure to a semisolid mass and was made free from solvent. The final obtained extract was weighed; percentage yield was calculated and stored in a cool place.

Table 1: List of chemical and drugs.

S.No.	Drugs/Chemical	Manufacturer/Supplier
1.	Indomethacin	Cayman chemical company.
2.	Fruends adjuvant	Sigma-Aldrich chemicals Ltd.
3.	Carboxy methyl cellulose	Merck specialties Pvt. Ltd
4.	Formaldehyde	CDH private Ltd.
5.	Diclofenac sodium	Day' Pharmaceutical Ltd.
6.	Levamisol	Sigma Aldrich Ltd.
7.	Sodium bi carbonate	Merck specialties Pvt. Ltd
8.	Indian ink	Camel Ltd.
9.	Cyclophosphamide	Biochem pharmaceutical Ltd.
10.	Glacial acetic acid	Merck specialties Pvt. Ltd
11.	Leishman's stain	HD fine Ltd.
12.	Methanol	Merck specialties Pvt. Ltd.
13.	Petroleum ether	Merck specialties Pvt. Ltd
14.	Di ethyl ether	Merck specialties Pvt. Ltd
15.	Chloroform	Merck specialties Pvt. Ltd

Experimental Methods

Acute oraltoxicity

The dose limit were selected on the basis of previously performed oral acute toxicity studies in albino mice in accordance with the OECD guidelines. Acute toxicity studies on Quisqualisindica aerial parts extract were performed in mice containing 6 animals in each group, the graded doses of the methanolic and hydroalcoholic

extracts of Quisqualisindica aerial parts extract doses selected for the study were 100 mg/kg, 200 mg/kg, 400 mg/kg, 800 mg/kg, 1600 mg/kg, 2000 mg/kg were administered orally and the animals were observed for 2 weeks following administration, change in body weight gain, food consumption, any kind of behaviorally changes and mortality were noted. It was found that the methanolic extract has produced significant toxicity at the dose of 2000 mg/kg as 2 animal of this group was died. Thus the extract was highly tolerable up to 1500 mg/kg for methanolic extract and 2000 mg/kg for hydroalcoholic extract.

ANTI-ARTHRITICACTIVITY

Acute non-immunological arthritic activity[18]

Formaldehyde induced arthritis

Male Wistar rats weighing between 150-200 g. was randomly selected. They were grouped in a group of 4 animals each into 4 groups. On the 0th day, the basal paw volume of left hind paw of each animal was measured using Plethysmometer. On day 1 and day 3, they were injected into the sub-plantar region of the left hind paw with 0.1 ml of 2 % v/v formaldehyde in normal saline. Dosing with standard drug, Diclofenac sodium and extracts were started on same day and continued for 10days.

Experimental design

Animals were divided into four groups. Group I served as - Arthritis control

Group II - Diclofenac Sodium (standard drug, 13.5mg/kg) treated Group III & IV - Plant extract-100, 200mg/kg respectively.

Parameter monitored

a. Paw volume of injected paw was measured daily.

Chronic immunological arthritis: Freund's complete adjuvant arthritis[19]

Procedure

The albino rats of either sex were selected and divided into four groups, each comprising of four animals. Group I, served as control received vehicle 2ml/kg (0.3% carboxy methyl cellulose in distilled water), Group II, served as standard received indomethacin (100 mg/kg), Groups III, IV, extract respectively. The experimental protocol was of 21days and on the day one, Fruends adjuvant 0.1ml (1ml contains 1mg mycobacterium Tuberculosis (H37Ra, ATCC25177) heat killed and dried, 0.25 ml mineral oil and 0.15ml mannide mono oleate) was administered into the sub plantar region of right hind paw. The individual extracts were administered to respective groups at a dose of 100mg/kg and 200mg/kg for 21 days. The paw volume and paw thickness was measured at day 4, day 8, day 14 and day 21.

