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Analgesic and anti-inflammatory activities of methanol tuberous root extract of *Decalepis salicifolia* (Bedd. *ex* Hook.f.) Venter

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ABSTRACT

The objective is to study the analgesic and anti-inflammatory activities of methanol extract of tuberous root of *Decalepis salicifolia.* The analgesic effect was studied using acetic acid-induced writhing and hot plate method in mice and anti-inflammatory effect was investigated using carrageenan, formalin and histamine induced paw edema in rats. In the acute toxicity study, oral median lethal dose (LD_{50}) was found that the extract was non-toxic up to 2000 mg/kg, p.o. The *Decalepis salicifolia* root extract (50 and 100 mg/kg b.w.) was found to possess, analgesic and anti-inflammatory activities in a dose-dependent manner and related inhibition were achieved in histamine, followed by formalin and the effect was compared with standard drug. The above results will be the supporting evidence for the potential anti-rheumatoid activity of *D. salicifolia* in our Indian traditional medicine system.

Keywords: *Decalepis salicifolia*, anti-inflammatory, analgesic, anti-rheumatoid.

INTRODUCTION

Decalepis salicifolia (Bedd. *ex* Hook.f.) Venter popularly known as swallow root in English, belongs to the family Apocynaceae. It is endemic woody shrub and native of the forests Anaimalai hills, Southern Western Ghats of Tamilnadu, India. It is woody shrub with stout, smooth branches and aromatic tuberous roots. The name *Decalepis salicifolia* is synonym of *Utleria salicifolia* is critically endangered medicinal plant due to habitat loss, poor sexual reproduction and germination. Recently some researcher had taken necessary action to conservation management by micropropagation techniques (Saradha and Samydurai, 2018; Khan *et al.,* 2019). Its tuberous root is consumed as pickles and juice for its alleged health promoting properties. The tuberous root of *D. salicifolia* medicinal properties and bioactive compounds are like *D. hamiltonii* and *Hemidesmus indicus* having sweet taste, contains aldehyde, amyrins,

lupeols and volatile flavour compounds such as 2-Hydroxy 4-methoxybenzaldehyde, vanillin and essential oil like 4-methylresorcyladehyde, atlantone, terpinene, geraniol. Many researchers have been recently reported that the plant possessing nutritional, antioxidant, anti diabetic, antiatherosclerotic, anti-inflammatory and hepatoprotective properties (Chidambara Murthy *et al.,* 2006; Naveen and Khanum, 2010; Thangavela *et al.,* 2011 and Samydurai and Thangapandian, 2012).

Pain is an unpleasant sensation that is consequence of complex neurochemical processes in the central and peripheral nervous system (Howland and Mycek, 2006). Non-steroidal anti-inflammatory drugs and opioids are used in the management of mild to moderate and severe pains respectively. Opioids may cause respiratory depression, euphoria, tolerance and dependence while non steroidal drugs produce gastrointestinal irritation and renal damage (Howland and Mycek, 2006). The World Health Organization (WHO) encourages the inclusion of herbal medicines of proven safety efficacy in the healthcare programmes of developing countries (Amos *et al*., 2001). To the best of our knowledge, its activities were seldom examined. The aim of the study was to investigate the potential analgesic and anti-inflammatory activities of *Decalepis salicifolia* tuberous root extract in animals. We expect that it will support the evidence for the possible anti-rheumatoid properties of this plant root.

MATERIALS AND METHODS

Plant materials

The fresh plant roots of *Decalepis salicifolia* were collected from Southern Western Ghats of Coimbatore district, Tamilnadu, India. The collected flowered plant material was identified and they were authentically confirmed by self and Botanical Survey of India, Coimbatore, Tamilnadu, India. Freshly harvested plant tubers were cleaned for adhering soil particles and then dried under shade. The dried samples were powdered and used for further studies.

Preparation of extracts

The shade dried, tuber powder was extracted with methanol using soxhlet apparatus at room temperature for 48 h. The obtained residue was lyophilized (VirTis Benchtop K, USA) and stored at room temperature at 27°C. That yielded about 17–20 g per 100 g of tuber powder. The dried extract was used for assessing the analgesic and anti-inflammatory activity.

