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Evaluation of Anti-inflammatory activity of leaf and bark extracts of Cipadessabaccifera (Roth) Miq

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Abstract

Inflammation is a fundamental defensive reaction of the body to invasion of pathogens or injury. Traditional practice of administering anti-inflammatory drugs is known to produce multiple side effects in human body. To address this gnawing issue researchers are taking deeper interest in finding plant based medicines. Several commonly available medicinal plants like neem, rosemary etc. are being used to formulate plant based drugs that have the potential to curtail the use of conventional anti-inflammatory drugs. *Cipadessa baccifera* is a wild grown plant which is considered to be a storehouse of nutritional as well as medicinal values. It is fortified with extraordinarily important minerals (calcium, phosphorus, potassium) and nutrients (carbohydrate, fibre) that are indispensable for healthy and complete growth of our body. This study pivots around the anti-inflammatory properties of leaf and bark extract of *Cipadessa baccifera.* The results of the investigation (acute toxicity study and carrageenan-induced rat paw edema test) clearly depict that leaf and bark extract of *Cipadessabaccifera*possess significant anti-inflammatory properties that can be considered indispensable for side effect free treatment of inflammation.

Key words: *Cipadessabaccifera*, Anti-inflammatory property, Acute toxicity study, Carrageenan-induced rat paw edema test

Introduction

Inflammation is a fundamental pervasive form of defensive reaction of the body in response to a noxious stimuli, injury, trauma or infection (Calixto*et al.*, 2004), which is induced by physical, chemical and immunological agents. It involves a complex array of enzyme activation, mediators release, extravasation, cell migration, tissue breakdown and repair (Vane and Bolting, 1995). Conventional non-steroidal anti-inflammatory drugs are

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known to produce undesired side effects (Oluwafemi, 2018). Role of chronic infection is prominently manifested in development of various lifestyle diseases in developing as well as developed countries (Oluwafemi,2018). Development of new drugs with little or no side effects is needed as some of the anti-inflammatory drugs cause undesired side effects. Hence interest in phytopharmaceuticals to treat chronic diseases is gaining momentum.

The classic cardinal signs which characterize an inflammation viz., redness, swelling, pain and loss of function are produced by inflammatory agents such as nitric oxide, prostaglandins, bradykinin, serotonin, leukotrienes and histamine (Banasik, 2000; Chandrasoma and Taylor, 2005). Persistent and uncontrolled inflammation could lead to the progression of many chronic diseases such as atherosclerosis, rheumatoid arthritis, psoriasis, multiple sclerosis and inflammatory bowel disease (Talwar*et al.*, 2011).

The first anti-inflammatory drug to be synthesized was Aspirin, the origin of which can be traced to the serendipitous discovery of salicylates in the bark of willow tree, *Salix alba*(Rainsford, 1984; Brune and Hinz, 2004; Rainsford, 2004). Since the development of the Aspirin, the progenitor of anti-inflammatory drugs, significant progress has been made in this field which has deepened the understanding of the mechanism involved in the inflammatory process. Successful identification of novel anti-inflammatory targets also revealed the underlying pathology of inflammatory diseases. In the last few decades several novel non-steroidal anti-inflammatory drugs (NSAIDs), chemical analogs and a variety of anti-inflammatory therapeutics have been synthesized **(**Rainsford, 1984; Rainsford, 2004**)**. However, these NSAID cause adverse side effects like renal dysfunction, cardiovascular ill effects, adrenal suppression, gastrointestinal tract mucosal erosion and asymptomatic ulcers (Okoli*et al.*, 2003; Pountos*et al*., 2011). Therefore the search for new formulations with minimal side effects is gathering great momentum and medicinal plants possess immense potential to inhibit chronic inflammatory diseases.

