EFFECT OF ALCOHOLIC EXTRACT OF *TINOSPORA CORDIFOLIA* ON ACUTE AND SUBACUTE INFLAMMATION.

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Summary

To study the anti inflammatory activity of alcoholic extract of T. Cordifolia on carrageenan – induced hind paw oedema and cotton pellet granuloma models in male wistar rats. The hind paw oedema was produced by subplantar injection of carrageenan and the paw volume was measured plethysmographically at 0, 1, 2, 3, 4 and 5 hr. In sub acute model, Cotton pellet granuloma was produced by implantation of 50 ± 1 mg sterile cotton is axilla under ether anaesthesia. The animals were fed with ethanolic extract at various dose levels (125, 250, 375 and 500 mg / kg). Diclofenac sodium was used as a standard drug. The alcoholic extract (375 and 500 mg/kg showed maximum inhibition of oedema by 66.72% and 83.21% at the end of 3 hr in acute model of inflammation, respectively using a chronic test, the granuloma pouch in rats, the extract exhibited a 51.25% and 60.21% reduction in granuloma weight. Tinospora cordifolia possesses anti-inflammatory effects in both acute and sub – acute inflammation.

Keywords: *Tinospora cordifolia,* Anti-inflammatory activity, diclofenac sodium, paw oedema, cotton pellet.

Introduction

In the last decade natural herbal products have received maximum attention owing to their wide utility in traditional medicine system like ayurveda. A number of herbal preparations both in crude form and their fractionated components or combination of herbel preparations from different plants have been shown to render anti inflammatory property both invivo and invitro mammalian systems.

Tinospora Cordifolio meirs (Menispermiaceae) commonly known as Guduchi (Sanskivit) and heart leaf moonseed (English), and Giloy (Hindi) is a glaborous, Climbing succulent herb, commonly found in hedges and in a native of India and thrives easily tropical regions. It is widely used in Ayurveda as antidiabetic, antistress, antiuleer, anti oxidant[1][12][13]. Immunomodulatory and in the treatment of fever, urinary disorders. General debility. It is also used in the treatment of rheumatism, Jaundice and antipyretic agent[2][3]. It was therefore considered that anti inflammatory properties of T. Cordiofolia may be responsible for these medicinal effects substantially. The active adoptogenic constituents are diterpene compounds including alkaloid, berberins, Giloin, arabinogalactan Polysaccharide (ISP).

In view of this its anti inflammatory property of alcoholic extract of T. Cordifolia on acute and subaccute inflammation was studied in experimental animals. The present study has been undertaken to understand probable mode of action.

Materials and Methods

Preparation of extract

Dried stem of T. cordifolia, supplied by Institute of Himalayan Bioresource Technology, CSIRS Palampur, H.P. India. The dried stems were powdered in a pulveriser and passed through a 80 mesh sieve. The powdered Plant was packed into a soxhlet apparatus (350 g) and dewaxed with Benzene. The dried dewaxed powder was extracted sequentially with 50% ethanol. After completion of extraction, filtered and the solvent was removed by distillation under reduced pressure. The dried Benzene and alcoholic extract were subjected qualitative chemical tests for the detection of Phytoconstituents[4] [5]. Preliminary Phytochemical studies showed the presence of flavanoid glycosides, polyphenols, flavones in alcoholic extract. The alcoholic extract was suspended in 0.75% carboxy methyl cellulose (CMC) and employed for evaluation of anti inflammatory activity.

Animals used

Adult male rats (wistar albino strain) weighing, 130-150g were purchased from M/s Reeta ghosh and Co.Ltd., Kolkata, west Bengal (India), and the animals were acclimatized in the Laboratory for two weeks before experimentation. They were fed on standard diet and water *adlibitum*. Ethical clearance were obtained from the institute's Animal ethics Committee for using animals in the presence study method.

Drugs and Chemicals

Ethanol (Baroda Chemicals Industries Ltd., Dabhoi) All other Chemicals were of analytical grade.

Carrageenan – induced paw oedema

The rats were divided into six groups (n=8) and the first group served as negative control (received 0.75% CMC, 5 ml/kg). Second group was administered Diclofenac sodium (5mg/kg) as a standard drug. Group 3 to 6 were fed with alcoholic Extract (125, 250, 375 and 500 mg/kg)[6] Oedema was produced by the method described by winter et al (1962)[4]. The paw volume was measured plethysmographically at 0, 1, 2, 3, 4 and and 5 hr after the injection of carrageenan. Drug – pre treatment was given 1 hr before the injection of carrageenan Mean increase in paw volume was measured and % inhibition was calculated.

Cotton pellet granuloma

Sub – acute inflammation was produced by the method described by winter et all (1957)[7][8]. Sterile cotton (50 ± 1mg) Soaked in 0.2 ml of distilled water containing penicillin (0.1mg) and streptomycin (0.13mg) was implanted Subcutaneously bilaterally in axilla under ether anaesthesia. Animals are divided into six groups (n=6). Extract (125, 250, 375 and 500 mg/kg) diclofenac sodium and control vehicle were administered daily for 10 days. On the 10^{th} day the pellets were dissected out, dried at 60^0C and the dry weight was determined.

The weight of the cotton pellet before implantation is substracted from the weight of the dried granuloma pellets. The results were expressed as mean ± SEM. The differences were compared using one-way ANOVA. Followed by Dunnett's test. P Values < 0.05 were considered significant.

