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Anti-inflammatory Effect of *Quassia indica* leaf extract And Role of Antioxidant activity

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ABSTRACT

The plant Quassia indica belongs to the family Simarubiaceae is found mainly in India and Srilanka. This plant has been widely used in traditional system of medicine in India. The in-vitro antioxidant activity of methanolic extract of Quassia indica leaves were estimated by DPPH radical scavenging method. Anti inflammatory activity of Quassia indica leaves was tested against carrageenan induced paw edema in wistar albino rats. Here diclofenac used as standard drug for comparison. The study reveals that it possesses significant anti-inflammatory and antioxidant activity.

Key words: *Quassia indica, Antioxidant, Anti-inflammatory, carageenan, diclofenac.*

INTRODUCTION

Herbal drugs constitute a major part of therapeutics in all the traditional systems of medicine. There are approximately 1250 Indian medicinal plants, which are used in formulating therapeutic preparations according to Ayurveda and other traditional systems of medicine. Free radicals can also react with DNA, proteins or lipids in the cell membrane and cause cellular damage which is the root cause of many diseases. Flavonoids are phytochemicals known to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, neuroprotective, anticancer activities. *Quassia indica* (simarubiaceae) is one such bitter plant which is abundantly grown and used as important medicinal plant. However much of its medicinal importance is not assessed. By literature survey it is found that the leaves of the plant contain main phytochemical constituents such as quassinoids, samaderin b, samaderin c. Earlier studies shows that its related species shows anti-oxidant and anti-inflammatory activity, due to the presence of flavanoids [1-5].

MATERIALS AND METHOD

The fresh leaves of *Quassia indica* was collected from the locally growing area of Allapuzha, Kerala in February 2012. The plant was identified and authenticated by Dr. K.V.George, Department of Botany, C.M.S College Kottayam, Kerala. A herbarium specimen is deposited in our college museum. (UCP/MGU/RIMSR/2012/herb9).

Extraction:

The leaves were shade dried and coarsely powderd; 100 gm of powder were soaked in 500ml of methanol for 3-5 days with intermittent shaking. At the end of the extraction, it was passed through whattman filter paper. This filtrate was concentrated under reduced pressure on rotary evaporator .The yield was 16.4 % w/w and subjected to phytochemical screening to identify the various phytoconstituents.

Experimental animals:

Female rats of thirty healthy weighing 150-200gms and three female albino mice weighing between20-25gms were obtained from the animal house of UCP, Regionial institute of medical sciences and housed in polycarbonate cages. The animals were housed under standard conditions. Approved at the Institutional Animal Ethics Committee (IAEC) of UCP, RIMSR was taken for conducting Anti-inflammatory activity.

Acute toxicity study:

Acute toxicity study was performed to ascertain safe dose by Organization of Economic Cooperation and Development (OECD) 423 guidelines [6].

Antioxidant activity of *Quassia indica* leaf extract: *Invitro*
DPPH free radical scavenging assay:

The free radical scavenging capacity of the extracts was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. Methanolic extract of *Quassia indica* was mixed with 95% methanol to prepare the stock solution. DPPH solution was taken in test tubes and *Quassia indica* extracts was added followed by serial dilutions from100 µg to 500 µg to every test tube, made up to final volume 3 ml, the absorbance was read at 515 nm using a spectrophotometer after 10 min. Ascorbic acid was used as a reference standard which dissolved in distilled water to make the stock solution with in the same concentration 5mg/ml [7, 8].
% scavenging of the DPPH free radical was measured using the following equation = (Absorbance of the control - absorbance of the test sample) / (absorbance of the control) *100

Anti-inflammatory activity by Carrageenan induced paw edema in rats: *Invivo:*
Experimental procedures:

Wistar albino rats weighed around 150 to 250 were used for this study. The initial right hind paw volume of the rats was measured using a plethysmometer. They were divided into 5 groups consists of 6 rats each.

Group-1: Negative control which received vehicle distilled water only.

