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ANTI-INFLAMMATORY ACTIVITY OF EXTRACTS OF *COMMIPHORA* SPECIES AND ITS POLYHERBAL FORMULATION

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ABSTRACT

Objective: The present study is to evaluate anti-inflammatory activity of ethanolic extracts of *Commiphora* species. Materials and Methods: The *in-vivo* anti-inflammatory activity was evaluated in rats by using carrageenan-induced paw edema. The ethanolic extracts of *Commiphora* species and the formulated polyherbal capsules were screened for their anti-inflammatory activity at different dosages in Carrageenan induced inflammatory oedema of hind paw in Wistar albino rats. Results: The anti-inflammatory activity were observed at different doses i.e., 100 mg/kg/i.p and 200 mg/kg/i.p., of plant extracts and for formulated polyhedral capsules were given at the dose of 300 mg/kg/i.p. In carrageenan induced method *EECB* showed (65.18%, 73.44%), *EECC* showed (62.33%, 65.35%), *EECP* showed (57.49%, 59.04%) and formulation showed (76.64%) inhibition (24th hour). Conclusion: From the current study it reveals that the combination of multiple herbs found as a better insight in the treatment of inflammation.

Key words: Anti-inflammatory activity, *Commiphora* species, Polyherbal capsules.

INTRODUCTION

Commiphora berryi, *Commiphora caudata* and *Commiphora pubescens* which were included in the study belongs to the family Bruseraceae. *Commiphora berryi* commonly known as mudgiluvai, it is the traditional folk medicine as an astringent, antiseptic, diuretic, uterine stimulant and emmenagogue. *Commiphora caudata* (Common name Kiluvai) is a traditionally medicinal plant used in the treatment antidiabetic, ulcer, inflammation and diarrhea and spasms. Pharmacological evidence reports that *Commiphora caudata* have antidiabetic, antimicrobial, antibacterial and anticancer activities. Hepatoprotective activity was previously reported for *Commiphora berryi*, *Commiphora caudata* and *Commiphora pubescens*. The bark of *Commiphora berryi*, leaves of *Commiphora caudata* and *Commiphora pubescens* were used in the study. The work was aimed at scientific validation of anti - inflammatory properties of *Commiphora* species by pharmacological methods based on ethanopharmacological information.

The *Commiphora berryi* which has been predominantly grown in arid places was procured from Tirunelveli and Pudhukottai district of Tamil Nadu; *Commiphora caudata* was grown in arid places were procured from Tuticorin and Perambalur district of Tamil Nadu; *Commiphora pubescens* was grown in arid places were procured from Pudukottai district in Tamil Nadu. The freshly collected plants were then authenticated by the botanists of Botanical Survey of India, Coimbatore, Tamil Nadu, India and a voucher specimen of all the plants were kept in our laboratory, Department of Pharmaceutical Technology, Anna university Tiruchirappalli, Tiruchirappalli, Tamil Nadu (voucher number: *C. berryi*-BSI/SC/5/23/07-08/Tech.669., *C. caudata*-BSI/SC/5/23/06-07/Tech.1821., and *C. pubescens*- BSI/SC/5/23/06-07/Tech.1664.). The required plant parts were then spread over trays and dried under shade, with regular sifting of collected plant materials every day. Such shade dried bark/leaves/aerial portions of plant were ground to powder (1kg) and passed through a 10-mesh sieve.

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MATERIALS AND METHODS

Extraction

The coarsely powdered material (1 kg) was extracted with petroleum ether thrice to remove the fatty material and further plant material was further extracted thrice with ethanol (99.9%, v/v). The ethanolic extracts of *Commiphora caudata* (EECC), ethanolic extract of *Commiphora berryi* (EECB) *and* ethanolic extract of *Commiphora pubescens* (EECP) was then filtered, pooled and concentrated at reduced temperature (5ºC) on a rotary evaporator (Buchi, USA) and then freeze dried (Freezone 4.5, Labconco, USA) (Brain KR and Turner TD, 1975) at high vacuum and at temperature 40±2ºC (yield 6.12%, w/w). The preliminary investigations for the anti-inflammatory activity of ethanolic extracts of *Commiphora* species was evaluated by carrageenan induced paw edema method.

