



## Anti-inflammatory activity of hydroalcoholic extract of *Quisqualis indica* Linn. flower in rats

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### Abstract

Inflammation plays an important role in various diseases with high prevalence within populations such as rheumatoid arthritis, atherosclerosis and asthma. The aim of the present context to evaluated to the anti-inflammatory activity of hydroalcoholic extract of *Quisqualis indica* in wistar rats. It's also known as Rangoon creeper or, madhumalati. The anti inflammatory activity was test using acute inflammatory models like; acetic acid-induced vascular permeability and chronic models like; cotton-pellet induced granuloma. Oral administration of the extract at the doses 100 and 150 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in acute (acetic acid-induced vascular permeability,  $p < 0.0001$ ) and chronic (cotton pellet granuloma  $< 0.0001$ ) Hence, present investigation established some pharmacological evidences to support the folklore claim that *Quisqualis indica* L. is use as anti-inflammatory agent.

**Key-Words:** *Quisqualis indica*, Flower, Hydroalcoholic extract, Anti-inflammatory

### Introduction

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation<sup>1</sup>. During the inflammatory process, large amounts of the proinflammatory mediator's nitric oxide (NO) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) are generated by the inducible isoforms of NO synthase (iNOS) and cyclooxygenase-2 (COX-2), respectively<sup>2</sup>. NO is produced in large amounts from the amino acid, L-arginine. In mammalian cells, three isoforms of NOS, types I, II and III, have been identified on the basis of the physical and biochemical characteristics of the purified enzymes.

Types I (neuronal NOS, nNOS) and III (endothelial NOS, eNOS) have been classified as constitutive NOS (cNOS) because they are continuously present in the cells, whereas type II, an iNOS, is expressed only after exposure to specific stimulants such as cytokines, bacterial endotoxin lipopolysaccharide (LPS), and calcium ionophore in some cells. COX is the enzyme which converts arachidonic acid (AA) to prostaglandins (PGs). Nonsteroidal anti-inflammatory drugs (NSAIDs) produce their therapeutic activities through inhibition of COX. They share, to a greater or lesser degree, the same side effects, including gastric and renal toxicity. Recent research has shown that there are at least two COX isoenzymes. COX-1 is constitutive and makes PGs that protect the stomach and kidney from damage. COX-2 is induced by inflammatory stimuli, such as cytokines, and produces PGs that contribute to the pain and swelling of inflammation<sup>3</sup>.

COX-2 is mainly an inducible enzyme and is involved primarily in the regulation of inflammation. PGs, and in particular PGE<sub>2</sub> are regarded as a potent pro inflammatory molecule; however, increasing evidence has indicated that they can also exert anti-inflammatory functions which are important for the resolution of the inflammatory response<sup>4</sup>. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g.

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opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations<sup>5</sup>.

*Quisqualis indica* 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. Flower numerous, pendent, 7.5 cm long, 3.8 cm wide. At first they are white in color then they become deep red<sup>6</sup>. In Philippines the fruit is used as a vermifuge. The plant is also used as a cough cure. In Amboina the leaves are given in a compound decoction for flatulent distension of abdomen. In India the leaves are given in a compound decoction for flatulent distension of abdomen, seeds are given with honey as an electuary for the expulsion of entozoa in children. Leaves contains rutin, trigonelline, L-proline, laspargine and quisqualic acid whereas flower gum contains pelargonidin-3-glucoside. Seed Oil contains linoleic, oleic, palmitic, stearic and arachidic acids. ellagitannins, quisqualin A and quisqualin B is present in fruits of this plant and flower contains linalool oxides (furanoid and pyranoid), 2,2,6-trimethyl-6-vinyl-3-oxo tetrahydropyran, (E,E)-alpha-farnesene, (Z)-3-hexenyl benzoate and benzyl benzoate<sup>8</sup>. Four Diphenyl propanoids were isolated from stem bark of *Quisqualis indica*. However, the anti-inflammatory activity of galls has never been evaluated<sup>6</sup>.

### Material and Methods

Selection and Collection of plant on the basis of ethno botanical survey, traditional use and literature survey. The mature flower of *Quisqualis indica* Linn were collected in the morning locally from Bhopal District, M.P, India, in the month of November 2009 The flower of *Quisqualis Indica* was collected from Bhopal, Madhya Pradesh, India. The powdered drug packed in a paper bags & stored in air tight container until use. Identification and Authentication of herb by Dr. Zia ul Hassan, Professor of Botany, Saifia College of Science, Bhopal, Madhya Pradesh, India (Voucher. No 138/Bot/Saifia/2010)

### Extraction of plant material

About 180 gm of dry powder was taken in a closed bottle and it was defatted with Petroleum ether. The

deffating was continued for 9-10 days with occasional shaking. The Petroleum ether extract was filtered. The marc left after Petroleum ether deffating 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.