Group-2: Positive control which received carrageenan 0.1ml 1% intra plantar.

Group-3: Standard which received only diclofenac sodium 5mg/kg p.o+ carrageenan 0.1ml 1% intra plantar.

Group-4: Test group which has received the extract 200 mg/kg p.o+ carrageenan 0.1ml 1% intra plantar.

Group-5: Test group which has received the extract 400 mg/kg p.o+ carrageenan 0.1ml 1% intra plantar.

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Procedure:

Anti-inflammatory activity was assessed by test group's methanolic leaf extact 200,400 mg/kg and negative control vehicle, standard group diclofenac sodium was given orally. After 30 min, the rats were challenged with intra plantar injection of 0.1 ml of 1% w/v solution of carrageenan into the sub plantar region of paw. The paw was marked with ink at the level of lateral malleolus. The paw volume was measured at 1, 3, 6 hours after carrageenan injection using a Plethysmograph. The difference between initial and subsequent reading given the actual edema volume. The anti-inflammatory activity in animals that received *Quassia indica* extracts and diclofenac (5mg/kg) was compared with that of vehicle control groups [9, 10].

Statistical analysis:

Results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Dunnett's t test. *P<0.05, **<0.01 and ***<0.001, show statistical significance when compared treatment group with control group.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis revealed the presence of alkaloids, tannins and phenolic compounds, triterpenes, carbohydrate, flavanoids in methanolic leaf extract. Acute toxicity studies for methanolic leaf extracts of *Quassia indica* belonging to the family Simarubiaceae were conducted as per OECD guidelines 423 using albino Swiss mice. The extracts were found to be safe up to 2000 mg/kg body weight since no death and signs of toxicity were observed.

Free radicals involved in the process of lipid peroxidation are considered to play a cardinal role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others, and are implicated in the ageing process. Methanolic leaf extract of *Quassia indica* have demonstrated dose dependent increase in the DPPH radical scavenging activity. **Table. 1**. The IC50 value of MEQI extract was found to be 190µg/ml. Therefore, the extract were assessed against scavenging of free radicals like DPPH and compared against standard shows good antioxidant activity **Fig.1**.

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants. In Carrageenan-induced rat paw edema, the injection of carrageenan, the phlogistic agent, caused localized edema starting at 1 hour after injection. In pre-treated with diclofenac (5 mg/kg, p.o) had a significant reduction of rat paw edema at 1 hour after the diclofenac administration. Methanolic leaf extract at a dose of 200, 400 mg/kg, p.o exhibited the anti-inflammatory effect to reduce the rat paw edema at 1 h after administration and continued up to 6 hours. The inhibition of rat paw edema of the MEQI at the dose of 200 mg/kg, p.o shows 43, 36 and 6.7% respectively while the MEQI at the dose of 400 mg/kg, p.o decreased the rat paw edema by 43, 31, 11% respectively when compared to the control it shows higher level of significance at the 3rd hour (p<0.001) as shown in **Table. 2.** In the present study, the methanolic extract of *Quassia indica* at doses of 200,400 mg/kg significantly decreased the rat paw edema induced by carrageenan, suggesting methanolic extract may involve inhibition of these inflammatory mediators release in all phases showing a good anti-inflammatory activity **Fig.2**.

Table No. 1: Comparison of the DPPH free radical scavenging activity

S. No.	Concentration µg/ml	% scavenging Activity	
		MEQI	**Ascorbic acid**
1	100	34.55±0.1102***	65.013±0.013
2	200	52.86±0.0585***	73.14±0.0100
3	300	65.65±0.0105***	80.06±0.0200
4	400	69.86±0.0152***	83.92±0.0152
5	500	76.29±0.0458***	93.16±0.1528

Values are mean ± SD, n=3, p<0.001=***, indicates extremely significant increase in Scavenging of DPPH radical by MEQI as compared with Ascorbic acid.