Chemicals

Diclofenac, carrageenan was purchased from sigma. Other solvents and reagents of analytical grade were obtained from Himedia.

Animals

Wistar rats obtained from the Veterinary University, Madhavaram, Chennai and King Institute, Guindy, Chennai, Tamil Nadu, India were used in the present study. Weight of the rats ranged from 180-200g. All animals were housed individually and were maintained at 22ºC (±3ºC) with a dark/light cycle of 12:12 hours. Unlimited supply of water was provided and feeded with conventional rodent laboratory diet and was fasted 16 hours prior to the experiment.

Phytochemical screening

Phytochemical screening of *Commiphora berryi Commiphora caudata and Commiphora pubescens* was done to evaluate the presence of alkaloids, glycosides, flavanoids, saponins, carbohydrates, protein, amino acids, lipids and steroids. Preliminary phytochemical screening was done the according to the standard procedures given in Trease and Evans (1983).

Acute toxicity testing

Acute toxicity testing was performed according to OECD guidelines 432 (Somchit MN and Nur SMH, 2003).

Formulation of Polyherbal Capsule

Commiphora berryi, Commiphora caudata, and Commiphora pubescens were finely powdered and capsules were formed by the wet granulation method using lactose (20%) as binder compound. The wet mass of powdered plant material mixed with lactose was passed through mesh #20 to obtain granules. The granules were dried at 45°C in dryer. The granules were lubricated with 5% magnesium stearate. These granules were then filled into capsules.

Paw edema induced by carrageenan

Six rats in ten groups were included in the study in order to determine the anti-inflammatory activity.

Group 1was treated with only Normal saline (0.9% NaCl w/v, 5 ml/kg)

Groups II act as a positive control group (0.1 ml of carrageenan in isotonic saline)

Group III treated orally with Diclofenac sodium as standard anti-inflammatory drug (10 mg/kg), before carrageenan injection

Groups IV and V received EECC; 100 mg/kg and 200 mg/kg, before carrageenan injection.

Groups VI and VII received EECB; 100 mg/kg and 200 mg/kg, before carrageenan injection.

Groups VIII and IX received EECP; 100 mg/kg and 200 mg/kg, before carrageenan injection.

Group X received polyherbal capsule formulation; 300mg/kg, before carrageenan injection (Charles Risley *et al.*, 1962).

Thirty minutes after oral treatment, group I received 0.05 ml saline, while groups II–X received 0.05 ml carrageenan (1% solution in saline) on the plantar surface of the right hind paw. The right hind paw volume was measured immediately after carrageenan injection by water displacement using modified digital plethysmometer (Tokyo, Japan) (Sofowora A, 1993). The volume was measured again sequentially 1, 2, 3, 4 and 24^{th} h after carrageenan injection and immediately. The results were expressed as percentage inhibition in relation to the control group. Then the paw volume was tabulated and found the average volumes and the percentage inhibition was calculated by mean paw.

Statistical analysis

The data are expressed as mean±SD. The difference among means has been analyzed by one-way ANOVA. A value of $P < 0.05$ was considered as statistically significant (Dutta Sarmistha and Das Swarnamoni, 2010).

RESULTS

Phytochemical screening

The phytochemical screening includes the tests for alkaloids, amino acids, flavanoids, glycosides, proteins, reducing sugars, starch, tannins and terpenoids. Phytoconstituents present in *Commiphora berryi* were flavonoids, glycosides, reducing sugar, saponins, steroids, starch, tannins and terpenoids; amino acids, flavonoids, glycosides, proteins, reducing sugar, steroids, starch, tannins and terpenoids were present in *Commiphora caudata*; amino acids, glycosides, proteins, reducing

sugar, steroids, starch and terpenoids were present in *Commiphora pubescens* and alkaloids, amino acids, flavonoids, glycosides, proteins, reducing sugar, steroids, starch and terpenoids were present in capsule formulation. These are the common class of phytoconstituents reported in other species of plants belonging to *Commiphora* genus which have been reported to possess various therapeutic properties. Comparison with the previous study confirms the presence of various constituents which was previously reported (Ozaki Y, 1990).

