International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.2, pp 763-768, April-June 2011

Influence of Methanolic Extract of *Avicennia officinalis* leaves on Acute, Subacute and Chronic Inflammatory Models

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Abstract: The present study was designed to investigate anti-inflammatory activity of crude methanolic extract of *Avicennia officinalis* leaves on acute (carrageenin), Subacute (Formalin) and chronic (Freunds Complete Adjuvant) rat paw odema model. The extract of dose 200 and 400 mg/kg b.wt given orally and acetyl salicylic acid as standard was given intraperitonially. Biochemical parameters like SGOT, SGPT, ALP, total cholesterol were also estimated as supportive studies. The extract showed its effect on dose dependent manner. Also the extract restored the biochemical parameters when compared to control group and were statistically significant. Preliminary phytochemical screening of the extract showed the presence of alkaloids, triterpene and tannins. The chemical test of the extract showed the presence of triterpene - betulinic acid. Since the literature review proved the anti-inflammatory activity of betulinic acid in other medicinal plants. The anti-inflammatory activity of methanolic extract of *Avicennia officinalis* may be due to the presence of the phytoconstituent, betulinic acid.
Keywords: *Avicennia officinalis*, Freunds adjuvant, Carageenin, Formalin, betulinic acid.

Introduction

Avicennia officinalis forms part of mangrove vegetation in the form of halophytic shrubs or small trees.It is an ever green tree found sporadically on the banks of river and rarely found near the sea. Fruits are plastered on to boils and tumours in India. a poultice of unripe seed and leaves stop inflammation Roots are aphrodisiac. bark is used to treat skin problems especially scabies, resin for snake bite, seed for ulcers and bitter resin used as contraceptive by women. The Phytochemicals reported were pentacyclic triterpenoids [1]such as lupeol, betulin, betulinaldehyde,betulinic acid,beta-sitosterol.Iridoid glucosides having c-11 carboxylic acid group were also present. Other compounds present were flavanoids ,alkaloid, steroids, tannins,wax esters.[2]

Materials and Methods

Collection and extraction

The plant was collected from machilipatnam port area of Krishna District of Andhra pradesh. The botanical identity of the plant material was confirmed with the help of Dr. Jayaram, Professor of national institute of herbal science . Leaves were shade dried and cextracted with methanol by simple maceration.

Toxicity studies

According to OECD guide lines 423 female rats were selected and proceded. There was no signs of toxicity up to 4000mg /kg body weight.Based on the results obtained from this study, the dose for antii-nflammatory activity was fixed to be 200mg/kg b.wt. and 400mg/kg/b wt for dose dependent study.

Grouping of animals
Totally 5groups of 6 animals in each group which were maintained at laboratory conditions and provided with pellets adlibitum.
Group-1

Inflammatory studies

ACUTE MODEL -Carrageenin-induced paw edema[3,4]
Rats were divided into 4 groups (n=6 each). One group was used as a negative control and received 0.25%sodium carboxymethylcellulose (CMC) solution (4 ml/
kg). The positive control group received indomethacin(10 mg/kg, p.o.), while the other groups received AO extract in different doses of 200,400 mg/kg orally in 0.25% CMC. Inject 0.1 ml of 0.3% carrageenin in saline solution in the subplantar aponeurosis of the right hind paw according to Winter.et.al as modified by Sugishita.et.al. The increase in paw volume was measured at1-h interval until 5 h after injection of carrageenin by using a plethysmometer.

SUBACUTE MODEL -Formalin induced edema in rat paw[5]
Formalin 0.1 ml (2% in distilled water) was injected into the subplanter area of right hind paw of wistar rats (Chau,1989). extract at doses of 200 and 400 mg/kg or diclofenac
sodium 10 mg/kg were given 30 min prior to formalin injection and subsequently for 7 consecutive days. The paw volume was determined by plethysmographic method initially
and on seventh day to measure degree of inflammation

CHRONIC MODEL -Adjuvant induced arthritis[6]
Experimental induced arthritis was induced in rats according to the method of Newbould..The right foot pad of each rat was injected subcutaneously with 0.1 ml of Freund's complete adjuvat agent(FCA).The extract at two dose levels(200,400m/kg/b.wt), distill water and acetyl salicylic acid at 10mg/kg were given daily for 16 consecutive to concern groups respectively. Treatment started from day 8th after FCA injection. The edema of the left and right hind paws was evaluated at 2h,8,12,16,and 24 days post injection of FCA

Biochemical investigational procedures[7,8,9]
Animals were sacrificed on 24th day and blood collected by retroorbital plexus and the biochemical parameters like SGOT, SGPT, ALP, and total cholesterol were measured according to kit procedure in serum of the normal, CFA induced rats, and extract treated rats.

