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Anti-inflammatory and analgesic activities of roots and leaves of *Chassalia curviflora* (Wall.) Thwaites (*Psychotria curviflora* Wall.)

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ABSTRACT

Chassalia curviflora (Psychotria curviflora (Wall; Family Rubiaceae) is frequency used in folklore medicines to treat a number of clinical disorders. In this study the ethanolic extract of leaves and roots of this plant was screened for in vivo anti-inflammatory and analgesic and in vitro anti lipidperoxidative activites. Both leaf and root extracts at 50,100 and 200 mg/kg doses reduced significantly the formation of oedema induced by carrageenan. The results were comparable to that of indomethacin, the reference drug used in the study. In the acetic acid induced writhing, the extracts showed significant analgesic activity characterized by reduction in the number of writhes when compared to the control. The extracts also inhibited MDA production in $FeCl_2$–AA treated rat liver in vitro, exhibiting, thereby, a significant anti-lipid peroxidative efficacy. Acute toxicity study data suggested a nontoxic nature of the extracts within the range of doses tested.

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INTRODUCTION

Herbal formulations have been frequently used in traditional systems of medicine and health care since antiquity. The use of medicinal plants in the treatment of various clinical disorders has been associated with their folklore knowledge which is often not well documented & validated scientifically. *Chassalia curviflora* (Family: Rubiaceae; tribe: Psychotrieae), formerly known as *Psychotria curviflora,* is an erect shrub with white or tinged pinkish flowers and shiny black fruits (Fig. 1) and is commonly distributed in ever green forests [2]. The genus *Chassalia* includes more than 110 species with paleotropic distribution [21]. It is distributed from India to South China and Philippines. In India, the plant is widely distributed in Sikkim, Darjeeling, Meghalaya, Maharashtra, Karnataka, Andaman and Western Ghats. The plant is commonly called curved flower chasalis and is also known as Vellakurinji, Yamari or Mundanchedi in Malayalam. In Peninsular Malaysia, a decoction of the roots is used to treat malaria, cough and as a remedy in phlegm, rheumatism and pneumonia [3, 9]. Roots and leaves are used to cure wounds and ulcers. It is also externally applied to relieve headaches. Root paste in water is applied topically on affected places to heal

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Fig. 1. Plants of *Chassalia curviflora* (Wall.) Thwaites (*Psychotria curviflora* Wall.) in bloom.

wounds and pimple [9]. Chakma tribes use this plant for treating snake and insect bites [5]. It is also widely used by Kani tribes as an effective medicine in the treatment of jaundice [34].

Inflammation is a non-specific immune response through which the body responds to infection, irritation or injury. It is characterized by redness, warmth, swelling and pain. Chronic inflammatory diseases remain one of the world's major health issues [6]. Inflammatory and infectious diseases are efficiently treated by several traditional herbal medicines. In the present study, the anti-inflammatory, analgesic and anti-lipidperoxidative effects of ethanolic extract of the roots and leaves of *C. curviflora* were investigated.

MATERIALS AND METHODS

Plant material

C. curviflora leaves and roots were collected from the Medicinal Plant Garden of the University Campus, Kariavattom, Thiruvananthapuram. The plant was identified by Dr.G.Valsala Devi, Curator, Department of Botany, University of Kerala. A voucher specimen was deposited at the herbarium of JNTBGRI (TBGT 57053 23/9/11).

Preparation of plant extract

50g of the powdered leaves and roots was separately extracted with 500ml of 95% ethanol with continuous stirring. The aliquots were gradually concentrated in vacuum and allowed to dry. The root and leaf extracts were labelled Chr and Chl, respectively.

Phytochemical screening

Preliminary phytochemical analysis of crude extracts of roots and leaves was carried out to test the presence of major chemical constituents such as alkaloids, flavanoids, saponins, tannins, terpenoids, steroids, reducing sugars, resins, sterols, aminoacids and cardiac glycosides as described in the literature [37].