Acute toxicity studies

Acute toxicity study was carried out for ethanolic extracts of *Commiphora species* according to OECD [Organization of Economic Cooperation and Development] Guidelines No. 423. The ethanolic extract was safe up to a dose of 2000 mg/kg body weight so 100 mg/kg and 200 mg/kg were used as moderate dose for the evaluation.

Anti-inflammatory activity of *Commiphora* species and its Capsule Formulation

The effects of ethanolic extracts of *Commiphora* species plants and their percentage inhibition were depicited in the Table 1 and 2.

Table 1. Percentage Inhibition of *Commiphora* species and its Capsule Formulation

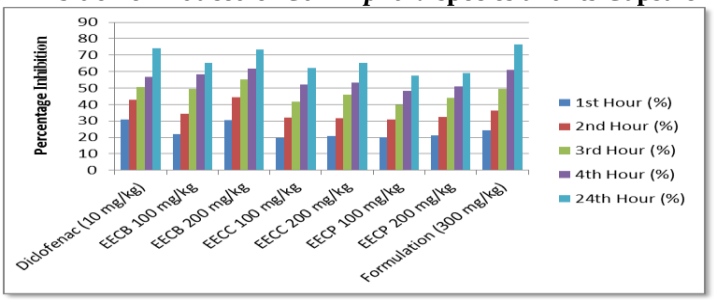
Groups	Normal Paw Volume	0th Hour	1st Hour	2nd Hour	3rd Hour	4th Hour	24th Hour
Negative Control	0.57±0.06	0.57±0.06	0.57±0.06	0.57±0.06	0.57±0.06	0.57±0.06	0.57±0.06
Positive Control	0.54±0.04	1.43±0.04	1.53±0.06	1.66±0.05	1.77±0.03	1.82±0.02	1.93±0.03
Diclofenac sodium10mg/kg	0.49±0.01	1.12±0.11	1.06±0.11	0.94±0.06*	0.87±0.04	0.78±0.05*	0.49±0.01*
EECB 100mg/kg	0.53±0.03	1.29±0.02	1.19±0.04*	1.09±0.04*	0.89±0.07*	0.75±0.06*	0.67±0.03*
EECB 200mg/kg	0.49±0.01	1.19±0.05	1.07±0.04[a]	0.92±0.05[a]	0.79±0.05[a]	0.69±0.03[a]	0.51±0.01[a]
EECC 100mg/kg	0.53±0.04	1.29±0.07	1.23±0.08*	1.13±0.10*	1.03±0.11*	0.87±0.09*	0.72±0.07*
EECC 200mg/kg	0.55±0.05	1.28±0.02	1.21±0.03*	1.13±0.04*	0.96±0.04*	0.85±0.04*	0.67±0.03*
EECP 100 mg/kg	0.64±0.04	1.26±0.04	1.22±0.05*	1.15±0.05*	1.06±0.06*	0.94±0.06*	0.82±0.05*
EECP 200 mg/kg	0.56±0.05	1.25±0.04	1.21±0.05*	1.12±0.06*	0.99±0.08*	0.89±0.06*	0.79±0.08*
Formulation (300 mg/kg)	0.52±0.03	1.23±0.05	1.16±0.05*	1.09±0.06*	0.86±0.09*	0.84±0.07*	0.58±0.05*
P Value	-	-	<0.05	<0.05	<0.05	<0.05	<0.05

Values are mean ±SD. (N=6), * p<0.05 with respect to control.