The history of use of plants to curtail inflammatory process started with the use of the bark of willow tree more than 2400 years ago by Greek, Roman and Egyptian civilizations. One of the best examples of traditional medicines forming the basis for the synthesis of modern drugs has been the most effective plant derived anti-inflammatory drug; acetyl salicylic acid (aspirin), derived from the natural product salicin, isolated from the bark of the willow tree *Salix alba* in 1899 (Vane and Bolting, 1987; Vane, 2000). In the year 1960 Indomethacin and Ibuprofen was developed (Pountos*et. al*., 2011). The extensively used non-steroidal anti-inflammatory drugs (NSAIDs) such as antipyrine, fenmates, phenacetin,

phenylbutazone, naproxen and indomethacin which have the ability to inhibit the enzymatic production of prostaglandins were formulated (Vane, 2000).

Literature is overflowing with studies on anti-inflammatory potential of *Curcuma longa, Rosmarinusofficinalis, Azadirachtaindica, Zingiberofficinale* and many more (Naik*et al*., 2014;Ghasemian et al., 2016) while no such attempt has been made with *C. baccifera* which is being used in the folkloric medicines for treating inflammatory diseases viz., psoriasis and rheumatism (Malarvannan*et al.,* 2009).

Material and Methods

***In vivo* Anti-inflammatory activity Animals:**

Male albino Wistar rats of approximately 12 to13 weeks of age, weighing around 150-200 g were procured from a registered breeder and used for animal studies. The animals were kept in polypropylene cages with husk bedding material and standard environment conditions like controlled temperature (23±1°C), relative humidity (55±5%) and 12±1 hour light and dark cycles. The animals were acclimatized to the lab condition for a week before the commencement of the experiments. They were fed with standard food pellet diet and water *ad libitum*. The animals were deprived of food 24 hours prior to conduction of the experiment; however they had free access to water. The animals were taken care of in accordance with the norms of Good Laboratory Practice (GLP) and procedures complied with CPCSEA guide lines.

The anti-inflammatory activity experiment was designed and conducted after obtaining the approval from Institutional Animal Ethical Committee (IAEC) [DSCBS/PhD/IAEC/5/14-15 date: 07-01-2015].

Acute toxicity studies

Acute oral toxicity study of methanolic extracts of leaves and bark of *Cipadessa baccifera* was performed according to Organization for Economic Co-operation and Development (OECD) guidelines, Test No. 423; acute oral toxicity-acute toxic class method (OECD- 2000; Prema, 2001).

***In vivo* Carrageen induced paw edema test**

The anti-inflammatory activity of methanolic extracts of leaves and bark was evaluated using standard protocol (Winter *et al*., 1962). Male albino Wistar rats weighing between 150-200 g were used for the study. They were fasted overnight prior and during the

experiment, but had free access to water. The albino Wistar rats were divided into 6 groups of n= 6 animals.

Groups	Treatment
Group I	Control
Group II	Sodium diclofenac -50 mg/kg
Group III	Leaf Extract 100 mg/kg PO.
Group IV	Leaf Extract 200 mg/kg PO
Group V	Bark Extract 100 mg/kg PO
Group VI	Bark Extract 200 mg/kg PO

Low (100 mg/kg) and high dose (200 mg/kg) of methanolic extracts of the leaf and bark of *Cipadessa baccifera* were administered orally 60 min prior to carrageenan challenge. After one hour of drug administration, paw edema was induced by injecting 0.1 ml of 1% of carrageenan in 0.9% sodium chloride into the sub plantar region of the right hind paw of each rat. Paw volumes were measured using plathysmograph immediately after injection and at hourly time intervals of 60, 120, 180, 240 and 360 minutes after the administration of carrageenan. The percentage inhibition of carrageen induced paw edema in malealbino Wistar rats by methanolic extracts of leaf and bark were evaluated and compared to that of the standard diclofenac sodium.

The anti-inflammatory effect of extract was calculated using the formula:

Percentage of anti-inflammatory activity = (1-D/C) × 100

(Where, D represents the percentage difference in paw volume after the administration of drugs to the rats and C represents the percentage difference of volume in the control groups).

The data is represented as mean ± SD for each group at different time intervals. Within each group the change in mean value from the 1st hour to other time intervals was compared using a paired t test. The level of significance was set at p value of less than 0.05. The percentage inhibition of paw edema is presented using mean and SE of mean.

