International Journal of Technical Research and Applications e-ISSN: 2320-8163,
www.ijtra.com Volume 1, Issue 3 (july-august 2013), PP. 103-106

EVALUATION OF ANTI-INFLAMMATORY ACTIVITIES WITH AERIAL PART EXTRACTS OF CASSIA SOPHERA (LINN) IN WISTAR RATS

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***Abstract*—Cassia sophera (Caesalpiniaceae) has a variety of ethnic medicinal uses along with antioxidant activity. In present study anti-inflammatory activities with alcoholic (AEACS) and aqueous (AQEACS) extracts of aerial part of C. sophera are evaluated. Aerial parts powder successively extracted with alcohol and water was subjected for phytochemical screening to identify different phytoconstituents. LD50 studies for both the extracts were conducted up to the dose level of 2 g/kg following OECD guidelines No. 425. The anti-inflammatory activity was studied in carrageenan and formalin induced paw oedema (acute) models in rats. Phytochemical investigations revealed the presence of carbohydrates, amino acids, fixed oils, fats, glycosides and sterols in AEACS and AQEACS. LD50 studies for alcoholic and aqueous extracts up to maximum of 2g/kg dose level no mortality was observed in any of the animals that indicated their practically nontoxic nature. Both the extracts significantly reduced the paw oedema volume in carrageenan and formalin induced (acute) paw oedema models in rats**

***Index Terms*— Cassia sophera, anti-inflammatory, carrageenan, formalin, oedema.** *(key words)*

I. INTRODUCTION.

Most of the patients suffering from disease conditions complain of pain and inflammation as a common. Inflammation is a protective response of our body to stop the invasion of microbes so as to inhibit its spread. There are several categories of drugs for treating inflammation among which commonly prescribed are the non-steroidal anti-inflammatory drugs (NSAIDs). Currently available anti-inflammatory agents are associated with unwanted side effects and have their own limitations. NSAIDs usually cause some gastrointestinal damage due to the inhibition of the protective cyclooxygenase enzyme in gastric mucosa1. The added advantages of indigenous medicinal treatment would include its complementary nature to the conventional treatment making latter safer, well tolerated and economical remedy for acute and chronic inflammatory conditions.

Cassia sophera (Caesalpiniaceae) known as 'Kasondi' is an important drug in Unani Medicine. ''Kasondi' is described in Unani literature to be repulsive of morbid humours (specially phlegm), resolvent, blood purifier, carminative, purgative, digestive, diaphoretic and reported to be useful in epilepsy, ascites, dyscrasia of liver, skin disorders, piles, jaundice, fever, articular pain and palpitation. In ethno botanical literature it is mentioned to be effective in the treatment of pityriasis, psoriasis, asthma, acute bronchitis, cough, diabetes and convulsions of children. In the present study alcoholic and aqueous extracts of the aerial parts of Cassia sophera, Linn were screened for anti-inflammatory activity2.

II. METHODOLOGY

A. Preparation of Different Extracts:

The powder was packed in a soxhlet apparatus and extracted with 95% alcohol for 18 h. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50oC to get alcoholic extract.

About 100 g of powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 24 h with shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at 50oC to get aqueous extract.

B. Pharmacological activities

Experimental animals:

Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice 20-30g were procured from National Centre for Laboratory Animal sciences, C/0 Sri. Venkateswara Enterprises, Bangalore for experimental purpose and were maintained under standard husbandry conditions (temperature of 25 10C; RH 45 to 55% and 12: 12 light/dark cycle). The animals were fed with a synthetic standard diet from Amrut laboratories & Pranav Agro Industries Ltd. Sangli. Water was allowed ad libitum under strict hygienic conditions. All animal studies were performed in accordance to guidelines of CPCSEA and Institutional Animal Ethical Committee (IAEC) of V.L. College of Pharmacy, Raichur (Karnataka).