Table 2. Percentage Inhibition of *Commiphora* species and its Capsule Formulation

Groups	1st Hour (%)	2nd Hour (%)	3rd Hour (%)	4th Hour (%)	24th Hour (%)
Diclofenac (10 mg/kg)	30.94	43.01	50.66	56.81	74.19
EECB 100 mg/kg	22.14	34.33	49.62	58.51	65.18
EECB 200 mg/kg	30.29	44.51	55.36	61.98	73.44
EECC 100 mg/kg	19.67	31.92	41.52	51.96	62.33
EECC 200 mg/kg	20.84	31.62	45.76	53.17	65.35
EECP 100 mg/kg	20.19	30.72	39.94	48.2	57.49
EECP 200 mg/kg	21.17	32.53	44.06	50.87	59.04
Formulation (300 mg/kg)	24.42	36.33	49.61	61.04	76.64

Figure1. Percentage Inhibition of induced of *Commiphora* species and its Capsule Formulation



DISCUSSION

Inflammation is a protective and defensive mechanism of the body which is indicated by various pathological changes like accumulation of fluid in body tissues. A dosage of 100 and 200 mg/kg/i.p., of *EECB, EECC, EECP* and 300 mg/kg/i.p., of capsule formulation showed anti-inflammatory activity when compared with positive control and standard drug diclofenac sodium (10 mg/kg/i.p.). Carrageenan is an exudative phase of inflammatory pathology (Ozaki Y, 1990) that involve the action of vasoactive amines, such as histamine, serotonin, and kinins on Vascular permeability (Whittle BA, 1964; Vinegar R *et al*., 1978; Green KL, 1972). Subcutaneous injection of carrageenan into the rat paw produces plasma extravasations and inflammation characterized by increased tissue water and plasma protein exudation with neutrophils extravasations and metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways (Gamache DA *et al*., 1986). The increased paw volume under the digital plethysmometer in experimental rats in this study suggested that the early hyperemia of carrageenan-induced oedema results from the release of histamine and serotonin (Winter CA and Porter CC, 1957). Thus Carrageenan-induced paw oedema in rats appears to be a biphasic events and the early phase (2.5–3 h) of the inflammation is due to the release of vasoactive amines such as histamine and serotonin. In the later phase (4.5–6h) is due to the activation of kinin-like substances such as prostaglandins, proteases and lysosome (Olajide OA *et al*., 2000). The first phase showed that the *EECB, EECC, EECP* and formulation reduced the inflammation by control the proliferation of the histamine and serotonin and in second phase controlled the stimulation of kinin like substances was reported that leukocyte adhesion represents one of the first steps in the inflammatory response initiation and it is essential for accumulation of active immune cells at sites of inflammation. The extracts reduced the paw volume in which first phase the *EECB* showed (49.62%, 55.36%), *EECC* showed (41.52%, 45.76%), *EECP* showed (39.94%, 44.06%) and formulation Showed (46.61%) inhibition as that the histamine and serotonin like substances reduced. In the second phase the *EECB* showed (65.18%, 73.44%), *EECC* showed (62.33%, 65.35%), *EECP* showed (57.49%, 59.04%) and formulation Showed (76.64%) inhibition, those results showed that the reduced kinin like substances level.

Statistical analysis showed that the anti-inflammatory activity of preparations containing extract and formulation are significantly different from the control group at all the tested concentrations and the activity is dose-dependent i.e., 200 mg/kg/i.p. These methods revealed that the elevated percentage inhibition of *EECB, EECC, EECP* and formulation. Although direct evidence of the mechanism of action of extract is not clear. Previous study states that flavonoids exhibit anti-inflammatory activity. Flavonoids may deliberately reduce the mediators on inflamed area. On the basis of the study conducted it was found that the anti-inflammatory activity of the compounds and their polyherbal capsule preparation were dose dependent and varied their activity in higher dosages. Comparison of the combination ratio variation and individual dosage of plant extracts showed that polyherbal formulations have more approaching anti-inflammatory activity.

CONCLUSION

The study confirms the presence of various phytoconstituents in the ethanolic extracts of the three plants of *Commiphora* species. Alkaloids, flavonoids, steroids, tannins and terpenoids are the most active phytoconstituents were present in crude ethanolic extracts of *Commiphora* species. Flavonoids are one of the most efficient phytoconstituents was present in *Commiphora* extracts. This may activate the immune cells to control the inflammation in the inflamed area by the paw volume assessment and its percentage inhibition. Data evaluated showed a significant reduction in paw volume when compared to the standard drug Diclofenac Sodium in carrageenan induced animal models. From the study, the *Commiphora* Species and its formulation has confirmed the presence of active phytoconstituents having anti-inflammatory activities.

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