Animals

Swiss albino mice (18-25g) and Wister rats (170-230g) were purchased from Small Animal Breeding Station, Munnuthy, Trissur, Kerala. The animals were housed under standard conditions of temperature at 23˚C, relative humidity (55±1) %, 12/12 h light/dark cycles and fed with standard pellet. All the animal experiments were conducted with permission from Institutional Animal Ethical Committee.

Chemicals and drugs

Carrageenan, acetic acid, formaldehyde, histamine and standard drug Pentazocine, Acetyl salicylic acid and Indomethacin were purchased from Sigma Aldrich Chemical Company, Steinheim, Germany. All the chemicals and drugs used were of the highest purity and analytical grade.

Acute oral toxicity studies

Acute oral toxicity studies were performed according to OECD No.423 guidelines (OECD, 1996 and Ecobichon, 1997) and its median lethal dose (LD_{50} value) was determined. Three rats of either sex (2 female and 1 male) were selected for the study. The *D. salicifolia* methanol root extract (suspended with 0.5%, w/v, Carboxyl Methyl Cellulose) was administered with higher dose 2000 mg/kg p.o. to the rats which were made to fast overnight for food with free access for water prior to test extract. Individual rat was observed after dosing at least once during the first 30 min, periodically during the first 4 hr and daily thereafter, for a total of 14 days. The rats were observed for systemic and behavioural toxicity patterns as described in OECD/OCDE test Guidelines. At the end of toxicity study, all survived animals were sacrificed.

EVALUATION OF ANALGESIC ACTIVITY

Hot plate method

The hot plate test was used to measure analgesic activity by the method (Eddy NB, Leimback, 1953), with minor modifications. In this experiment, the hot plate was maintained at 55±0.5˚C. All animals were selected 24 hr prior to experimentation on the basis of their normal reaction time i.e., pain response to the hot plate to the minimum and maximum of 2-15 sec. respectively. In order to avoid damaging the paws of the animals, the time standing on the plate was limited

to 25 sec. All the rats were divided into four groups each comprising six animals. Group I normal rat served as a control. Pentazocine 10mg/kg was administered intraperitoneally (Group II) as a reference standard. Group III and IV received *D. salicifolia* methanol tuber extract at concentrations of 50 and 100mg/kg body weight respectively. Thirty minutes after administration of standard drug and extract, animals were placed individually onto the hot plate and the time from placing the animal on the hot plate to jumping of the animal from the hot plate was reordered as the reaction time or latency of the pain response.

Acetic acid-induced writhing test in mice

This test was conducted using the method (Koster *et al.,* 1959). Swiss albino mice, weighing 18-25g, were randomly divided into four groups, each group consisted six animals. Control Group (Group I) received 10mL/kg distilled water orally. The reference group (group II) received acetyl salicylic acid (10mg/kg dissolved in distilled water, p.o) and group III and IV orally pre-treated with 50 and 100mg/kg methanolic tuber extract respectively. All drugs were administered orally 30 minutes prior to intraperitoneal administration of 0.75% v/v acetic acid solution (0.1mL/10mg). 30 minutes later the animals were placed on an observation table and observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. The number of writhing and stretching was recorded and compared with the control drug. A reduction in the number of writhes is an indication of analgesic property.

EVALUATION OF *IN VITRO* ANTI-INFLAMMATORY ACTIVITY

Carrageenan-induced paw edema in rats

The anti-inflammatory activity was evaluated by carrageenan induced rat paw edema model (Winter *et al.,* 1962). Wister rats of either sex were weighed (170-230gm) and normal paw volumes of all the rats were measured initially and then divided into four groups each comprising six animals. Inflammation was induced in all rats by single sub plantar injection of 0.1mL freshly prepared 1% carrageenan in normal saline. Group I treated with carrageenan alone served as control. The group II rats were treated with 10mg/kg Indomethacin (Dharmasiri *et al.,* 2002), group III and IV received *D. salicifolia* methanol root extract at concentrations of 50 and 100mg/kg body weight orally one hour before the carrageenan injection. The change in paw thickness (mm) was measured using Plethysmometer (Vogel, 2002) at 0, 1, 2, 3, 4 and 5 hr after carrageenan injection. The change in paw thickness was considered as a measure of inflammation and was calculated as % inflammation inhibition.