Parameter monitored

A: Percentage inhibition of paw volume was calculated by the formula,

i = (1-ΔV / -ΔV)*100

Treated Control

Where, ΔV represents the mean change in paw volume.

RESULT AND DISCUSSION

Anti-arthritic activity

1. Effects of Hydroalcoholic and Methanolic extract of Quisqualisindica on Formaldehyde induced arthritis

Effect of Hydroalcoholic and Methanolic extract of Quisqualisindica on Formaldehyde induced arthritis is shown in Table 3.1.1.The assessment made on the 10th day showed that, treatment with hydroalcoholic extract (low dose 100 mg/kg, high dose 200 mg) and methanolic extract (low dose 100 mg/kg, high dose 200 mg) significantly reduced (P<0.01) the swelling in the injected (left) hind paw as compared to Diclofenac sodium treated group. On 2nd day onwards Diclofenac sodium showed significantly reduction in paw edema. Effect of hydroalcoholic and methanolic extract showed much similar effect on 9 and 10 day.

Table 2: Effect of Hydroalcoholic and Methanolic extracts of Quisqualisindica in Formaldehyde induced arthritis model.

Day	Control	Standard (Diclofenacsodium 13.5mg/kg)	Low dose hydroalcoholic (100mg/kg)	High dose hydroalcoholic (200mg/kg)	Low dose methanolic (100 mg/kg)	High dose methanolic 200mg/kg)
1st day	0.35±0.003	0.29±0.002***	0.30±0.002***	0.29±0.002***	0.30±0.002***	0.30±0.002***
2nd day	0.37±0.002	0.28±0.002***	0.30±0.002***	0.29±0.002***	0.31±0.002***	0.3±0.004***
3rd day	0.38±0.003	0.28±0.004***	0.30±0.004***	0.29±0.002***	0.30±0.002***	0.3±0.004***
4th day	0.39±0.004	0.27±0.002***	0.30±0.004***	0.29±0.002***	0.30±0.002***	0.29±0.002***
5th day	0.39±0.002	0.26±0.004***	0.29±0.002***	0.29±0.004***	0.29±0.002***	0.29±0.002***
6th day	0.40±0.004	0.26±0.004***	0.29±0.004***	0.28±0.002***	0.29±0.002***	0.29±0.002***
7th day	0.41±0.004	0.25±0.002***	0.29±0.004***	0.27±0.005***	0.29±0.002***	0.29±0.004***
8th day	0.40±0.004	0.24±0.002***	0.28±0.002***	0.27±0.002***	0.28±0.004***	0.28±0.002***
9th day	0.38±0.004	0.23±0.004***	0.28±0.004***	0.27±0.004***	0.28±0.002***	0.28±0.004***
10th day	0.37±0.006	0.23±0.002***	0.27±0.004***	0.26±0.002***	0.28±0.002***	0.27±0.004***

The values are Means ±S.E.M. (n=4), * $P < 0.05$, ** $P < 0.01$, ***P<0.001.ascompared with control group (one-way ANOVA followed by Dunnet's test)

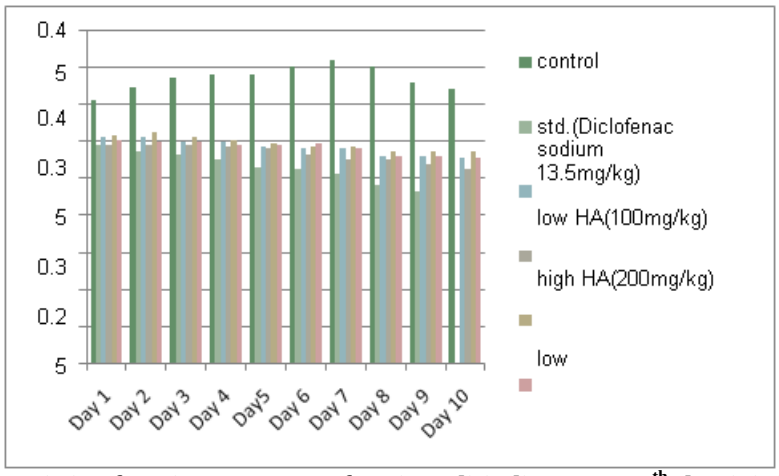

Fig 2: Column statistic of various extracts of Quisqualisindica on 1-10th day ininjected hind paw.