Preliminary phytochemical investigation:

Alcoholic (AEACS) and aqueous (AQEACS) extracts of aerial part of C. sophera were subjected for the qualitative

preliminary phytochemical identification by the standard methods described in practical Pharmacognosy 3,4.

Determination of acute toxicity (LD50)5:

The acute toxicity of AEACS and AQEACS was determined in albino mice of either sex weighing between 18-22 g by following "up and down" (OECD guideline no.425) method of CPCSEA. 1/5th, 1/10th, 1/20th of the lethal dose of the individual extracts was taken as effective doses ED50 and was used throughout the experimental studies.

Grouping of animals:

Group I : Control (10 ml/kg distilled water p.o)
Group II : Standard drug (Ibuprofen 40 mg/kg, p.o)
Group III : AEACS (100 mg/kg p.o)
Group IV : AEACS (200 mg/kg p.o)
Group V : AEACS (400 mg/kg p.o)
Group VI : AQEACS (100 mg/kg p.o)
Group VII : AQEACS (200 mg/kg p.o)
Group VIII : AQEACS (400 mg/kg p.o)

C. Carrageenan induced rat paw oedema [6], [7]:

Albino rats of either sex weighing 150 – 200 g were selected. The animals were divided into 8 groups each having 6 animals. The various groups were treated mentioned as above. Initial paw volume of individual rats (right paw) was noted and vehicle/extract/standard were administered accordingly. One hour after the administration of vehicle or extracts or standard drug, all the rats were injected with 0.1 ml of 1% carrageenan suspension in normal saline in the sub-plantar region of the right hind paw and the left hind paw served as reference. Immediately thereafter the paw oedema volumes were measured plethysmographically at fixed time intervals8.

D. Formalin induced paw oedema

Albino rats of either sex weighing 150 – 200 g were selected. The animals were divided into 8 groups each having 6 animals. The various groups were treated mentioned as above. Initial paw volume of individual rats (right paw) was noted and vehicle/extract/standard was administered accordingly. One hour after the administration of vehicle or extracts or standard drug, all the rats were injected with 0.05 ml of formalin (2.5%) in normal saline in the sub-plantar region of the right hind paw and the left hind paw served as reference. Immediately thereafter the paw oedema volumes were measured plethysmographically at fixed time intervals9.

The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated. Percentage reduction in oedema volume was calculated by using the formula,

$$\text{Percentage reduction} = \frac{Vo - Vt}{Vo} \times 100$$

Where,

Vo = Volume of the paw of control at time't'.

Vt = Volume of the paw of drug treated at time't'.

Results:

1. Anti-inflammatory activity of aerial parts extracts of C. sophera in carrageenan induced rat paw oedema:

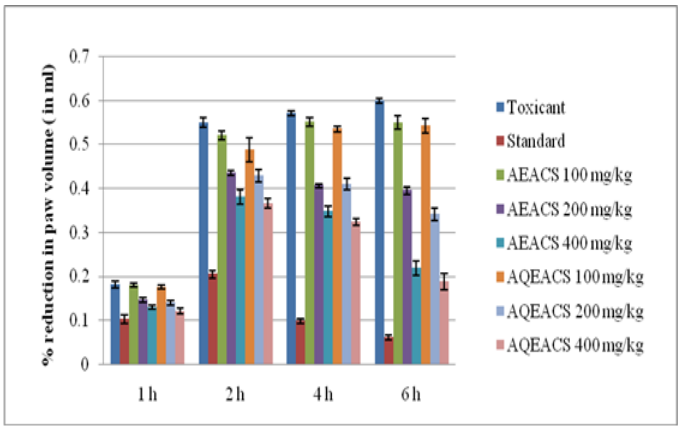
The alcoholic and aqueous extracts of aerial part of C. sophera with three dose levels tested i.e. 100, 200 and 400 mg/kg had exhibited a significant reduction in paw oedema volume in carrageenan induced paw oedema (acute model) in rats. Results are tabulated in Table 1 and graphically represented in Fig-1. Ibuprofen was used as standard reference and it has reduced paw oedema volume to 90.00% at 6th hr. Alcoholic and aqueous extracts with medium and higher doses i.e. 200 mg/kg and 400 mg/kg have reduced oedema volume 34.16%, 63.66% and 43.16%, 68.66% respectively at 6th hr. ANOVA indicates a significant difference among the extract treated groups. Dunnet's't' test confirms a significant anti-inflammatory activity with both the extracts, but more with alcoholic than aqueous extract.