% inflammation inhibition = [Control group mean-test group mean]/ (Control group mean) ×100

Formalin-induced paw edema

Wister albino rats of 180-200g weight were used for the study and divided into four groups each group contains six rats. Vehicle (distilled water 10mg/kg), Indomethacin (10mg/kg) and tuber extract (50 and 100 mg/kg) were administered orally for respective groups. Thirty minutes after the treatment inflammation was produced by sub planter injection of 0.1mL of (1% w/v) freshly prepared formalin in the right hind paw of rats. Before formalin injection, the paw volume for each rats were measured separately by means of digital calibrated vernier caliper. Edema caused by formalin was measured at 0, 1, 2, 3, 4 and 5 hr. The increase in paw thickness and percentage inhibition are calculated and compared with control group (Turner, 1995).

Histamine-induced paw edema

Using the method of Perianayagam *et al.,* (2006), the paw edema was produced by sub-plantar administration of 0.1% freshly prepared solution of histamine in to the right hind paw of the rats. Rats were divided into four groups of six rats per group were used just like in Carrageenan test. The paw volume was recorded before 0, 1, 2, 3, 4 and 5 hr after the histamine injection. The four groups of the rats were pretreated with the plant extract (50 and 100 mg/kg) 2mL/kg of normal saline (vehicle control) or 10 mg/kg Indomethacin. These were administered intraperitoneally 1hr before eliciting paw edema. The anti-inflammatory activity was calculated as described for carrageenan-induced edema.

Statistical analysis

Values are expressed as mean (*n=6*) of each groups analysis of the samples (*n=3*) standard deviation (SD). Analysis of variance and significant differences (*P<0.01*) among means were tested by Duncan multiple range test.

RESULTS AND DISCUSSION

ACUTE ORAL TOXICITY

The *Decalepis salicifolia* methanol tuberous root extract did not produce any mortality at the highest dose employed. Selected doses of this plant methanol extract were found to be safe. Two doses (50 and 100 mg/kg, p.o.) of this extract were selected for further pharmacological studies.

ANALGESIC ACTIVITY

Effect of methanol tuberous root extract of *D. salicifolia* on thermally-induced pain in mice

The *D. salicifolia* methanol root extract exhibited a dose dependent manner on thermally induced pain in mice. This inhibition was statistically significant ((*P*<0.01)) compared to the standard drug Pentazocine (Table 1).

Effect of methanol tuberous root extract of *D. salicifolia* on acetic acid induced writhing response in mice

Figure 1 showed that *D. salicifolia* root extract (50mg and 100mg/kg b.w.), at 30 minutes beforehand, produced a dose related inhibition of acetic acid induced writhing effect in mice. The writhing reductions were statistically significant (*P<0.01*) relatively to the standard drug, acetyl salicylic acid, at the highest dose, 20mg/kg and also produced marked inhibition of treated doses of tuber extract of 50mg and 100mg/kg respectively.

Table 1 Analgesic activity of methanol extract of *D. salicifolia* tuberous root using hot plate test in mice.

Treatment	Doses (mg/kg)	Reaction time after oral administration of control/ standard/extract in minutes			
		30 min	60 min	90 min	120 min
Control	-	4.14±0.19^{d}	3.66±0.10^{c}	3.16±0.07^{d}	2.45±0.07^{d}
Pentazocine	10	6.40±0.17^{c}	6.28±0.13^{ab}	5.61±0.18^{b}	5.25±0.14^{b}
Extract	50	7.75±0.15^{b}	7.10±0.11^{ab}	6.40±0.10^{c}	5.13±0.09^{c}
Extract	100	8.35±0.19^{a}	7.91±0.15^{a}	7.19±0.12^{a}	6.71±0.10^{a}

Mean in a column followed by a same letter(s) are not significantly (*P<0.01*) different according to Duncan's Multiple Range Test.