Table 1: Effect of *Avicennia officinalis* on Carragenan induced paw edema in rats

Treatment	Dose(mg/kg)	Increase in paw volume at time(ml)					% inhibition
		1hr	2hr	3hr	4hr	5hr	
Control	4 ml/kg	0.616± 0.01	0.61± 0.01	0.661± 0.02	0.732± 0.02	0.766± 0.01	-
Indomethacin	10	0.441± 0.02***	0.50± 0.03**	0.57± 0.03**	0.5± 0.02***	0.466± 0.02***	39.16
AO Extract	200	0.641± 0.01(ns)	0.625± 0.01(ns)	0.641± 0.01(ns)	0.64± 0.01**	0.608± 0.01***	20.62
AO Extract	400	0.60± 0.02(ns)	0.585± 0.02(ns)	0.56± 0.02**	0.56± 0.02***	0.53± 0.01***	30.80

Values are expressed as mean± S.E.M (n=6)
Statistical significance was calculated by ANOVA followed by Kruskal-wallis test.
** $p < 0.01$, *** $p < 0.001$ significant as compared to the control group
Ns = non- significant
AO = *Avicennia officinalis*

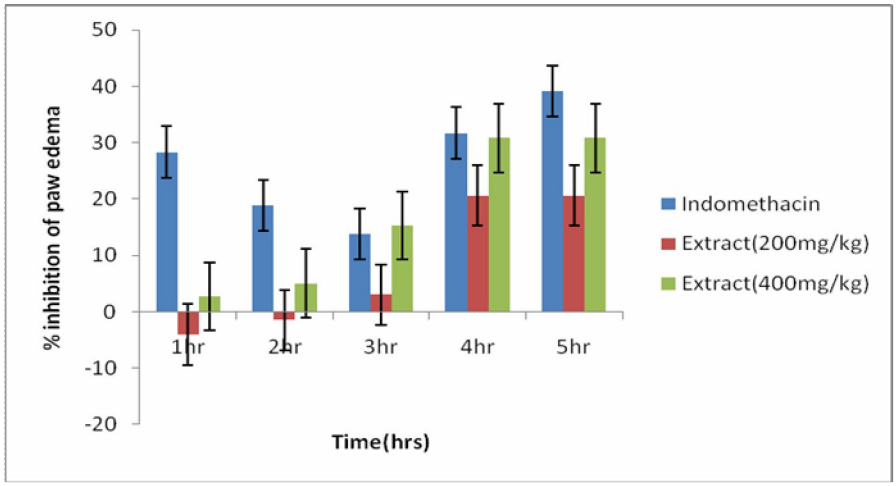

Table 2: Effect of *Avicennia officinalis* on Formalin induced hind paw edema in rats

Treatment	Initial paw volume (ml)	Paw volume on 7th day	% inhibition
Control(cmc) (4ml/kg)	0.776±0.01	0.816±0.01	-
Diclofenac sodium (10 mg/kg)	0.548±0.01***	0.501±0.01***	38.52
AO extract (200mg/kg)	0.631±0.02***	0.596±0.01***	26.88
AO extract (400mg/kg)	0.55±0.01***	0.535±0.01***	34.43

Values are expressed as mean± S.E.M (n=6)
Statistical significance was calculated by ANOVA followed by Kruskal-wallis test
*** $p < 0.001$ significant as compared to the control group
AO = *Avicennia officinalis*