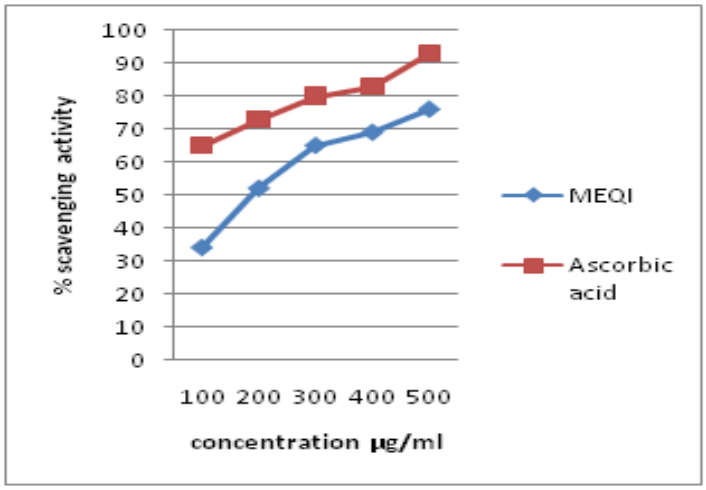

Fig.1: Comparison of the DPPH free radical scavenging activity

Table No. 2 : Carragenan induced paw edema in rats

Groups	Paw volume (ml)			% Inhibition		
	1h	**3h**	**6h**	**1h**	**3h**	**6h**
Normal	0.625 ± 0.11	0.633 ± 0.10	0.625 ± 0.11			
control	1.625 ± 0.01	1.325 ± 0.02	0.875 ± 0.02			
standard	0.908 ± 0.01	0.733 ± 0.01	0.625 ± 0.01	44	39	15
MEQI 200	0.933 ± 0.01	0.780 ± 0.02***	0.691 ± 0.02	43	36	6.7
MEQI 400	0.916 ± 0.61	0.785 ± 0.02***	0.658 ± 0.01	43	31	11

All values are Mean±SEM, n=6, **P<0.01, *** P<0.001 represents statistical significance of treatment group Vs control.

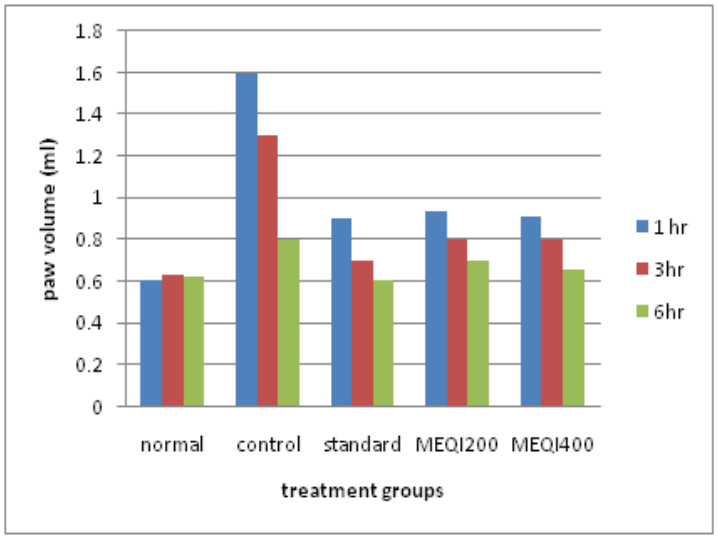

Fig. 2: Effect of MEQI in carragenan induced paw edema in rats

CONCLUSION

In summary, in the present investigation it could be concluded that MEQI at tested doses exhibited the pharmacological activities; it shows the anti-inflammatory activity in acute inflammation. The possible mechanism of action may involve inhibition of inflammatory mediators release (histamine, serotonin, bradykinin, prostaglandins and TNF-α in both phases. The DPPH free radical scavenging activity results are indicating that antioxidant principles are also having role in this leaves. Future studies are focusing on the isolation of active constituent responsible for the activity formulate and compare with the commercially available preparation.

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