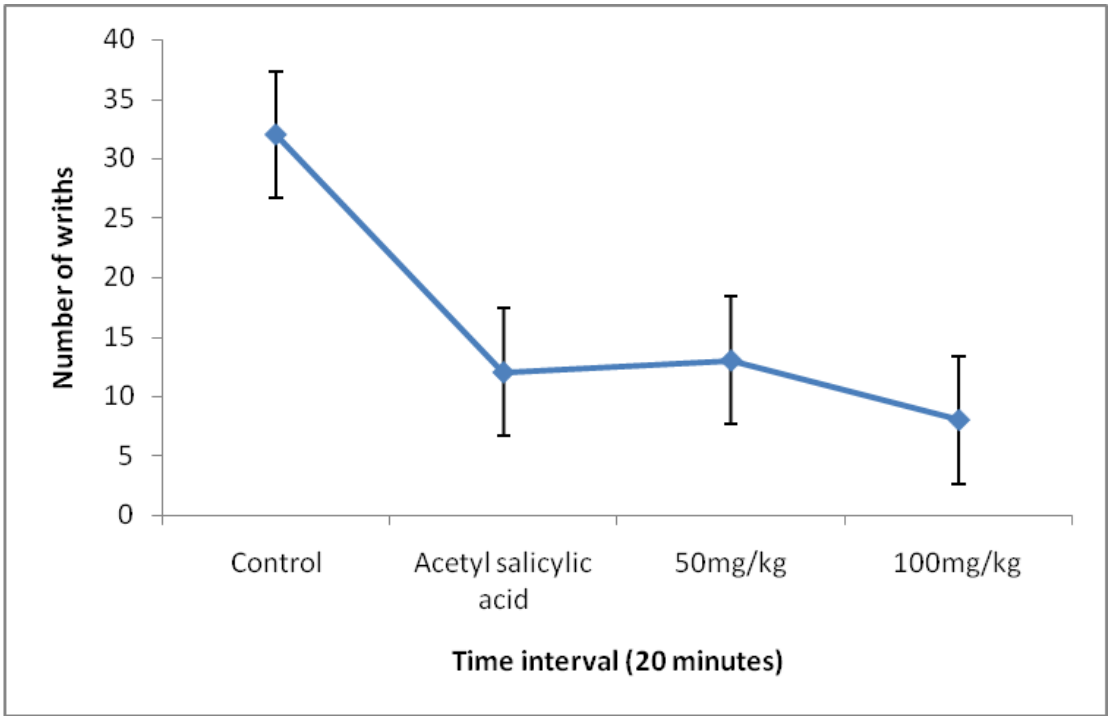

Figure 1. Effect of *D. salicifolia* tuberous root extract on acetic acid induced writhing in mice

Table 2. Effect of *D. salicifolia* tuberous root methanol extract on carrageenan-induced edema and formalin induced edema in rats.