2. Effect of Hydroalcoholic and Methanolic extract of Quisqualis indica in Freund Adjuvant induced arthritis model

The anti-arthritic activity was evaluated by using Complete Freund Adjuvant induced arthritis model in wistar albino rats. The assessment made on the 21st day showed that, treatment with hydroalcoholic and methanolic extract significantly reduced (P<0.01) the swelling in the injected (right) hind paw as compared to Indomethacin treated group (Table 3.1.2). On the 21st day the % inhibition of paw oedema exhibited by low dose hydroalcoholic, high dose hydroalcoholic, low dose methanolic, high dose methanolic extract were 36.29, 44.28, 37.38, 46.09 respectively; while Indomethacin treated animals showed maximum % of inhibition of paw oedema 49.01 on 21st day.

Table 3: Effect of Hydroalcoholic and Methanolic extracts of Quisqualisindica in Freund Adjuvant induced arthritis model.

Treatment	Day 4	Day 8	Day 14	Day 21	%inhibitionofpaw swelling on 21st day
Control	0.525±0.01322	0.532±0.0125	0.540±0.0108**	0.551±0.0108***	0
Standard(Indomethacin 100mg/kg)	0.312±0.0085***	0.310±0.0070***	0.291±0.0070**	0.281±0.0070***	49.01
Low dose hydro Alcoholic (100mg/kg)	0.382±0.0085***	0.371±0.0081***	0.361±0.0081**	0.351±0.0081***	36.29
Highdosehydroalcoholic(200mg/kg)	0.342±0.0085***	0.330±0.0108***	0.317±0.0131***	0.307±0.0131***	44.283
Lowdosemethanolic (100mg/kg)	0.377±0.0137***	0.367±0.0137***	0.355±0.0119***	0.345±0.0119**	37.38
Highdosemethanolic (200mg/kg)	0.331±0.0108***	0.317±0.0131***	0.307±0.0131***	0.297±0.0131**	46.09

The values are Means ±S.E.M. (n=4), * $P < 0.05$, ** $P < 0.01$, ***P<0.001 ascompared with control group (one-way ANOVA followed by Dunnet"s test)

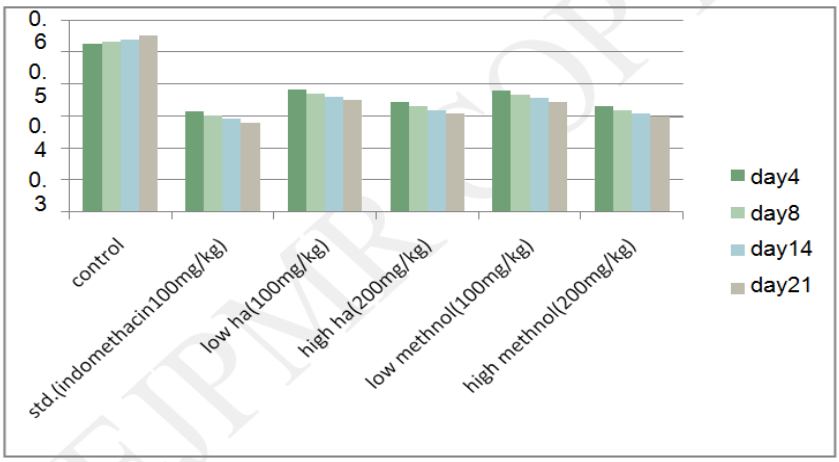

Fig 3: Column statistic of various extracts of Quisqualisindica inAdjuvant induced arthritis model.

