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Anti-inflammatory Activity of Methanol Extract of *Syzygium Alternifolium* in Experimental Rats

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ABSTRACT

The *Syzygium alternifolium* (SA) is a popular medicinal plant used in traditional systems of medicine in India and belongs to family Myrtaceae. It is mainly used in inflammation conditions by local tribal's as folklore medicine. This study was intended to evaluate the anti-inflammatory activity of methanol extracts of Syzygium alternifolium leaves in carrageenan induced paw edema in Wister rats at the dose level of 200 and 400mg/kg administrated per orally. Both the extracts exhibited significant anti-inflammatory activity, which supports the folklore claims of the traditional system of medicinal usage of the plant. This study established anti-inflammatory activity of Methanol extract of *Syzygium alternifolium* (MESA).

Key words: Anti-inflammatory, Syzygium alternifolium, Carrageenan.

INTRODUCTION

Syzygium alternifolium (myrtaceae), is a endemic plant and available in Tirumala hills Chittoor district, Andhrapradesh 'was pharmacologically proven to possess hypoglycemic, antibacterial, anti-HIV activity and anti-diarrhoeal effects (Bhuiyan MA *et al*., 1996; Indira G *et al*., 1993; Kusumoto IT *et al*., 1995; Mossa JS *et al*., 1995; Muruganandan S *et al*., 2001; Ravi K *et al*., 2004; Slowing K *et al*., 1994) reported the anti-inflammatory activity of leaf and barks. Hence the present study has been made to investigate the anti-inflammatory effects of Syzygium alternifolium leaves in Wister rats.

METHODS AND MATERIALS

Plant materials

The fully matured Syzygium alternifolium leaves were collected in the months of June-Jully 2008 from Tirumala Hills, Chittoor District, and Andhrapradesh, India from a single Tree. The leaves are identified and authenticated by Botany department of Sri Venkateswara University, Tirupati, AP, India.

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Animals

Wistar rats of either sex weighing 160-180g were purchased from DRDE Gwalior for experimental study. They were acclimated to animal house conditions fed with commercial pelleted rats chow (Hindustan lever ltd., Bangalore, India), and had free access to water. The experimental protocol was approved by the IAEC (institutional animal Ethical Committee of CPCSEA and supervision of Experiments on Animal).

Chemicals

Diclofenac sodium, ethyl acetate and methanol.

Preparation of extracts

The Syzygium alternifolium leaves were first washed several times with distilled water. The leaves were shade dried at room temperature and coarsely powdered by using mechanical grinder and sieved with the sieve No.40 to get uniform sized powder. The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with methanol using cold percolation method. The percentage yield is 10.46% in methanol.

Preparation of the Drug for the experimental study

Extracts and the standard drugs were administered in the form of suspension in water with 1% sodium carboy methyl cellulose (SCMC) as suspending agent.

Qualitative phytochemical analysis

The qualitative phytochemical analysis was carried out for the presence of detection of the following compounds. The leaf extract was subjected to detect the alkaloids by using with Mayers test, flavanoids with alkaline reagent, carbohydrates with Molish reagent, Glycosides with legal's test, saponins using sodium bicarbonate, tannins using lead acetate, Phytosterols with Salkowsk's test, phenols with ferric chloride, Triterpenoids with libermann bucherd test, anthroquinones with concentrated sulfuric acid, benzene and ammonia and amino acids with Ninhydrin test. These were identified by characteristic color changes by using standard procedures laid down in the Khandalwal.

Acute toxicity studies

Acute oral toxicity (Ecobichon DJ, 1997), study was performed as per OCED-423 guidelines (acute toxic class method).Wister rats (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 5mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals then the same dose was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50,300 and 2000mg/kg body weight.

Anti-Inflammatory activity

The animals either sex was divided into six groups each composed of six animals.

Group I -control animals received 1% SCMC 10ml/kg p.o

Group II -Animals received ethyl acetate extract the dose of 200mg/kg p.o

Group III -Animals received ethyl acetate extract at the dose of 400mg/kg p.o

Group IV - Animals received Methanolic extract at the dose of 200mg/kg p.o

Group V - Animals received Methanolic extract at the dose of 400mg/kg p.o

Group VI -Standard diclofenac sodium 5mg/kg,p.o

The percent inhibition was calculated by using the following formula.

Percentage inhibition of oedema= $V_C - V_t / V_C$ X100

Where, V_C is the inflammatory increase in paw volume in control group of animals and V_t is the inflammatory increase in paw volume in drug-treated animals. Paw oedema was induced injecting 0.1ml 1% carrageenan in physiological saline into the sub plantar tissues of the left hind paw of each rat (Winter CA *et al*., 1969). The extracts (Ethyl acetate and methanol) were administered orally 30 min prior to carrageenan administration. The paw volume was measured at intervals of 60,120,180 and 240 min by the mercury displacement method using plethysmograph. The percentage inhibition of paw volume in drug treated group was compared with the carrageenan control group (Group- I).Diclofenac sodium (5mg/kg/p.o) was used as reference drug.