Anti-inflammatory model	Treatment	After injection of paw edema thickness (h)						
		0	0.5	1	2	3	4	5
Carrageenan induced paw edema	Control	4.42 ±0.13^{b}	5.10 ±0.10^{d}	5.32 ±0.08^{d}	5.58 ±0.06^{c}	5.89 ±0.05^{c}	5.61 ±0.11^{c}	5.33 ±0.10^{b}
	Indomethacin 10mg/kg	4.11 ±0.13^{a}	4.34 ±0.15^{a}	5.05 ±0.16^{a}	4.88 ±0.13^{a}	4.44 ±0.12^{a}	4.14 ±0.10^{a}	4.09 ±0.08^{a}
	Tuber extract 50mg/kg	4.25 ±0.16^{b}	4.61 ±0.18^{c}	4.99 ±0.19^{c}	4.71 ±0.16^{b}	4.51 ±0.18^{b}	4.22 ±0.11^{b}	4.03 ±0.10^{a}
	Tuber extract 100mg/kg	4.14 ±0.12^{a}	4.35 ±0.14^{b}	4.49 ±0.16^{a}	4.37 ±0.15^{a}	4.17 ±0.14^{a}	4.09 ±0.11^{a}	4.0 ±0.08^{a}
Formalin induced edema	Control	4.33 ±0.17^{a}	5.28 ±0.23^{c}	5.47 ±0.25^{a}	5.61 ±0.28^{b}	5.80 ±0.31^{d}	6.06 ±0.32^{c}	5.94 ±0.18^{d}
	Indomethacin 10mg/kg	4.15 ±0.17^{a}	4.88 ±0.25^{a}	5.15 ±0.21^{a}	5.04 ±0.17^{a}	4.71 ±0.19^{a}	4.26 ±0.15^{a}	4.15 ±0.12^{a}
	Tuber extract 50mg/kg	4.29 ±0.17^{a}	4.76 ±0.21^{b}	5.08 ±0.23^{a}	4.88 ±0.16^{b}	4.73 ±0.15^{c}	4.35 ±0.13^{ab}	4.11 ±0.11^{c}
	Tuber extract 100mg/kg	4.19 ±0.19^{a}	4.49 ±0.21^{b}	4.71 ±0.23^{a}	4.66 ±0.17^{ab}	4.47 ±0.14^{b}	4.26 ±0.13^{ab}	4.11 ±0.09^{b}

Mean in a column followed by a same letter(s) are not significantly (*P<0.01*) different according to Duncan's Multiple Range Test.

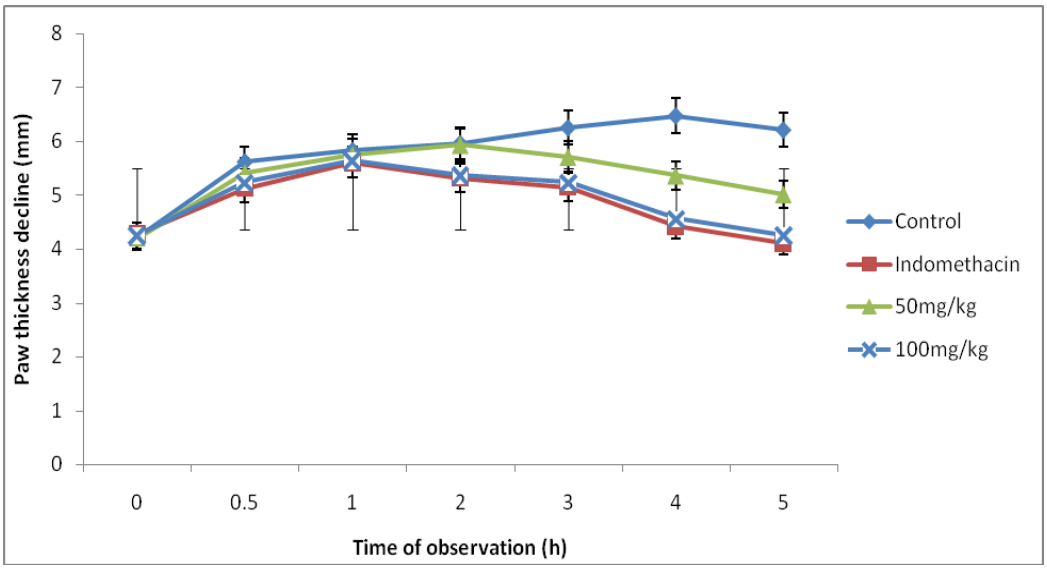

Figure 2. Effect of *D. salicifolia* methanol extract of tuberous root extract on histamine induced paw edema in rat

ANTI-INFLAMMATORY ACTIVITY

Effect of methanol tuberous root extract of *D. salicifolia* on carrageenan induced paw edema in rats

The results of carrageenan induced rat paw edema model were employed for anti-inflammatory assessment of *D. salicifolia* methanolic tuberous root extract was shown in Table 2. The extract exhibited statistically significant (*P<0.01*) at the dose of 50 and 100mg/kg, the reduction of paw volume were quite comparable to standard drug i.e. Indomethacin (10 mg/kg) (Table 2).

