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Evaluation of Antiinflammatory Activity of the Aerial Parts of *Cocculus hirsutus.Linn* in Rats

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ABSTRACT

The present study was carried out in wister rats for evaluation of anti-inflammatory activity of four extracts (pet. ether, chloroform, ethanol & aqueous) of the aerial parts of *Cocculus Hirsutus.Linn* (C.H.L) .The LD_{50} studies were carried out by up & down method in albino mice following OCED guide lines (No: 425 of CPCSEA) up to the dose limit of 2000mg / kg, p.o. All extracts were administered 1/5th of the tested dose for LD_{50} dose to study the anti-inflammatory activity by carrageenan induced rat paw edema model. All the extracts of C.H.L were found to decrease the acute inflammation of rat paw significantly. Therefore, the present study suggests that the aerial parts of plant possess significant anti inflammatory property.

Keywords: *Cocculus Hirsutus Linn,* carrageenan, rat paw edema.

1. INTRODUCTION

Inflammation is a complex pathophysiological process. Inflammatory diseases including different types of rheumatic disorders are very common throughout the world [1].Prolong use of presently available synthetic agents have been associated with many side effects like gastro intestinal irritation; such possibility is negligible with medicinal plants. Medicinal plants have been used as remedies for various diseases from ancient time. In ancient civilization, people made use of different part of plants to cure various diseases including rheumatism. In India, several natural medicinal plants are available for the management of inflammatory diseases.

The plant *Cocculus Hirsutus Linn* (CHL) (Syn:kattukodi), Family: Minispermaceae is a widely growing plant found in the plains of India, in dry localities and is used medicinally by Indian tribes for wide range of ailments[2,3].The aqueous extract of the aerial parts and the roots are used for the treatment of rheumatism, Fever and also as laxative. Roots of CHL have been mention in traditional literature as bitter, acrid, alternative, laxative, demulcent, anti periodic in fever, tonic and diuretic [4]. The juice of leaves coagulates in water and forms mucilage, which is used externally as a cooling and soothing application in prurigo, eczema and impetigo [5]. Based on the literature survey this study was first time undertaken with objective to evaluate the anti-inflammatory activity of various extracts of aerial parts of plant.

2. MATERIALS AND METHODS

2.1. Plant material

The plant C.H.L was collected in and around Cumbum valley, Theni (District), Tamilnadu, India during the month of July 2011 & authenticated at The American College, Madurai by expert taxonomist (Dr D.Stephen, Prof, Botany Dept) where a voucher specimen has been retained.

2.2. Preparation of plant extract

The aerial parts were separated from plant, dried in shade and powdered in grinder. The obtained powder (600g) was subjected to successive soxhlet extraction with solvents with increasing order of polarity i.e., pet.ether (60°C-80°C), chloroform (59.5°-61.5°C), ethanol (64.5°-65.5°C) and water. Yield 3.29, 4.10, 6.29 & 5.71 % respectively. All extracts were concentrated by Buchi rotary evaporator (JSGW 118,800ml) under reduce pressure at 40°C

2.3. Test Animals

Wister rats (150-200gm) and Albino mice (20-25gm) of either sex were obtained from the animal house, Vasavi institute of pharmaceutical sciences, Kadapa. They were properly housed in separate cages and fed with standard diet (Amruth, Bangalore, India) and water *ad libitum*.Experiments on animal were performed by the following guidelines of Institutional Animal Ethical Committee.

2.4. Chemicals & Drugs

Diclofenac sodium (Dr.Reddy's Labs, Hyd), Tweens 80(S.D fine chem. Ltd, Mumbai), Carrageenan (Sigma, Mumbai), Pet. ether, Chloroform & Ethanol (Modern scientific, Nashik) were purchased for this study. The used solvent and chemicals were analytical grade.

2.5. Phytochemical studies

Preliminary phytochemical studies of various extracts of the aerial parts of the C.H.L were conducted according to the method of Trease & Evans [6].