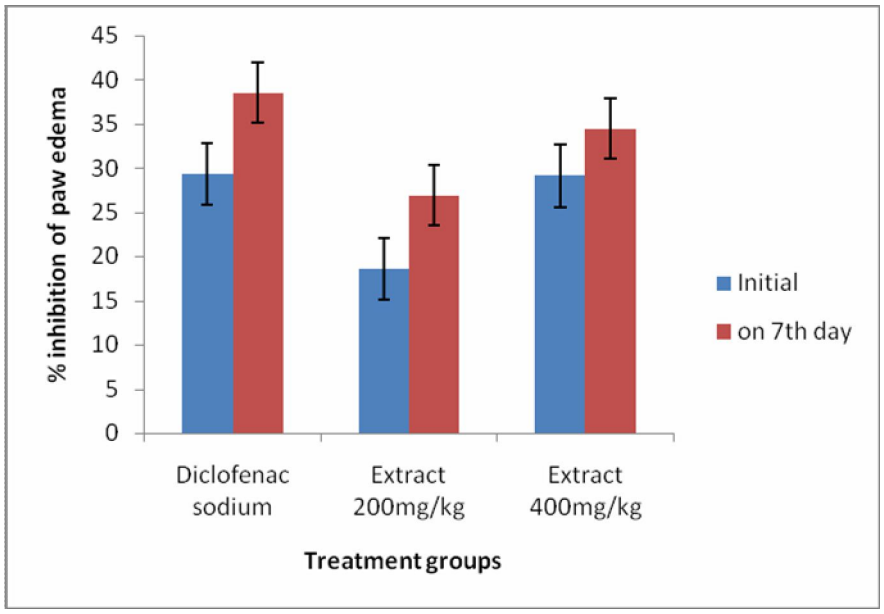

Table 3: Effect of *Avicennia sofficinalis* on FCA-induced paw edema in rats

treatment	paw volume at(ml)						% inhibition
	2hrs	8thday	12thday	16thday	20thday	24thday	
CFA	0.66± 0.01	0.69± 0.02	0.73± 0.01	0.80± 0.02	0.85± 0.01	0.88± 0.01	-
ASA	0.61± 0.02**	0.53± 0.01(ns)	0.485± 0.01**	0.43± 0.01***	0.39± 0.01***	0.392± 0.01***	55.45
Extract (200mg/kg)	0.64± 0.01**	0.66± 0.01(ns)	0.60± 0.01**	0.53± 0.01***	0.47± 0.03***	0.47± 0.03***	46.59
Extract (400mg/kg)	0.67± 0.01**	0.66± 0.01(ns)	0.57± 0.01**	0.51± 0.01***	0.43± 0.01***	0.41± 0.01***	53.40

Values are expressed as mean± S.E.M (n=6)
Statistical significance was calculated by ANOVA followed by followed by Kruskal-wallis test
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Ns = non- significant
AO=*Avicennia officinalis*

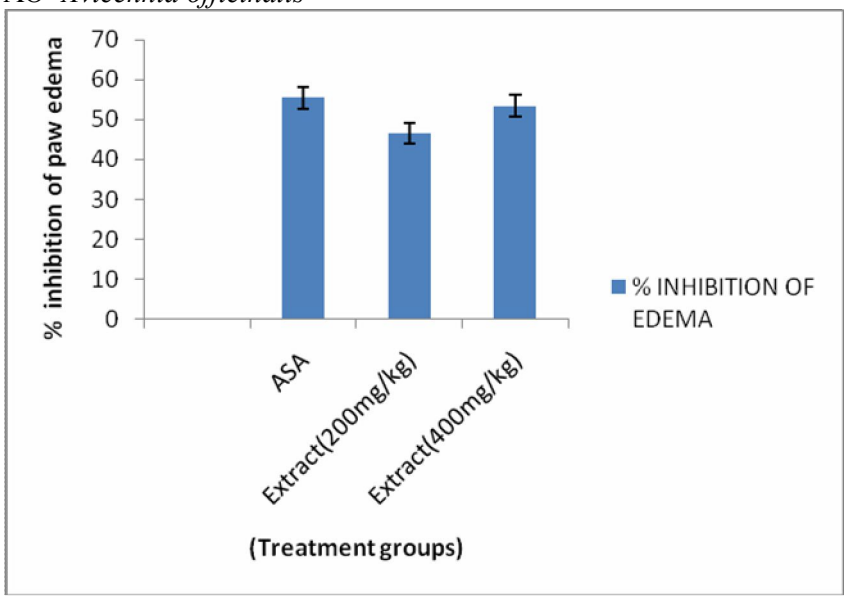
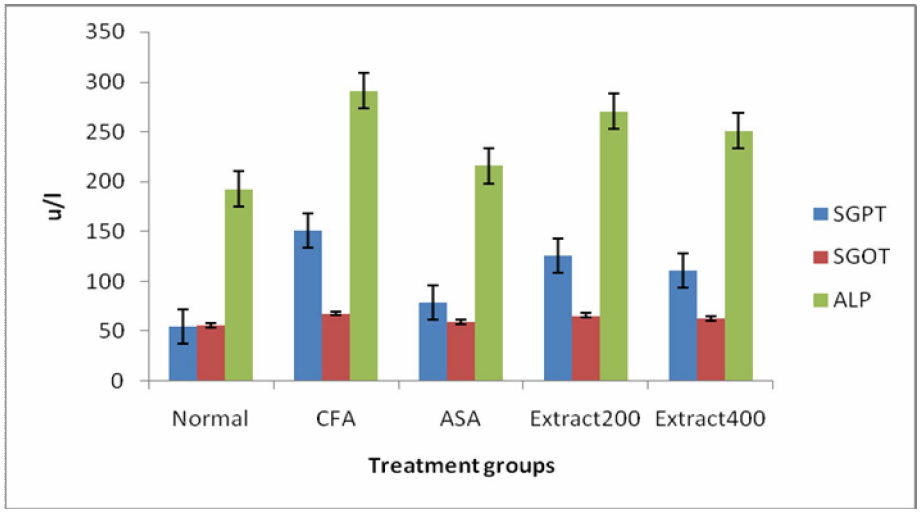
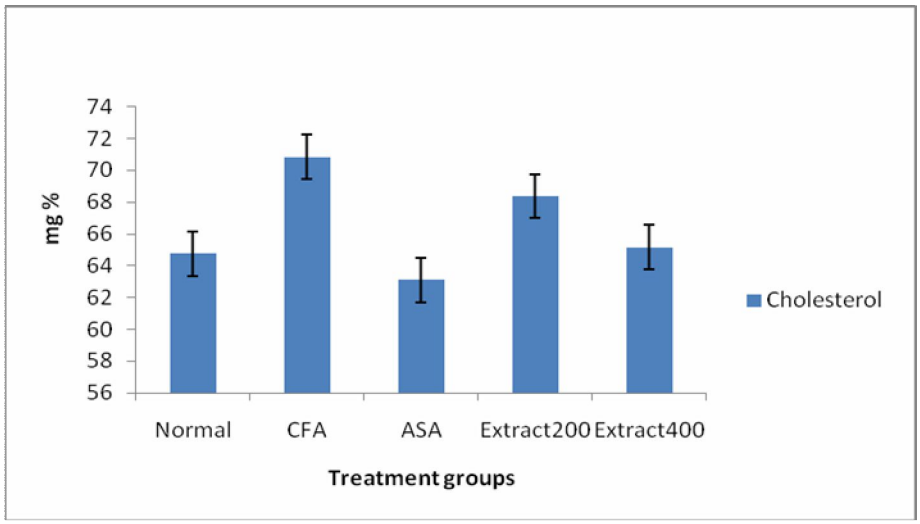