Effect of methanol tuberous root extract of *D. salicifolia* on formalin induced paw thickness

Formalin induced hind paw edema model, the oral administration of plant root extract in dose dependent (50mg and 100mg/kg) reduction in paw volume statistically significant (*P<0.01*). The formalin induced paw thickness decline was compared to Indomethacin, at the dose of 10mg/kg b.w were recorded (Table 2).

Effect of methanol tuberous root extract of *D. salicifolia* on histamine induced paw edema

Histamine induced paw edema model was employed for inflammation paw volume decline in methanol root extract displayed statistically significant (*P<0.01*) at dose dependent manner of 50 and 100mg/kg respectively. The histamine induced paw magnitude reduction was compared to standard drug, at the dose of 10mg/kg b.w were recorded (Figure 2).

Tuberous root of *D. hamiltonii* is traditionally used as pickling along with curds or lime juice (Anon, 1952) and the earlier reports of the phytochemical analysis of the root revealed that 2-hydroxy 4-methoxy benzaldehyde is the major flavonoid compound (Nagarajan *et al.,* 2001). Tuberous roots are used as health tonic, food additives and the treatment of appetite, blood purifier, indigestion, constipation, pain relief and gas troubles (Vedavathy, 2004). So, these properties drive to evaluate the analgesic and anti-inflammatory properties of the methanol tuberous root extract of *Decalepis salicifolia*.

In acute toxic examination, the median lethal dose (LD_{50}) was determined up to 2000mg/kg b.w and the crude extract was relatively safe (Ecobichon, 1997). *D. salicifolia* root extract may not cause any toxic effects, because aromatic tuberous roots are traditionally used as food and food additives (Samydurai *et al.,* 2012). In this evident that carrageenan induced hind paw edema is widely used as an experimental model for inflammation and is believed to be biphasic; the first phase is attributed to the release of histamine, serotonin, kinin and the second phase is related to the release of prostaglandin and bradykinins (Castro *et al.,* 1968; Vane, 1987). The methanol root extract was found to be steroids and flavonoids (Samydurai and Thangapandian, 2012). So, the anti-inflammatory activity of this plant may be presence of these bioactive chemical constituents. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception (Bittar *et al.,* 2000; Santa-Cecilia *et al.,* 2011).

The extract significantly increased the reaction time and reduced skipping time on hot plate induced pain and acetic acid induced writhing response as well as reaction time of mice to thermally induced pain. This model is selective for centrally acting analgesics and indicates narcotic involvement with opioids receptors (Turner, 1965). Acetic acid causes inflammatory pain by inducing capillary permeability and in part through local peritoneal fluid concentration of PGE_2 and $PGE_2{}^a$ (Bentley *et al.,* 1983). However, the central acting drugs inhibit both acetic acid induced writhing and thermally induced pain (Amanlou *et al.,* 2005).

The system of the anti-inflammatory effect of *D. salicifolia* root extract in formalin induced paw edema in rats may be depend on the neutralization of active globulins which are non-steroidal anti-inflammatories (Suleyman *et al.,* 1999. Generally histamine is released following the mast cell degranulation by the number of inflammatory mediators including substances P and IL-1. It could be induced and release of neuro peptides as well as liberate prostaglandins and monohydroxy eicosatetranoic acid from endothelial cell leading to hyperalgesia and other pro-inflammatory effects (Suralkar *et al.,* 2008; Ved Prakash, 2017; Ibrahim *et al.,* 2017). The *D. salicifolia* root extracts may act on inflammatory mediators and inhibit the release of prostaglandins and histamine mediators which causes mucus secretion and mucosal edema. In conclusion, the *Decalepis salicifolia* tuberous root extract had noticeably inhibitory effects of analgesic and anti-inflammatory activities. These kinds of inhibitory effects could be clearly proved to be novel therapeutic strategies for the treatment of analgesic and inflammatory properties of traditional medicinal plant *Decalepis salicifolia*.

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Conflict of interest

The author declares that there is no conflict of interest.

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