Table 1: Anti-inflammatory effect of aerial part extracts of C. sophera in carrageenan induced paw edema in rats at different time intervals

SlNo	Groups	Treatment	1 hr	% ROV	2 hr	% ROV	4 hr	% ROV	6hr	% ROV
I	Control	VEHICLE (10ml/kg)	0.181 ± 0.0070	--	0.55 ±0.011	--	0.57 ±0.0057	--	0.6 ± 0.0057	--
II	Standard	Ibuprofen 40 mg/kg	0.10 ±.0098**	43.09	0.205 ±0.009**	62.72	0.098 ±0.0060**	82.80	0.06 ±0.0047**	90.00
III	AEACS	100 mg/kg	0.18 ±0.0044	0.55	0.521 ±0.0094	5.27	0.551 ±0.010	3.33	0.55 ±0.016	8.33
IV	AEACS	200 mg/kg	0.146 ±0.004**	19.33	0.436 ±0.005**	20.72	0.406 ±0.0049**	28.77	0.395 ±0.008**	34.16
V	AEACS	400 mg/kg	0.13 ±0.003**	28.17	0.38 ±0.017**	30.72	0.34 ±0.012**	38.94	0.218 ±0.016**	63.66
VI	AQEACS	100 mg/kg	0.176 ± 0.0042	6.07	0.488 ±0.027*	11.27	0.53 ±0.0061*	6.14	0.54 ±0.016*	9.5
VII	AQEACS	200 mg/kg	0.14 ±0.005**	22.65	0.428 ± 0.094**	22.18	0.41 ±.0131**	28.07	0.34 ±0.015**	43.16
VIII	AQEACS	400 mg/kg	0.121± 0.006**	33.70	0.36 ±0.011**	34.54	0.323 ±0.0088**	43.85	0.188± 0.0192**	68.66

AEACS-Alcoholic extract of Aerial parts of *C sophera* **AQEAS**- Aqueous extract of Aerial parts of *C sophera* , n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant

Fig 1: Percentage reduction in paw volumes at different intervals in carrageenan induced paw edema model in rats.



III. Anti-inflammatory activity of aerial part extracts of C. sophera in formalin induced rat paw oedema:

The alcoholic and aqueous extracts of aerial part of C. sophera with three dose levels tested i.e. 100, 200 and 400 mg/kg had exhibited a significant reduction in paw oedema volume in formalin induced paw oedema (acute model) in rats. Results are tabulated in table 2 and graphically represented in Fig-2. Ibuprofen was used as standard reference and it has reduced paw oedema volume to 96.42% at 6th hr. Alcoholic and aqueous extracts with medium and higher doses i.e. 200 mg/kg and 400 mg/kg have reduced oedema volume 46.42%, 73.21% and 51.78%, 80.35% respectively at 6th hr. ANOVA indicates a significant difference among the extract treated groups. Dunnet's 't' test confirms a significant anti-inflammatory activity with both the extracts, but more with alcoholic than aqueous extract

