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Research Article

EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF SYMPLOCOS RACEMOSA

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ABSTRACT

In Indian System of Medicine the bark of *Symplocos racemosa* belonging to family Symplocaceae is used for the treatment of asthma, bronchitis, dropsy, arthritis, inflammation, ulcers, menorrhagia and hepatic ailments. The objective of the present study was to investigate the analgesic and anti-inflammatory potential of the bark of *Symplocos racemosa.* The formalin induced paw licking and tail flick models were used to study the analgesic activity and carrageenan induced hind paw edema model was used to study anti-inflammatory activity of ethanolic and aqueous extracts of the bark. Wistar strain albino rats with 200 mg/kg dose were used for both studies. Diclofenac sodium (5 mg/kg) was used as the standard drug. In tail flick test the increase in the reaction time was significant ($P < 0.01$) with ethanolic extract of the bark of *Symplocos racemosa* as compared to the control group. Acute edema in the left hind paw of the animals was induced by sub plantar injection of 0.1 ml (1%) carregeenan suspension in normal saline. The ethanolic extract of the bark of *Symplocos racemosa* very significantly ($P <0.001$) reduced the paw edema in carrageenan treated rats. The effect was maximum at 3 hr after the carrageenan injection. The significant suppression of inflammation during the whole experimental period indicates the long duration of action of the ethanolic extract of the bark.

Keywords: *Symplocos racemosa*, pain, inflammation, Paw edema

INTRODUCTION

The world is facing an explosive increase in the incidence of many systemic diseases and hence cost effective complementary therapies are needed. Medicinal plants have been the subject of intense research due to their potential as sources of commercial drugs or as lead compounds in drug development. Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agent. There are various components of an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation[1]. The drugs which are used presently for the management of pain and inflammatory conditions are either narcotics or non narcotics (NSAIDS), and have known toxic and lethal effects[2]. About 34-46% of the users of NSAIDs usually sustain some gastrointestinal damage due to the inhibition of the protective cyclo-oxygenase enzyme in gastric mucosa [3]. On the contrary, herbal medicines with good absorption, less toxicity, and easy availability have been used since ancient times[4]. According to WHO about 80% of the world population still rely mainly on herbal remedies[5-6]. It is therefore, essential that efforts be made to introduce new medicinal plants, to develop cheaper, effective and safe analgesic and anti-inflammatory drugs[7].

Symplocos racemosa (Symplocaceae) commonly known as "Lodhra" in Sanskrit or "Rodhra, is a small, evergreen tree upto 6 m tall. It is found in the plains and lower hills throughout North and East India [8]. The bark is dark grey and rough; and is useful in diarrhea, dysentery, eye diseases, fever, ulcer, scorpion sting, diabetes, and liver disorders[9]. It has been scientifically reported as an antimicrobial [10], anticancer[11] and has beneficial effects in gynaecological disorders[12]. Phytochemical investigations of the bark of *Symplocos racemosa* have revealed the presence of monomethyl pelargonidin glucosides (I &II), loturine, colloturine, loturidine, reducing sugars, oxalic acid, phytosterol and flavon glycosides. The present study was therefore aimed at evaluating the analgesic and anti-inflammatory activity of the bark of *Symplocos racemosa*.

MATERIALS AND METHODS

Animals

Male / Female Albino rats of Wistar strain weighing 120 – 200g obtained from the Department of Pharmacy, MJRP, were used for the experiment. The animals were housed in cages under standard lab conditions (12:12 hr light / dark cycles at 25±2˚C, RH55±10%). They had free access to standard pellet diet and water ad libitum. The animals were acclimatized at least one week prior to experiment. All experiments were approved by Institutional Animal Ethics Committee.

Plant Materials and Extracts

The bark of *Symplocos racemosa* was procured from Shantikunj, Haridwar and was authenticated by the Department of Botany, Ambah Post Graduate College, Jiwaji University, Gwalior. The authenticated plant materials were shade dried and powered coarsely. The coarsely powdered drugs were extracted separately in soxhlet apparatus in sufficient volume of redistilled water and ethanol at 45-48˚C for 18 hrs. The filtrates were collected and evaporated to dryness at 40˚C on rotary evaporator. The solid masses were collected carefully and weighed. Their yields were calculated and then stored in sealed (air tight) glass bottles at 4˚C for further experimental work.

Treatment protocol

The animals were divided into four groups of six animals each. Group I served as control and received the suspension of 1% CMC in distilled water. Group II received the reference drug diclofenac sodium 5mg/kg. Group III and IV received 200 mg/kg of ethanolic and aqueous extracts of SR by oral route (p.o.). All the test drugs were suspended in1% CMC and were administered in a volume of 5 ml/kg body weight.

Formalin induced paw licking model

The Formalin test comprises the early and late phase assessment of the analgesic effect as described by Hunskaar

& Hole[13]. One hour after drug administration by the oral route, 20 µl of 1% formalin was injected subcutaneously sub-plantar in the right hind paw. Then the duration of paw licking as an index of nociception was recorded in periods of 0 to 5 minutes (early phase) and 15 to 30 minutes (late phase) after formalin injection.