Results

In-vivo anti-inflammatory activity Acute toxicity study

Acute oral toxicity study was performed as per OECD guidelines, where the limit dose of methanolic extracts of leaf and bark of *Cipadessa baccifera* was determined to be 2000 mg/kg.

The methanolic extracts of bark and leaf at a dose level 500, 1000 and 2000 mg/kg body weight were administered orally to the Swiss albino micewhich were fasted overnight with access only to water *ad libitum*. It was observed that there was no test substance related mortality at this limit dose of 2000 mg/kg. As the samples were practically nontoxic at 2000 mg/kg, further testing with higher doses was not found necessary. It was observed that at 2000 mg/kg dose the animals did not show any stereotypical behavioral changes or morbidity symptoms associated with toxicity such as hyperactivity, tremors, salivation, coma, sleep, convulsion, diarrhea and ataxia during the experimental period. Therefore it was noted that a maximum dosage of up to 2000 mg/kg of both the extracts were safe, which is the median lethal dose; LD_{50} for methanolic extracts of leaf and bark of *C. baccifera*.

Determination of carrageenan-induced rat paw edema

The anti-inflammatory activity of methanolic extracts of leaves and bark at two different concentrations viz., 100 and 200 mg/kg and of the standard diclofenac sodium (50 mg/kg b.w.) were assessed using carrageenan-induced paw edema model in rats. Percentage inhibition of the paw edema determined at various time intervals are presented in Table 1 and Figure 1.

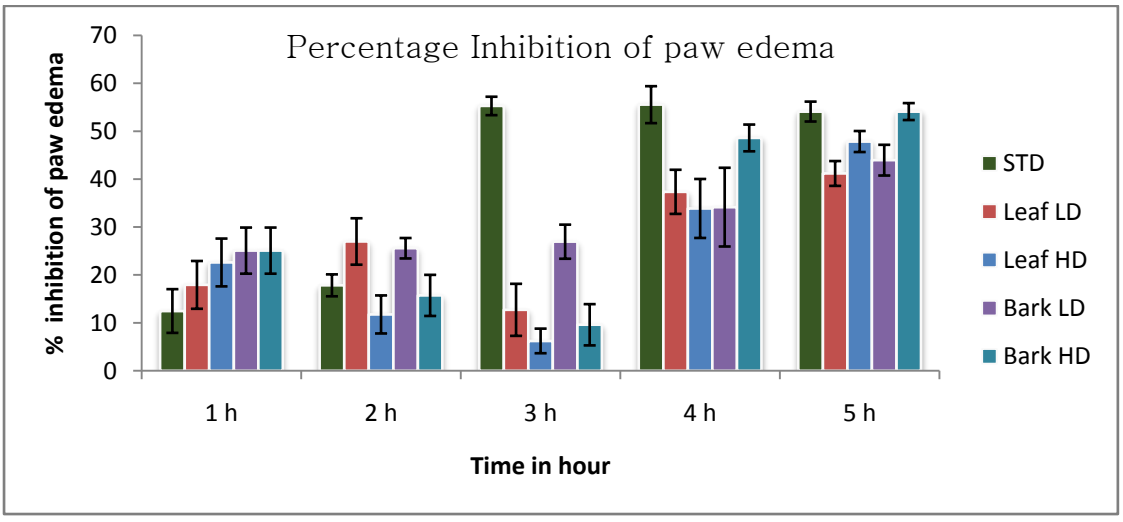

Figure 1. Percentage of inhibition of paw edema by methanolic extracts of leaf and bark

The percentage inhibition of paw edema in rats is presented as mean ± S.E.M. of n=6. Std=diclofenac sodium; LD=low dose, 100 mg/kg body weight; HD=high dose, 200 mg/kg body weight

Table 1. Effect of methanolic extracts of leaf and bark of *C. baccifera* on carrageenan - induced paw edema in rats