DISCUSSION

Arthritis was induced in left hind limb of rats using formaldehyde and CFA in right hindlimb of rats. The most objective measurement that can be made to assess the anti-arthritic activity is the determination of magnitude of swelling of hind paws. The injected paw is used to assess the acute inflammatory response to the injection of the adjuvant. In CFA induced arthritis model, rats develop a chronic swelling in multiple joints with influence on inflammatory cells, erosion of joint cartilage and bone destruction. These inflammatory changes ultimately result in the complete destruction of joint integrity and function in the affected animal. It appears from our results that hydroalcoholic and methanolic extract of Quisqualisindica significantly reduced ($P<0.01$) the CFA induced paw edema on 21st day as compared to standard drug (Indomethacin), which may be due to inhibiting the response of inflammatory cells or protecting erosion of joint cartilage and bone destruction in chronic arthritis model. It also appears from result that the hydroalcoholic and methanolic extract of Quisqualisindica significantly reduced ($P<0.01$) the formaldehyde induced paw edema on 10th day as compared to standard drug (Diclofenacsodium). The results obtained in the present study indicate that *Quisqualisindica (QI)* flower extract is a potent Immunostimulants, stimulating specific and non-specific immune mechanisms. The phagocytic activity of the reticulo-endothelial system is generally measured by the rate of removal of carbon particles from the blood stream *Quisqualisindica*extract appeared to enhance the phagocytic function by exhibiting a clearance rate of carbon by the cells of the reticulo- endothelium system. The effect of *Quisqualisindica*flower extract. on the phagocytic activity by the carbon clearance test. In carbon clearance test, QI flower extract treated all groups, exhibited significantly high phagocytic index. The phagocytic index of (100 mg/kg) and QI flower extract (200 mg/kg) showed significant ($p<0.05$) increased in phagocytic index when compared to control group. This indicates stimulation of the reticuloendothelial system. Cyclophosphamide atthe dose of 30 mg/kg, caused a significant reduction in total WBC count in rat as compared to control group. The rise in the total WBC count lowered by Cyclophosphamide was observed at 100 mg/kg and 200 mg/kg of *Quisqualisindica*flower extract. The total WBC count was significant restoration of white blood cell count. There was a significant decrease, Neutrophils lymphocytes, eosinophils and monocytes in animals treated with Cyclophosphamide (group II) as compared to control group (groupI) because Cyclophosphamide showed that in rats lymphocytes decrease due to immunotoxic effect as well as decreases in the activity of lymphoidcells especially the CD4+ lymphocyte.

CONCLUSION

The pharmacological screening included evaluation of anti-arthritic and immunomodulatory activity of hydroalcoholic and methanolic extract of Quisqualisindica at the dose of 100 mg/kg, 200mg/kg in rats with CFA-induced arthritis, formaldehyde induced arthritis, carbon clearance test and Cyclophosphamide induced myelosuppression. In injected (right) paw, low dose and high dose hydroalcoholic extract exhibited 36.29 %, 44.28 % inhibition and low dose, high dose methanolic extract exhibited 37.38 %, 46.09 % inhibition respectively against CFA induced paw edema on 21st day as compared to indomethacin (49.01 %). Administration of QuisqualisIndica was found to increase phagocytic activity by stimulation of macrophages, total WBC and differential leukocytescount.

The phytochemicals analysis revealed the presence of polyphenols and flavonoids. The polyphenols have potent anti-inflammatory activity by inhibiting prostaglandin synthesis. So anti inflammatory activity of Hydroalcoholic and methanolic extract of *Quisqualisindica*can be attributed to bradykinin and PG synthesis inhibition property of polyphenols.

The study also reveals that, hydroalcoholic and methanolic extract of *QuisqualisIndica*(HEQI*)* has Immunostimulants activity which strongly affected immune system seems to be bioactive fraction of this plant. However, the mechanism of action could be unfolded only after detailed investigations whereby the extract modulates the immune system however; the extract contains compounds which had Immunomodulatory activity. Besides, to isolate the active constituents and clarify its mechanism of action will be our auxiliary objective.

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