ANTINOCICEPTIVE AND ANTIPYRETIC ACTIVITY OF ETHANOLIC EXTRACT OF ROOTS OF *CAESALPINIA SAPPAN*

Arun K Thomas*, Jennifer Fernandes[2]

Corresponding author,

*Mr.Arun Thomas
Lecturer,
Dept.of Pharmacology
Nirmala college of Pharmacy,
Muvattupuzha, Ernakulam,Kerala-686 661

1. Dr.Jennifer Fernandes
Professor,
Dept.of Pharmachemistry
NGSM Institute of Pharmaceutical Sciences,
Paneer-575018,
Mangalore,India.

Summary

In the present study the analgesic and antipyretic activities of ethanolic extract of the roots of the plant *Caesalpinia sappan.* (Family: *Caesalpiniaceae*) was carried out. The preliminary phytochemical investigation revealed the presence of carbohydrates, proteins, triterpenoids, flavonoids, steroids and tannins. The ethanolic extract was subjected to analgesic study by Tail flick method and Acetic acid induced writhing method and antipyretic study by Yeast induced hyperthermia in rats. The results of analgesic and antipyretic activities of ethanolic extract of the roots of the plant *Caesalpinia sappan* were compared with control and found to be statistically significant ($P<0.01$) at graded dose levels of 100,200 and 400mg/kg.

Key words: analgesic, antipyretic, *Caesalpinia sappan,Caesalpiniaceae.*

Introduction

Caesalpinia sappan belongs to the family *Caesalpiniaceae,*commonly known as Brazil or sappan wood.Various pharmacological activities of the plant other than the roots were reported like anti-bacterial, antifungal, antiviral, antiinflammatory properties[1], analgesic and antipyretic activities of ethanolic extract of the roots of the plant *Caesalpinia sappan*, and the present study has been conducted.

Materials and Methods

Plant material and Extraction

In the present study the roots of the plant *Caesalpinia sappan* were collected from Muvattupuzha, Kerala, India, during the month of November- December 2005. It was identified and authenticated by Prof. Dr. J G Ray.Dept. of Botany, S.B college, Changanacherry,Kerala.The roots were then dried in shade and chopped into small pieces and powdered. The extraction was carried out using 95% ethanol by continuous soxhlet extraction method and extract were vacuum dried.

Animals

Healthy wistar albino rats of either sex weighing between 150 - 200 g and albino mice of either sex weighing between 20-30 g were obtained from KSHEMA, Deralakatte Mangalore. (Animal ethics committee, KS Hedge Medical Academy, Nithyanandanagar, Mangalore-575018 Ref. No. KSHEMA /AEC/037/2006). The animals were kept at constant temperature (25$\pm$ 2^0 C), humidity (55%), and light and dark cycle (12/12h light/dark). The animals were deprived of food for 24h before experiment but allowed free access to drinking water *ad libitum***.**

Preliminary phytochemical studies

The ethanolic extract was then sujected for preliminary phytochemical analysis as standard procedure[2] for testing the different chemical groups present in the ethanolic extract.

Acute toxicity studies

Acute toxicity was carried out in adult female albino rats as per staircase method[3] and OECD guidelines 425[4]. Animals were observed continuously for two-three hours for general behavioral, neurological and autonomic profiles[5] and death for a period of 24 hours.All the experiments were performed within the the guidelines of the Institutional ethical committee of KSHEMA,Deralakatte,Mangalore(KSHEMA/IAEC//).

Tail-flick method[6]

Acute nociception was assessed using a tail flick apparatus (Analgesiometer). Animals were divided into five groups containing six animals each.The I group served as control,II group served as standard and III,IV,V groups received 100,200 & 400 mg/kg body weight of test extract.Each animal was placed in a restrainer, before treatment the basal reaction time was measured by keeping distal one-third portion of the animal tail. The extracts were orally administered immediately after this step and reaction time was measured at every 30 minutes intervals after the drug administration for 2h. 10 seconds cut off time was used in order to prevent the tissue damage.

Acetic acid induced writhing method[7,8,9]

Divided into 5 groups containing 6 animals in each group. To the group I injected acetic acid 0.6 % v/v solution (1ml/b.wt) alone and noted the onset and severity of writhing response. To the second group injected acetyl salicylic acid. 15 min later, administered acetic acid solution to these animals. Noted the onset

and severity of writhing response. To the groups III, IV&V injected the test extracts of *caesalpinia sappan* at graded doses of 100,200 & 400mg /kg bodyweight. 15 minutes later administered acetic acid solution to these animals. Noted the onset and severity of writhing response. Calculated the mean writhing scores in control, standard and test treated groups and compared. Noted the % inhibition of pain response.

$$\text{Percentage inhibition} = \left(1 - \frac{Rt}{Rc}\right) \times 100$$

Rt=mean reaction time in treated group.
Rc=mean reaction time in control group

***Yeast induced hyperthermia in rats*[10]**

A 15% suspension of Brewer's yeast in 0.9% saline was prepared. The animals are fevered by injection of 10ml/kg body weight of Brewer's yeast suspension s.c in the back below nape of the neck. The site of injection was massaged in order to spread the suspension beneath the skin. The room temperature was kept at 22-24 ^{o}c. Immediately after yeast administration, food was withdrawn. After 18 h post challenge the rise in rectal temperature was recorded, by insertion of a thermocouple to a depth of 2cm into the rectum. Only animals with a rise in body temperature of at least 0.2 –0.5°C are taken into the test. The control group received 0.6% Na CMC, standard group received paracetamol at a dose of 135mg/kg body weight orally& test group received ethanolic extract of the roots of *Caesalpinia sappan* at a dose of 100, 200, 400mg/kg body weight orally.
Rectal temperature is recorded again at 30, 60, 90, and 120min post dosing. The mean temperature after the treatment were compared with those of 18 hour and expressed as a temperature index, which was sum of mean changes[11].