Treatment	**Rat hind paw volume in ml (percentage inhibition)**				
	1 hour	**2 hour**	**3 hour**	**4 hour**	**5 hour**
Group I Control	0.88±0.09	1.22±0.05	1.40±0.07	1.32±0.17	1.28±0.06
Group II Diclofenac Sodium (50mg/kg)	0.74±0.06 (12.5%)	1.00±0.07 (17.9%)	0.62 ±0.04 (55.3%)	0.54±0.07 (55.6%)	0.58±0.04 (54.1%)
Group III Leaf Extract (100mg/kg)	0.68±0.02 (17.9%)	0.88±0.10** (27.0%)	1.20±0.14*** (12.7%)	0.78±0.05** (33.9%)	0.74±0.05* (41.2%)
Group IV Leaf Extract (200mg/kg)	0.64±0.02 (22.6%)	1.06±0.06*** (11.8%)	1.30±0.05*** (6.2%)	0.76±0.02** (37.4%)	0.66±0.05 (47.9%)
Group V Bark Extract (100mg/kg)	0.62±0.03 (25.1%)	1.28±0.08*** (25.6%)	1.04±0.15*** (27.0%)	0.76±0.09** (34.2%)	0.70±0.05** (44.0%)
Group VI Bark Extract (200mg/kg)	0.78±0.04 (25.0%)	1.28±0.10*** (15.7%)	1.24±0.08*** (9.6%)	0.64±0.06** (48.6%)	0.58±0.03*** (54.1%)

Values are Mean± SD, n = 6; * P<0.05; **P<0.01; ***P<0.001, Paired t- test.

The results indicated that the sub plantar injection of carrageenan caused strong localized paw edema in rats, starting at 1 h in the Control Group I and the Groups treated with methanolic extracts of leaf and bark of *C. baccifera*. After 3 h a maximum increase in the volume of paw edema by about 1.5 folds was observed, which gradually decreased thereafter.

In the Control Group there was a time dependent increase in edema to reach a maximum volume of 1.4 mL at the 3rd hour. In the II Group of rats treated with standard diclofenac sodium drug the paw volume showed a slight increase of 1.0 mL in paw volume in the 2nd hour, but subsequently there was a gradual decrease with increase in time (Table 1).

In Group III animals treated with low dose of 100 mg/kg methanol extract of leaf, there was an increase in the paw edema volume initially which decreased after 3rd hour. However when compared to the 1st hour the decrease in the mean paw volume was significant in 2nd hour ($P<0.01$), 3rd hour ($P<0.001$), 4th hour ($P<0.01$) as well as the 5th hour ($P< 0.05$).

Similarly the Group IV animals which were treated with 200 mg/kg of leaf extract showed a gradual increase in paw edema volume which tapered down to the initial volume at the end of the 5th hour. The reduction in mean paw volume at the 5th hour was significant ($P<0.05$) when compared to the Group II animals treated with the standard diclofenac sodium.

In the Group V, bark extract at low dose of 100 mg/ kg demonstrated similar effect in paw edema volume as in the case of leaf extract, where there was an initial increase in the paw edema which showed decrease only at the end of the 3rd hour. When compared to the 1st hour the decrease in mean paw edema volume was found to be significant at the 4th hour ($P<0.01$) as well as 5th hour ($P<0.01$). At the 5th hour the mean paw edema volume was observed to be significant when compared to the Group II ($P<0.01$).

There was a significant decrease in the paw edema observed in Group VI animals treated with high dose of 200 mg/kg bark extract after the 4th hour ($P<0.01$) and 5th hour ($P<0.001$) of carrageenan injection. However statistically significant difference was not found between the Group II and Group VI at the end of 5th hour ($P\geq0.05$) indicating that the

inhibition of paw edema by high dose of bark extract was significant and comparable to the standard diclofenac sodium drug.

The percentage inhibition of paw edema was calculated for all the groups of animals at different time intervals (Table 1 and Figure 1). The percentage of inhibition induced by the standard drug was 12.5% at the 1st hour which progressively increased to a maximum of 54.1% after the 5th hour. The leaf extract at 100 mg/kg dose caused an increase in the percentage of inhibition from 17.9% in the first hour to a 27% in the 2nd hour, however in the 3rd hour there was a decline (12.73%), subsequently there was an increase to 41.2% noticed after the 5th hour. The high dose of leaf extract brought in a significant increase in the percentage of inhibition after the 5th hour (47.9%).