2.6. Acute oral toxicity study

Acute oral toxicity was performed by up & down method in albino mice following OECD guidelines (No.425 of CPCSEA) up to the dose limit of 2000mg for kg p.o for 24hrs[7]. Acute inflammation was produced by carrageenan according to the standard method [8].The rats were divided in to six groups, each group consist of five animals. Tween 80 was used as a vehicle for suspending the extracts as well as standard anti-inflammatory drug diclofenac sodium. The group I served as control & received only vehicle. The group II received standard drug diclofenac sodium (10mg/kg p.o). Group III to VI were treated with pet ether, chloroform, ethanol & aqueous extract of the aerial parts of C.H.L respectively at the dose of 400mg/kg p.o. After 30 minutes of sample treatment, acute inflammation was produced by sub planter injection of 0.1ml of 1% carrageenan in normal saline in the right hind paw of rat. The paw volume was determined by plethysmometrically (UGO Basile, Italy) at 0, 1, 2 & 3 hr after carrageenan injection and the % anti-inflammatory activity was calculated as shown in table (1).

2.7. Statistical analysis

All the data were expressed as mean ± S.E.M. Data were assessed by the method of ANOVA followed by Dennett's test [9]. *P<0.05 and **P<0.01 were considered statistically Significant.

3. RESULT AND DISCUSSION

3.1. Phytochemical screening

Preliminary phytochemical screening of various extracts revealed the presence of carbohydrates, sterols, tannins, alkaloids, flavonoids and glycosides.

3.2. Acute oral toxicity study

In acute oral toxicity study, no mortality was observed up to the dose of 2000mg /kg p.o for 24 hours and 1\5th dose tested for L.D50 i.e.,400 mg /kg p.o was administered to the experiment animals to study anti inflammatory activity.

3.3. Carrageenan induced rat paw edema

In acute inflammatory model i.e., Carrageenan induced rat paw edema, all the four extracts exhibited significant inhibition of paw volume when compared to control and standard group. Ethanol extract showed potent anti inflammatory activity among the four extracts (table 1) and maximum reduction in paw volume was observed at third hour after administration of Carrageenan.

Table -1: Effect of various extract of the aerial parts of C.H.L on carrageenan induced rat paw edema

Group	Dose (mg/kg p.o.)	Mean increases in paw volume (ml)			
		0 hr	1 hr	2 hr	3 hr
Control	--	0.834±0.012	1.508± 0.085	1.982±0.087	2.17±0.011
Pet Ether	400	0.910±0.037	1.246±0.084* (17.37)	1.369±0.003 ** (30.92)	1.30±0.05** (40.09)
Chloroform	400	0.964±0.049	1.286±0.013* (14.72)	1.480±0.013** (25.32)	1.410±0.097** (35.02)
Ethanol	400	0.848±0.066	1.220±0.086* (19.09)	1.348±0.081** (31.98)	1.224±0.012** (43.53)
Aqueous extract	400	0.964±0.066	1.328±0.033* (11.93)	1.658±0.059** (16.34)	1.519±0.003** (29.95)
Diclofenac sodium	10	0.926±0.065	1.090±0.059* (27.71)	1.302±0.093** (34.30)	1.184±0.049** (45.30)

Values are mean ±S.E.M.
*- P<0.05, **- P<0.01 Significant compared with control values.
Figures in parentheses indicate the % anti inflammatory activity.

4. CONCLUSION

Carrageenan induced inflammation is biphasic event. The initial phase which last for one hour is attributed to the release of histamine and serotonin. The second phase which occurs at 3rd hr is related to release of bradykinin and prostaglandins [10, 11].The second phase is sensitive to most chemically effective anti-inflammatory drugs [12, 13]. Pet ether, chloroform, ethanol and aqueous extracts showed reduction in paw volume from 1st hr to 3rd hr. But maximum reduction in paw volume was observed at 3rd hr after administration of carrageenan. From the data it is evident that, the ethanol extract showed a greater percentage of decrease in paw volume at second phase which is related to the release of bradykinin and prostaglandin. The ethanol extract showed greater percentage (43.53%) of decrease in paw volume among the four extracts. The anti inflammatory effect of various extract of the aerial parts of C.H.L can be attributed to the presence of phytochemical such as alkaloids phenolic compounds, flavonoids and glycosides that were detected in our preliminary phytochemical studies. These result also commensurate with earlier reported phytochemicls of C.H.L i.e. essential oil, β-sistosterol, ginnol, glycosides, sterol and alkaloids [14, 15]. Thus the presence of sterols, alkaloids and glycosides in various extracts might suppress the formation of bradykinin and prostaglandin and exhibit significant anti inflammatory property. In conclusion this study has shown that the aerial parts of the plant possesses anti inflammatory activity and also support that the traditional use of this plant in rheumatic condition. A further detailed study for anti inflammatory is currently underway to isolate and characterize the active principle of the plant.

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