Tail flick model

In this model Nichrome wire analgesiometer was used [14]. Individually the tail of each rat was placed over the radiant heat source of the apparatus and the tail withdrawal from the heat (flicking response) was taken as the end point. Analgesic activity was assessed by observing the reaction time in the treated groups. Following the administration of drugs, the reaction time was noted at 0, 30, 60, 90 and 120 min. A cut of time of 15 seconds was considered to avoid tissue injury.

Carrageenan induced paw edema

This test was performed as per the method described by Winter et al[15]. The animals were fasted for 16 hours but water was allowed ad libitum[16]. Acute edema in left hind paws of the rats was induced by the sub-plantar injection of 0.1 ml of freshly prepared (1% w/v) carrageenan suspension in normal saline 1h after the drug administration. The paw volume was measured before and 1, 2, 3 and 4 h after the carrageenan injection. The results were expressed as mean ± SEM. The statistical analysis of data was done by the Student's t-test and one-way Analysis of Variance (ANOVA) followed by Dunnet's test. The results were considered statistically significant at $P < 0.05$.

TABLE I: EFFECT OF ETHANOLIC AND AQUEOUS EXTRACTS OF *SYMPLOCOS RACEMOSA* ON EARLY AND LATE PHASE OF THE FORMALIN TEST

Group	Dose (mg/kg)	Early phase		Late phase	
		Licking time (s)	% inhibition	Licking time (s)	% inhibition
Control	-	72.33±3.80		89.50±7.62	
Reference Drug (Diclofenac sodium)	5	50.50±4.27**	30.18	35.83±3.37***	59.96
SRE	200	65.50±4.88	9.44	66.50±4.79*	25.69
SRA	200	69.33±5.20	4.14	69.00±4.64*	22.90

Values are expressed as mean±SEM, n = 6 in each group. *P< 0.05, **P< 0.01, ***P< 0.001 compared with control.

TABLE II: EFFECT OF ETHANOLIC AND AQUEOUS EXTRACTS OF *SYMPLOCOS RACEMOSA* ON RADIANT HEAT INDUCED TAIL FLICK RESPONSE IN RATS.

Groups	Dose (mg/kg)	After 0 min (sec)	After 30 min (sec)	After 60 min (sec)	After 90 min (sec)	After 120 min (sec)
Control	-	4.19 ±0.23	4.04±0.26	4.02±0.34	4.42±0.32	3.89±0.34
Diclofenac sodium	5	4.47±0.35	4.97±0.35	8.11±0.65***	7.19±0.50***	9.33±0.58***
SRE	200	4.16±0.28	4.27±0.31	4.72±0.36	6.64±0.50**	5.35±0.37*
SRA	200	3.91±0.27	4.17±0.26	4.54±0.29	5.48±0.47	4.63v0.35

Values are expressed as mean±SEM, n = 6 in each group. *P< 0.05, **P< 0.01, ** *P< 0.001 compared with control.

TABLE III: EFFECT OF ETHANOLIC AND AQUEOUS EXTRACTS OF *SYMPLOCOS RACEMOSA* ON CARRAGEENAN-INDUCED PAW EDEMA IN RATS

Groups	Dose (mg/kg)	1st hr	2nd hr	3rd hr	4th hr
Control		0.62±0.04	0.77±0.05	0.77±0.04	0.67±0.04
Diclofenac sodium	5	0.53±0.03	0.49±0.04**	0.45±0.03***	0.41±0.03***
SRE	200	0.55±0.03	0.61±0.04*	0.49±0.03***	0.53±0.03*
SRA	200	0.59±0.03	0.63±0.04	0.58±0.04*	0.62±0.04

Values are expressed as mean±SEM, n = 6 in each group. *P< 0.05, **P< 0.01, ** *P< 0.001 compared with control.

RESULTS

Formalin induced paw licking model

The formalin induced paw licking model was used to study the analgesic effects during early and late phase (Table 1). The administration of standard drug diclofenac sodium significantly (P<0.01) inhibited the licking response. The ethanolic and aqueous extracts of *Symplocos racemosa* failed to produce any significant suppression in the licking response during the early phase of formalin test. In the late phase of formalin test, the administration of standard drug highly significantly (P<0.001) inhibited the paw licking response as compared to the control group. The ethanolic and aqueous extracts of *Symplocos racemosa* significantly (P<0.05) suppressed the paw licking in the late phase as compared to control group.