Table 4: Effect of *Avicennia officinalis* on Biochemical parameters against CFA induced arthritis in rats

Treatment	Dose (mg/kg)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	CHOLESTEROL Mg%
Normal	-	54.51± 0.45	55.74± 0.69	192.4± 2.02	64.75±1.31
CFA	0.1ml	150.9± 0.86	67.84± 0.50	290.85± 0.82	70.85±0.74
ASA	10	78.96± 0.70***	59.31± 0.62***	216.01± 3.61***	63.11±0.764***
AO extract	200	125.96± 1.55***	65.71± 0.92(ns)	270.45± 1.34***	68.36±0.29(ns)
AO extract	400	111.21± 3.83***	63.1± 0.41***	250.86± 1.71***	65.18±0.29***

Values are expressed as mean± S.E.M (n=6)
Statistical significance was calculated by ANOVA followed by followed by Kruskal-wallis test
*** p < 0.001 significant as compared to the control group
Ns = non- significant
AO=*Avicennia officinalis*





Results and Discussion

In this plant preliminary phytochemical studies revealed that tri-terpenoids, iridoids glucosides, tannins, steroids and flavanoids were present. **Table 1,** showed the effect of extract on carrageenen induced model ,produced significant anti-inflammatory activity during 3-5 hours after administration in dose dependent manner compare to control and nearly equal to standard, this may be due to inhibition of prostaglandin, but it was not significant in initial phase (1-2 hours) [10]

In formalin induced model, the extract produces significant effect on 7th day **(Table 2)** compared to initial response and the extract at the dose of 400mg/kg showed good result compared to control but the exact mechanism is not clearly understood.

Freund's adjuvant –induced arthritis have been used as a model of sub-chronic or chronic inflammation in rats and is of considerable relevance after the study of patho-physiological and pharmacological control of inflammatory processes as well as the evaluation of anti-arthritic or anti-inflammatory effects of drugs[11,12]of the reasons for the wide utilization of this model is due to the strong correlation between the efficiency of therapeutic agents in this model and in rheumatoid arthritis in humans[13]. In **table 3** the effect of MEAO in FCA induced arthritis were tabulated. The report suggest that arthritis was significantly reduced in dose dependent manner and were statistically significant.

In table 4, the biochemical changes in CFA induced models were tabulated. As a result of arthritis induced by CFA, the level of the Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase(SGOT), and Alkaline Phosphate(ALP) is increased. Accordingly, the assessment of the level of SGOT, SGPT and ALP provides a good and simple tool to measure the anti-inflammatory activity of the target compounds. After

administration of *Avicennia officinalis,* the level of these enzymes reduced significantly when compared to arthritic control animals.

The MEAO showed better and good result in a dose dependent manner compared to control and standard and all the results are statistically significant. The extract possess good anti-inflammatory activity and was effective in chronic model than the acute model.

Conclusion

Our results shows that the methanolic extract of *Avicennia officinalis* possess significant anti-inflammatory, which may be due to betulinic acid (which had been proved for its anti-inflammatory activity) present in the leaves. Further study has to be carried out to isolate and characterise betulinic acid and precise mechanism based on receptor level to be determined.

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