### Preparation of dosage forms

For in vivo studies, the concentrated Hydroalcoholic extract of *Quisqualis indica* (HEQI) was administered orally after suspending in Distilled water The freshly prepared solution of *Quisqualis indica* extract was used in each experiment. 100mg/kg and 150 mg/kg per ml test doses were selected on the basis oral acute toxicity study in rat

### Phytochemical analysis

Preliminary Phytochemical studies of the hydroalcoholic extract of *Quisqualis indica* was performed for major classes of constituents like alkaloids, carbohydrates, protein and amino acid, Saponins, glycosides, steroids, tannins, flavonoid and phenolic compounds according to published standard methods<sup>7</sup>.

### Experimental animals and feeds

Albino wistar rats (100-150gm) of either sex were used for this study and these animal were obtained from animal house [CPCSEA Reg. No.1283/c/09/CPCSEA], Pinnacle Biomedical Research Institute, Bhopal (M.P.). Throughout the experiment, the animals were housed, four animal per cage, maintained at ambient temperature of (25 $\pm$ 2); 30-60% humidity, under 12 hr. light-dark cycle. They were fed with standard pellet diet and water *ad libitum*. The animals were habituated to laboratory conditions for 48 hr. prior to the experimental protocol to minimize any non specific stress.

### Acute toxicity study and dose selection

The dose limits were selected on the basis of previously performed oral acute toxicity studies in rat, in accordance with the OECD (420) guidelines<sup>8</sup>. The hydroalcoholic extract of *Quisqualis indica* (HEQI) was investigated for its acute toxicity studies according to the OECD guidelines (420). The extract was given at different doses to the group of six animals at 200 mg/kg, 500 mg/kg, 1000 mg/kg, 1500 mg/kg and 2000 mg/kg, orally. Animals were observed for regular three hours after the dose administration and after 24 hours and 48 hours for the changes in behavior, changes in

body weight and mortality. It was found that the extract has produced significant toxicity up to the dose of 1500 mg/kg. Thus the extract was highly tolerable up to 1500mg/kg.

#### Acetic acid-induced vascular permeability

The Animals were divided in 4 groups each having 4 animals. Group 1-control (Distilled Water vehicle 1% (w/v) 1ml/100 g, (p o) , Group-2, (HEQI) 100 mg/ kg, Treated, Group 3, (HEQI) 150mg/ kg, p.o Treated, Group 4 Standard (diclofenac) 10 mg/kg, intra peritoneal after 1h of administration of HEQI and diclofenac sodium, rat were injected with 0.25 ml of 0.6 % (v/v) solution of acetic acid intraperitoneally (ip). Immediately, 10 ml/kg of 10 % (w/v) Evans blue was injected intravenously via tail vein. After 30 min of Evan's blue injection, the animals were anaesthetized with ether anesthesia and sacrificed. The abdomen was cut open and exposed viscera. The animals were held by a flap of abdominal wall over a Petri dish. The peritoneal fluid (exudates) collected, filtered and made up the volume to 10 ml using normal saline solution and centrifuged at 3000 rpm for 15 min. The absorbance (A) of the supernatant was measured at 590 nm using spectrophotometer<sup>9</sup>.

#### Cotton pellet-induced granuloma

The rats were divided into four groups, each group consisting of four animals. After shaving of the fur, the animals were anaesthetized (80 mg/kg ketamine i.p) and an incision was made on the lumbar region by blunted forceps, a subcutaneous tunnel was made and a sterilized cotton pellet (100 ± 1 mg) was inserted in the groin area. All the animals received diclofenac sodium (10 mg/kg i.p) as standard, vehicle (distil water), and extract 100 and 150 mg of HEQI orally depending upon their respective grouping for seven consecutive days from the day of cotton pellet insertion<sup>10</sup>. On the 8th day, animals were anesthetized the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37 °C for 24 h and dried at 60 °C to constant weight. The difference between the initial weight and the final weight of the cotton pellet gives the amount of granulation tissue formed. The percentage inhibition of granulation tissue formation was measured by the following method, % Inhibition = (X - Y)/X \*100, Where X = mean increase in cotton pellet weight of rats in the control group = mean increase in cotton pellet weight of rats in the drug treated group.