Tail flick model

In the tail flick test the ethanolic extract of *Symplocos racemosa* produced a reaction time of 4.27±0.31, 4.72±0.36, 6.64±0.50, 5.35±0.37 seconds after 30, 60, 90 and 120 minutes respectively (Table 2). The reaction time for the same period with control group was found to be 4.04±0.26, 4.02±0.34, 4.42±0.32, 3.89±0.34 seconds respectively. The reaction time for diclofenac sodium treated group was found to be 4.97±0.35, 8.11±0.65, 7.19±0.50, 9.33±0.58 seconds after 30, 60, 90 and 120 minutes respectively. The reaction time with aqueous extract of S*ymplocos racemosa* for the same period was found to be 4.17±0.26, 4.54±0.29, 5.48±0.47 and 4.63±0.35 seconds respectively. The above results show that the increase in reaction time in ethanolic extract treated group was found to be significant after 90 min (P <0.01) and 120 minutes (P <0.05) respectively; and non significant after 30 and 60 minutes as compared to control groups. The increase in reaction time in standard drug treated group was found to be highly significant (P <0.001) after 60, 90 and 120 minutes. The increase in reaction time in aqueous extract of *Symplocos racemosa* treated group was found to be non significant after 30 and 60, 90 and 120 minutes respectively. The increase in reaction time was highest in standard drug treated group and was seen until the last phase observation (120 minutes). In the ethanolic extract and standard drug treated groups, the maximum effects were seen after 90 and 120 minutes respectively.

Carrageenan induced paw edema

Development of paw edema was observed in both control and treated groups after carrageenan injection. Thickness of the paw was found to be increased initially upon injection of carrageenan due to volume effect. Difference in the thickness of the rat paw edema was further increased during the time

interval of 1h – 4h in control group. When compared with the control, the treatment with ethanolic extract of SR was found to produce significant suppression of inflammation at 2, 3 and 4 h after carrageenan injection (Table 3). When compared with the control, the treatment with aqueous extract of SR showed significant ($P<0.05$) suppression of edema at 3 h.

When compared with control, the treatment with standard drug significantly suppressed the edema at 2h ($P<0.01$) and at 3 and 4h ($P<0.001$). When compared with ethanolic and aqueous extract of *Symplocos racemosa,* the anti-inflammatory activity with the standard drug treatment was more powerful and pronounced. When compared with aqueous extract, the ethanolic extract produced more powerful and pronounced anti-inflammatory activity. The aqueous extract exhibited significant ($P<0.05$) suppression of edema at 3h. The results clearly show that the anti-inflammatory activity of standard drug and ethanolic extract of *Symplocos racemosa* treated groups was seen at 2, 3 and 4h.

DISCUSSION

In the present investigation anti-inflammatory activity of ethanolic and aqueous extracts of *Symplocos racemosa* was studied by using inhibition of carrageenan induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. Carrageenan-induced inflammation is a useful experimental model of acute inflammation for detecting orally active anti-inflammatory agents[17]. Edema formation in the rat paw is a biphasic response. The first phase is mediated through the release of histamine, serotonin, and kinins, whereas the second phase is due to the release of prostaglandin and slow reacting substances[18].

The ethanolic and aqueous extract of *Symplocos racemosa* significantly reduced the paw edema. In this experiment the suppression of inflammation may be due to PG and kinin synthesis/release inhibition and antihistamine activities. The maximum inflammation is seen approximately three hours post the carrageenan injection, after which it begins to decline23.

The formalin model was developed >30 years ago to assess pain and evaluate analgesic drugs in laboratory animals. In this test, a dilute (0.5–5%) formalin solution (in which formaldehyde is the active ingredient) is injected into the paw of a rodent, and pain-related behaviors are assessed over two temporally distinct phases[19-20]. In the present study the analgesic activity of ethanolic and aqueous extracts of *Symplocos racemosa* was evaluated by formalin induced paw licking and the tail flick model. The persistent pain model of formalin induced hind paw licking was used in the study. The first phase of pain is attributed to the direct activation of nociceptors and primary afferent fibres by formalin, causing the release of bradykinin and tachykinins[21-22.] This phase is inhibited by opioid analgesics. The second phase is due to an inflammatory reaction caused by tissue injury leading to the release of histamine, serotonin, prostaglandin and excitatory amino acids [23-24]. This late phase is inhibited by non – steroidal anti inflammatory drugs and opioid analgesics

The tail flick model is used to evaluate the analgesic agents acting through central nervous system. In this method a nichrome wire is used to produce pain. In the present study ethanolic and aqueous extracts of *Symplocos racemosa* significantly inhibited the paw licking in the late phase. In the tail flick test the ethanolic extract of *Symplocos racemosa* *significantly* increased the reaction time. The results of analgesic and anti-inflammatory activities of ethanolic extracts of *Symplocos racemosa* are comparable with standard drug diclofenac sodium.

CONCLUSION

From the above findings it can be concluded that the ethanolic extract of *Symplocos racemosa* possesses promising analgesic and anti-inflammatory activities. The presence of alkaloids, glycosides, phenolic compounds, carbohydrates, sterols and flavonoids in the ethanolic extract of the plant under study may be responsible for these activities. Further pharmacodynamic investigations are required to understand the precise mechanism of action of *Symplocos racemosa*.

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