Statistical Analysis

The data obtained were expressed as mean ± SEM. The data of analgesic and antipyretic activities of ethanolic extract of the roots of *Caesalpinia sappan* were compared with control and analyzed by one way analysis of variance (ANOVA) followed by Dunnett's 't' test. P value less than 0.01 was considered as statistically significant.

Results & Discussion

The preliminary phytochemical screening of the ethanolic extract of the roots of *Caesalpinia sappan* revealed the presence of Carbohydrates, Proteins, Triterpenoids, Flavonoids, Steroids and Tannins.

The ethanolic extract of the roots of *Caesalpinia sappan* at a dose of 100,200,400mg/kg body weight significantly increased ($P<0.01$) the tail flick latency compared to control.The tail flick method is useful in detecting centrally acting analgesics[12]. Pretreatment of mice with the ethanolic extract of the roots of *Caesalpinia sappan* produced significant reduction ($P<0.01$) in writhing induced by acetic acid compared to control.It is a method for assessing the periperally acting analgesics. However in both instances exhibited dose dependent analgesic activity.The ethanolic extract of the roots was shown to possess anti-nociceptive activity at the doses tested. This was evident in the nociceptive models, which indicates that it possess both steroidal and non-steroidal type of analgesic activities[13] The extract (100,200,400mg/kg), administered orally, significantly inhibited the acetic acid induced writhings in the mice. The writhings are related to the increase in the peritoneal fluid level of PGE_2 and PG F [14]. In the tail flick test method the ethanolic root extract showed anti nociceptive activity simillar to pentazocine.

Table- 1: Effect of ethanolic extract of the roots of *Caesalpinia sappan* on tail-flick latency in rats

Treatment	Dose mg/kg	Tail-flick latency in sec at time (min) (Mean ±SEM)				
		0 min	**30 min**	**60 min**	**90min**	**120min**
Control		1.67±0.19	1.9±0.15	2.21±0.13	1.9±0.21	1.78±0.17
Pentazocine	10	1.57±0.18	6.67±0.11*	8.33±0.15*	10.00±0.0*	10.00±0.0*
extract	100	2.71±0.30	3.3±0.067*	5.4±0.11*	3.67±0.11*	3.42±0.05*
extract	200	2.51±0.14	4.12±0.33*	5.68±0.18*	4.57±0.19*	3.9±0.11*
extract	400	2.57±0.20	4.78±0.07*	6.32±0.09*	5.72±0.10**	4.5±0.07*

All values are expressed as mean ± SEM (n = 6),*P< 0.01 significant compared to control

Table-2: Effect of ethanolic extract of roots of *Caesalpinia sappan* on Acetic acid induced writhing in mice.

Treatment	**Dose mg/kg**	**Number of writhings Mean ±SEM**
Control	-----	84.17±0.7032
Diclofenac sodium	13.5	22.83±0.4014*
Extract	100	44.00±0.5774*
Extract	200	40.50±0.4282*
Extract	400	31.50±0.4282*

All values are expressed as mean ± SEM (n = 6) * $P < 0.01$ significant compared to control**.**

Table-5: Effect of ethanolic extract of roots of *Caesalpinia sappan on* yeast induced hyperthermia in rats.

Treatment	Dose mg/kg	Mean Rectal temperature (°C)					
		BeforeYeast Administration	18 h after yeast administration	Time after treatment (min) (Mean ± SEM)			
				30	60	90	120
Control	-----	38.2	38.7	39.0± 0.058	38.9± 0.058	38.87± 0.076	38.7± 0.058
Paracetamol	135	38.2	39.6	39.0± 0.058	38.55± 0.067*	38.3± 0.058*	38.2± 0.052*
Extract	100	38.17	38.75	38.75± 0.043	38.32± 0.048*	38.23± 0.042*	38.15± 0.043*
Extract	200	38.2	38.92	38.87± 0.042	38.43± 0.049*	38.33± 0.049*	38.2± 0.037*
Extract	400	38.28	38.97	38.83± 0.76	38.33± 0.076*	38.23± 0.076**	38.18± 0.079**

All values are expressed as mean ± SEM (n = 6).,*P < 0.01 significant compared to control

Treatment of rats with ethanolic extract of roots of Caesalpinia sappan at a dose of 100, 200 and 400mg/kg body weight produced a significant reduction ($P<0.01$) of elevated body temperature (hyperthermia) induced by the injection of yeast suspension compared to control. However it exhibited dose dependent antipyretic activity**.** Based on the results obtained, it is likely that the mechanism of action of the roots extract is similar to that of non-steroidal anti-inflammatory drugs, namely inhibition of prostaglandin biosynthesis.

This postulation is supported by the antipyretic effect of the extract, evidenced by its impact on pathogenic fever induced by the administration of a yeast injection. Its etiology includes production of prostaglandins in the central nervous system, which is the final common pathway responsible for fever induction. Inhibition of prostaglandin synthesis could then be the possible mechanism of antipyretic action as that of acetyl salicylic acid[15] . In conclusion the ethanolic, extract of the roots of *Caesalpinia sappan* is endowed with analgesic and antipyretic activities

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