Results

In acute inflammation model, the extract showed maximum inhibition of the Carrangeenan – induced rat paw oedemo at the end of 3 h (Table 1). Oedema suppressant effect of 375 and 500 mg/kg treated groups were found to be significant. (P<0.001) as compared to control. Diclofenac showed similar type of reduction (p < 0.001) as compared to the control rats.

In sub-acute inflammation model, the weight of the granulation tissue formation extract (375 and 500 mg/kg) and diclofenac sodium. The extract also showed dose independent inhibitory effect on granuloma weight. (500 mg/kg) was found to be almost similar to that of 5 mg / kg of diclofenac sodium (Table 2).

TABLE 1. EFFECT OF ALCOHOLIC EXTRACT OF TINOSPORA CORDIFOLIA ON CARRAGEENAN INDUCED RAT PAW OEDEMA

Group n = 8	Dose Mg / Kg	Oedema Volume (M1)				
		1 h	2 h	3h	4h	5h
Control 10.75% (MC)	5 ml	0.62 ± 0.05	0.68 ± 0.05	0.74 ± 0.05	0.69 ± 0.05	0.67 ± 0.06
Diclofenac Sodium	5	0.23 ± 0.03^{b} (59.30)	0.20 ± 0.01 C (74.06)	0.14 ± 0.02^{C} (81.09)	0.22 ± 0.02 C (72.51)	0.24 ± 0.02^{C} (69.68)
Alcoholic Extract	125	0.53 ± 0.04 ^{NS} (20.13)	0.46 ± 0.04^{b} (32.36)	0.46 ± 0.04^{b} (37.84)	0.45 ± 0.02^{b} (34.78)	0.50 ± 0.03^{a} (29.87)
Alcoholic Extract	250	0.47 ± 0.04 ^{NS} (19.96)	0.44 ± 0.04^{b} (41.24)	0.39 ± 0.04^{b} (47.30)	0.43 ± 0.04^{b} (37.68)	0.46 ± 0.04^{b} (34.84)
Alcoholic Extract.	375	0.42 ± 0.04 ^{a} (32.26)	0.40 ± 0.03^{b} (47.12)	0.26 ± 0.03^{C} (61.52	0.40 ± 0.03^{b} (38.58)	0.43 ± 0.04^{b} (35.83)
Alcoholic Extract	500	0.35 ± 0.05^{b} (43.55)	0.22 ± 0.04^{C} (69.18)	0.17 ± 0.03^{C} (81.73)	0.24 ± 0.03^{C} (65.22)	0.26 ± 0.03^{C} (61.20)
Oneway Anova		P < 0.001 F = 11.74	P < 0.001 F = 24.29	P < 0.001 F = 43.76	P < 0.001 F = 26.46.	P < 0.001 F = 18.12

Each value is the mean ± SEM of 8 rats.
Figures in Parentheses indicate the % anti-inflammatory activity.
^{a}P < 0.05 ; ^{b}P < 0.01 ; ^{C}P < 0.001 compared to control
NS : Statistically not significant ; degrees of freedom (5, 42)

Table 2. Effect of Alcoholic Extract of *Tinospora Cordifolia* on Sub-Acute inflammatory Model in Rats

Group (n=6)	Dose mg/kg	Weight of dry cotton pellet granuloma (mg) (Mean ± SEM)	Percentage of inhibition (Mean ± SEM)
Control 10.75% carboxy methyl cellulose)	5 ml	77.24 ± 4.82	---
Diclofenac Sodium	5	30.05 ± 2.66^{C}	61.09 ± 2.1
Alcoholic Extract	125	62.48 ± 2.25^{a}	19.28 ± 1.2
Alcoholic Extract	250	56.08 ± 4.13^{b}	27.97 ± 1.5
Alcoholic Extract	375	36.04 ± 1.99^{C}	53.76 ± 1.7
Alcoholic Extract	500	32.84 ± 3.12^{C}	57.49 ± 1.9
One way Anova		f(df) = 32.12 (5,30) P < 0.001	

^{a}P < 0.05 ; ^{b}P < 0.01; ^{c}P < 0.001 compared to control. n = 6 in each group.

Discussion

The probable mechanism of action of carrageenan induced oedema is bi-phasic. The first phase is attributed to the release of histamine, 5-HT and kinins in the first hour while the second phase is related to the release of prostaglandin in 2nd and 3rd hr[9] Effect of alcoholic extract at a dose of 375 and 500mg / kg are dose dependent and significant in inhibiting carrageenan induced oedema. On preliminary phytochemical screening the alcoholic extract showed the presence of terpenes, aminoacids, flavanoids and its glycosides[4][10]. The radio protective effect of antioxidant flavonoids may contribute to its anti-inflammatory activity. Hence the significant anti-inflammatory activity of T. Cordifolia could be due to presence of a flavanoid, which may exert predominant inhibition of inflammatory mediators. Alcoholic extract of T. Cordifolia has shown potential inhibitory action on exudates formation. Kinin is said to be main mediator of granuloma as it both vasodilates and increases vascular permeability in the early stages of inflammation. Sub-acute inflammation involves infiltration of macrophages, Neutrophils and proliferation of fibroblasts[11]. Hence the decrease in granuloma weight indicated the anti-inflammatory activity of alcoholic extract of T. Cordifolia in the treatment related to various types of inflammatory disorders.

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