#### Statistical analysis

The mean ± SEM values were calculated for each group. Significant difference between groups was determined using analysis of variance (ANOVA)

followed by Dunnett's t test. P<0.05 was considered as significant.

## Results and Conclusion

### Phytochemical investigation extract and toxicity

After extraction, the Physical analysis of hydroalcoholic extract of *Quisqualis Indica* (HEQI) was observed like Color-Dark blackish brown, Odor-Aromatic, Consistency-Slightly Sticky, State-Semisolid, Percentage yield-22.47% w/v and hydroalcoholic extract of *Quisqualis Indica* freely soluble in distilled water. Preliminary Phytochemical studies of the hydroalcoholic extract of *Quisqualis indica* was performed for major classes of constituents like alkaloids, carbohydrates Saponins, tannins, flavonoids and phenolic compounds are found positively in hydroalcoholic extract of *Quisqualis indica* whereas glycosides, steroids, protein and amino acid is negative The 1/10th of maximum tolerable dose 1500 mg/kg was selected for the present studies. The dose selected was 100 mg/kg and 150 mg/kg.

### Acetic acid-induced vascular permeability

Effect of HEQI extract (100 and 150 mg/kg), Diclofenac.sod. (10 mg/kg) and control vehicle on acetic acid-induced increased vascular permeability in rat was studied. Results of the activity showed that HEQI at dose (100 and 150 mg/kg) significantly inhibited (Table no.1) the vascular permeability (20.35%, 33.54 and 43.13% respectively) when compared with vehicle control group (Graph no.1) Acetic acid induced vascular permeability indicates acute phase of inflammation where there is increased vascular permeability and migration of leukocytes in to the inflamed area occurs<sup>11</sup>. Decreased concentration of dye with respected to absorbance indicates reduction in permeability

### Cotton pellet-induced granuloma

In cotton pellet induced granuloma formation study, regarded as an animal model for sub acute inflammation, there was a statistically significant (p<0.05) reduction in granuloma formation at all doses in comparison to the control group. (Table no 2)The extract exhibited 10.1% and 41.5 % inhibition of granuloma formation at the doses 100 and 1500mg/kg b.w respectively, whereas diclofenac sodium showed 57.30% when compared to control. (Graph no.2) The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation<sup>12</sup>. The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue. It is well known fact that diclofenac sodium act by inhibiting the prostaglandins synthesis at the late phases of inflammation. This effect

may be due to the cellular migration to injured sites and accumulation of collagen, an important mucopolysaccharide<sup>13</sup>. Decreasing granuloma tissue, prevention of occurring of the collagen fiber and suppression of mucopolysaccharids are indicators of the ant proliferative effect by NSAIDs

The present study reveals that, hydroalcoholic extract of *Quisqualis indica* (HEQI) has anti-inflammatory activity in acetic acid-induced vascular permeability and cotton pellet granuloma model. In both model they exhibited anti-inflammatory effect in a dose Dependent manner which can be comparable with that of Diclofenac.sod.. The phytochemicals analysis revealed the presence of polyphenols and flavonoids. The polyphenols have potent anti-inflammatory activity by inhibiting prostaglandin synthesis<sup>14</sup>. So anti inflammatory activity of Hydroalcoholic extract of *Quisqualis indica* can be attributed to bradykinin and PG synthesis inhibition property of polyphenols. Besides, to isolate the active constituents and clarify its mechanism of action will be our auxiliary objective.

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**Table 1: Acetic acid-induced vascular permeability**

Group	Treatment (n=6)	Dose mg/kg	Absorbance's 590 nm	Inhibition (%)
1.	Control (distil water)	-	0.1655±0.00205	-
2.	HEQI	100	0.13275±0.0023*	20.35
3.	HEQI	150	0.11075±0.0016*	33.54
4.	Diclofenac.sod.	10	0.09425±0.0014*	43.13

(n=4), \*p<0.05 as compared to vehicle treated group. (Statistically analysed by One-way analysis of variance (ANOVA) followed by (Dunnett) multip comparison test.

**Table 2: Cotton pellet-induced granuloma**

Group	Treatment (n=4)	Dose mg/kg	Granuloma dry wt mean±sem	Inhibition (%)
1.	Vehicle(distil water)	-	67.6675±0.6013	-
2.	HEQI	100	60.8275±0.613*	10.1
3.	HEQI	150	39.3075±0.541*	41.5
4.	Diclofenac.sod.	10	28.455±0.6213*	57.3

(n=4), \*p<0.05 as compared to vehicle treated group. (Statistically analysed by One-way analysis of variance (ANOVA) followed by (Dunnett) multip comparison test