Statistical analysis

Data obtained from pharmacological experiments are expressed as mean ±Sem. Difference between the control and the treatments these experiments were teased for significance using ANOVA followed by Dunnet's (Dixon WJ, 1990).

RESULTS AND DISCUSSION

The plant extracts did not exhibit any mortality up to then dose level of 2000mg/kg.so, the extracts safe for long term administration. The ethyl acetate and methanol extracts of s.cumini leaves at the dose level of 200 and 400mg/kg decreased the oedema significantly ($p<0.001$) at 3rd and 4th h after administration of the extract .When compared to the control group. The effect was compared to the activity ($p<0.001$) produced by standard drug diclofenac sodium at 3rd and 4th after administration (Table.1).

Table.1 Assessment of anti-inflammatory activity of MESA in experimental Rats

Group	Paw oedema volume (ml)			
	60 min	120 min	180 min	240 min
I	0.35 ± 0.14	0.49 ± 0.02	0.65 ± 0.01	0.67 ± 0.01
II	0.28 ± 0.16** (20 %)	0.39 ± 0.02* (20.4 %)	0.39 ± 0.02*** (40 %)	0.31 ± 0.02*** (53.7 %)
III	0.25 ± 0.01** (28.6 %)	0.38 ± 0.02* (22.4 %)	0.38 ± 0.02*** (41.5 %)	0.27 ± 0.03*** (59.7 %)
IV	0.28 ± 0.02** (20 %)	0.38 ± 0.03* (22.4 %)	0.38 ± 0.03*** (41.5 %)	0.30 ± 0.03*** (55.2 %)
V	0.24 ± 0.01 *** (31.4 %)	0.37 ± 0.01** (24.5 %)	0.35 ± 0.02*** (46.1 %)	0.25 ± 0.03*** (62.6 %)
VI	0.22 ± 0.01*** (37.1 %)	0.36 ± 0.01** (36.1 %)	0.27 ± 0.02*** (58.4 %)	0.17 ± 0.10*** (74.6 %

Values are mean ± SEM of 6 animals in each group. Comparisons were made between Group I Vs II, III, IV, V and VI. P-values: *p<0.05, **p<0.01, ***p<0.00., Percentage protection given on Parenthesis.

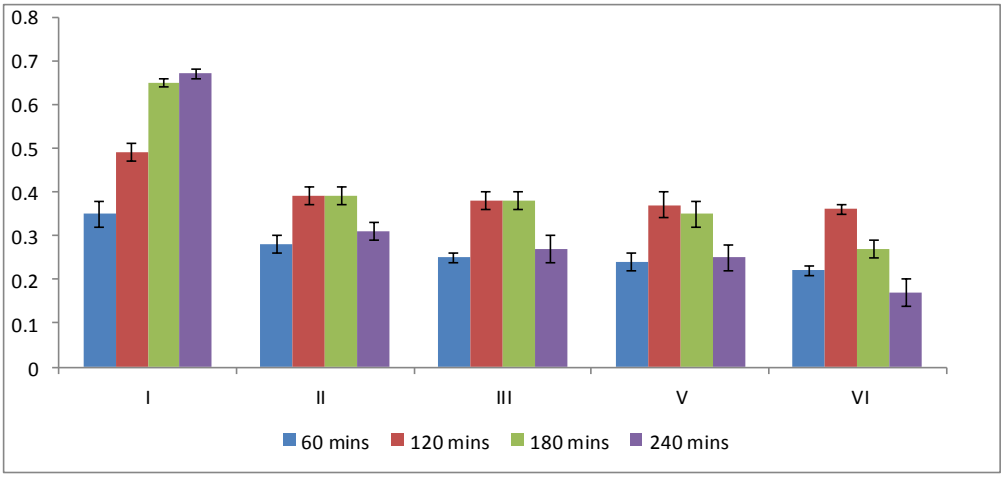

Fig.1 Assesment of anti-inflammatory activity of MESA in experimental Rats

In the present study, anti-inflammatory activity of the ethyl acetate and methanol extracts of MESA leaves has been established .The extracts were found to significantly inhibit the carrageenan-induced rat paw oedema, a test which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (Mossa JS *et al*., 1995). Carrageenan induced inflammation is useful in detecting orally active anti- inflammatory agents (Di Rosa M *et al*., 1971). Oedema formation due to carrageenan in the rat paw is a biphasic event (Vinegar R *et al*., 1969). The initial phase is attributed to the release of histamine and serotonin (Crunkhon P *et al*., 1971). The extracts of Syzygium alternifolium leaves possessed varying degree of anti- inflammatory activity when tested at various doses of 200 and 400 mg/kg .The methanol extract at the dose of 400 mg/kg showed high significant anti-inflammatory activity at 4 h ,where it caused 62.6% inhibition, as compared to that of 5 mg/kg of diclofenac sodium.

CONCLUSION

In conclusion, the results of the present study support to the traditional use of Syzygium alternifolium leaves extract, possessing significant anti- inflammatory activity. This may be due to the presence of triterpenoids, saponins and tannins which deserves further studies to establish its therapeutic value as well as its mechanism of action.

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