Sl.No	Groups	Treatment	1 hr	% ROV	2 hr	% ROV	4 hr	% ROV	6hr	% ROV
I	Control	VEHICLE (10ml/kg)	0.188 ±0.0047	-	0.561 ±0.0101	-	0.57 ±0.0051	-	0.566 ±0.0091	-
II	Standard	Ibuprofen 40 mg/kg	0.085 ±0.004**	54.78%	0.183 ±0.007**	67.37%	0.08 ±0.006**	85.96%	0.02 ±0.006**	96.42%
III	AEACS	100 mg/kg	0.18 ±0.0036ns	4.25%	0.51 ±0.008*	8.02%	0.5 ±0.010**	12.28%	0.33 ±0.007**	41.07%
IV	AEACS	200 mg/kg	0.161 ±0.004**	14.89%	0.44 ±0.013**	21.03%	0.36 ± 0.004**	36.84%	0.3 ±0.007**	46.42%
V	AEACS	400 mg/kg	0.11 ±0.006**	41.48%	0.371 ±0.014**	33.86%	0.23 ±0.007**	59.64%	0.15 ±0.010**	73.21%
VI	AQEACS	100 mg/kg	0.173 ±0.0061ns	9.57%	0.50 ±0.001**	9.80%	0.46 ±0.012**	19.29%	0.32 ±0.008**	42.85%
VII	AQEACS	200 mg/kg	0.158 ±0.004**	20.21%	0.42 ±0.007**	24.24%	0.34 ±0.012**	40.35%	0.27 ±0.006**	51.78%
VIII	AQEACS	400 mg/kg	0.105 ±0.007**	46.80%	0.32 ±0.008**	42.42%	0.22 ±0.007**	61.40%	0.11 ±0.006**	80.35%

AEACS-Alcoholic extract of Aerial parts of *C sophera* **AQEAS**- Aqueous extract of Aerial parts of *C sophera* , n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant

Table.2: Anti-inflammatory effect of aerial parts extracts of Cassia sophera in formalin induced paw edema in rats.

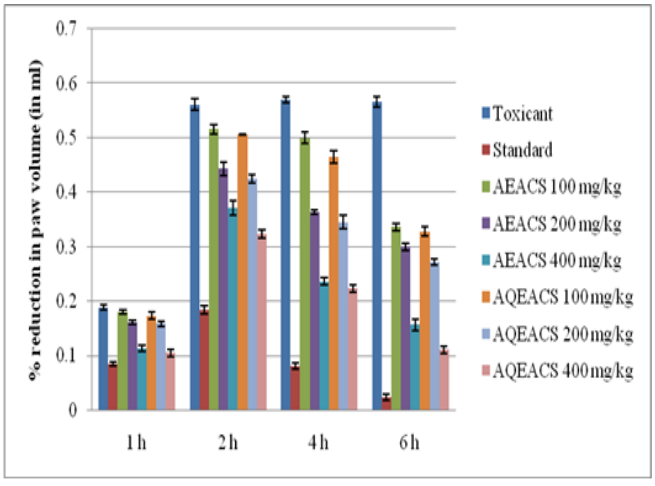


Fig.2 percentage recvuction in paw val at different intervals in formalin induced paw edema model in rats

IV. Discussion:

Inflammatory diseases affecting majority of the peoples is very common and are known to be as oldest diseases as that of mankind. No substantial progress has been achieved till today for their permanent cure. Anti-inflammatory activity was investigated in acute models of inflammation in rats as it was induced by sub-plantar injection of carrageenan or formalin. It was reported that carrageenan administration causes release of various mediators like histamine, serotonin (initial phase), kinins (middle phase) and PG (final phase) that play an important role in the development of inflammation 10. AEACS and AQEACS have inhibited the initial, middle and final phases suggesting that the extracts can block the mediators like histamine, kinins and PGs. In formalin induced paw edema model both extracts exhibited significant inhibitory action against formalin induced paw edema and this indicates that

these extracts exhibited their anti-inflammatory action by means of inhibiting the synthesis, release or action of various inflammatory mediators like kinins, histamine 10, 11. The aqueous extract was found to possess relatively better anti-inflammatory activity than alcoholic extract.

V. CONCLUSION:

Alcoholic and aqueous extracts (200, 400mg/kg) have shown significant anti-inflammatory activity against carrageenan and formalin induced paw edema in rats (acute model). The aqueous extract was found to be more potent than alcoholic extract, which is confirmed by its higher percentage reduction in paw oedema volume than the other in carrageenan and formalin induced paw oedema (acute) model in rats.

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