The animals (Group V) treated with 100 mg/kg of bark extract showed a steady increase in the percentage of inhibition. Significant anti-inflammatory effect of 100 mg/kg of bark extract started at the 4th hour after carrageenan injection and a maximum inhibition of 44.0% was seen after 5th hour. The bark extract at 200 mg/kg exhibited the maximum paw edema inhibition which was higher at the 1st hour ($P<0.001$) and on par with that of the standard Diclofenac after the 5th hour ($P\geq0.05$).

The anti-inflammatory effect of methanolic extracts of leaf and bark at a dose 200 mg/kg was observed to be more pronounced. However, the anti-inflammatory activity of bark extract (200 mg/kg) of *Cipadessa baccifera* was more effective and significant.

Discussion

In vivo anti-inflammatory activity

Inflammation is a fundamental defensive reaction of the body which involves several complex array of enzyme activation, release of mediators, extravasation, cell migration, tissues break down and repair (Vane & Bolting, 1995). The progression of inflammatory diseases could be due to persistent, uncontrolled inflammation and production of ROS in the body.

Acute toxicity test

Toxicity indicates the state of adverse effects caused by the interaction between toxicants and cells (Das *et al*., 2015). Determination of acute toxicity; LD_{50} is an initial step in the assessment and evaluation of toxic characteristics of a substance. According to the guidance document on Acute toxicity based on oral LD_{50} value recommended by OECD, the

crude methanolic extracts of leaves and bark of *C. baccifera* could be assigned under Category 4 with limit test dose (LD_{50}) of 2000 mg/kg.

In the present investigation, acute oral toxicity of leaf and bark extracts of *Cipadessa baccifera* was found to be significant when compared to findings of earlier reports on leaf and bark extracts of *Azadirachtaindica* (Okpanyi and Ezeukwu, 1981; Bakr in 2013).

Carrageenan-induced paw edema test

In the present study significant inhibition of paw edema by the methanolic extracts of leaf and bark was observed in the 2nd phase. The development of a characteristic edema in the rat hind paw following the injection of carrageenan is a biphasic event; dependent on the age and weight of the animal (Marsha *et al*., 2002). In the early phase of inflammation mediators such as histamine, serotonin bradykinin and possibly the cyclooxygenase products are produced, while in the second phase prostaglandins, leudotrienes, free radicals, proteases and lysosomes are released. It is the second phase which is sensitive to most of the clinical anti-inflammatory drugs (Purnima *et al*., 2010). The inhibition of paw edema may possibly be due to cyclooxygenase synthesis inhibition by the methanolic extracts of *C. baccifera* which is similar to the mode of action of non-steroidal anti-inflammatory drugs such as diclofenac. Non-steroidal anti-inflammatory drugs (NSAID) inhibit cyclooxygenase activity and block the synthesis of prostaglandins (Botting, 2006).

Significant anti-inflammatory activity of methanolic extracts of leaf and bark of *C. baccifera* was observed at a dose of 200 mg/kg. Similar findings have been reported in stem bark of *Khayasenegalensis* (Kolawole*et al*., 2013) and leaves of *Azadirachtaindica*.

The anti-inflammatory potential of methanolic extracts of leaf and bark of *C. baccifera* may be attributed to the presence of bioactive compounds such as polyphenolics especially phenols, tannins, and flavonoids which have been reported in literature to confer anti-inflammatory properties to plants (Handa*et al.*, 1992; Orhan*et al*., 2007).

The results obtained in the present investigation corroborate the use of the *Cipadessa baccifera* in traditional medicines to treat inflammatory related disorders such as, rheumatism, psoriasis, wounds, etc. Further investigation is required to discern specific active compounds exerting the anti-inflammatory activity for exploring the therapeutic uses in medicine.

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