Experimental animals

Wistar rats (150-200gm) and Swiss albino mice (20-30gm) of either sex were obtained from the animal house of the Jawaharlal Nehru Tropical Botanic Garden Research Institute, Palode. They were grouped and housed in poly-acrylic cages and maintained under standard laboratory conditions (temperature 24-28^{0}C, relative humidity 60-70% and 12 hours dark- light cycles). They were fed commercial rat feed (Lipton, Mumbai) and boiled water, *ad libitum*. All animal experiments were carried out according to NIH guidelines, with the approval of the Institute's Animal Ethics Committee (Registration No.25-1/99/AWD176/CPCSEA dtd.29/9/1999).

Anti-inflammatory activity measurement through Carrageenan- induced paw oedema

Anti–inflammatory activity of Chr and Chl was assessed by Carrageenan induced paw oedema method of Anuja et al., 2010 [4].The animals were divided into eight groups (six animals in each group). Group I animals (Carrageenan control) were orally administered 1 ml 0.5% of Tween-80. Group

II, the standard reference group was given 1 ml aqueous solution of indomethacin (10mg/kg) in normal saline, Groups III, IV, V received 50, 100 and 200 mg/kg of Chr, Groups VI,VII, VIII received 50, 100 and 200 mg/kg of Chl. After 30 min of Chr and Chl administration, 0.1 ml 1% carrageenan (Sigma Chemicals Company, USA) was injected into the right hind paw under plantar aponeurosis of all the animals. The paw volume was measured using a Plethysmometer just before and 3h after carrageenan injection. The difference in paw volume indicated the degree of inflammation. The percentage inhibition of oedema was calculated for each group in comparison to the vehicle treated control group.

Analgesic activity measurement through acetic acid induced writhing assay

Analgesie efficacy of Chr and Chl was assessed by the writhing test in mice [4]. Swiss albino mice were divided into eight groups (six animals in each group). Group I, acetic acid control group received a single dose of 0.5% Tween-80 (0.5ml) orally. Group II, the standard control group received a single oral dose of 25 mg / kg of aspirin as per the procedure of Bose *et al* [8]. Groups III, IV and V received a single dose of 0.5 ml of 50, 100 and 200 mg / kg Chr and groups VI, VII and VIII received a single dose of 0.5 ml of 50, 100 and 200 mg/kg Chl, respectively. All groups received intraperitoneally 0.5 % aqueous solution of acetic acid (0.25 ml) 20 min post drug administration. The number of writhes per animal was recorded during the 20 min period, beginning 5 min after the injection of acetic acid.

Anti lipid peroxidation assays

Anti lipidperoxidant effect of Chr and Chl was studied *in vitro* following the method of Suja et al. 2004 [41]. Briefly, 0.5 g of the rat liver tissue was sliced and homogenized in 10ml of 150 mM KCl-Tris-HCl buffer (pH 7.2). 0.25 ml of this liver homogenate was taken and added in control, induced and sample tubes. This was followed by addition of 0.15ml Tris HCl buffer (pH 7.2), 0.05ml of 1 mM ascorbic acid, 0.05 ml of 4mM $FeCl_2$ to the induced tubes and 25, 50, 100ìg/ml Chr or Chl in the sample tubes, respectively. The mixture was incubated at 37^{0}C for 1 hr in capped tubes. Then 0.5ml of 0.1N HC1, 0.2 ml of 9.8% sodium dodecyl sulphate (SDS), 0.9 ml of distilled water and 2ml of 0.6% thiobarbituric acid (TBA) were added to each tube followed by vigorous shaking. The tubes were then placed in a boiling water bath at 100^{0}C for 30 min. After cooling, 5 mL of butanol was added and centrifuged at 3,000 rpm for 25 min. The absorbance of the supernatant was measured at 532 nm.

Behavioral and toxicity assays

Behavioral and toxic effects of Chr and Chl extracts were studied following a previously reported method [36]. Fourteen groups of 10 mice each were administered orally 50, 100, 200, 500, 1000, 1500, 2000 mg/kg of Chr and Chl, respectively with appropriate controls. All the animals were observed continuously for the first 3hr and then 1h intermittently up to 24 h for recording behavioral changes like convulsions, hyperactivity, sedation, grooming, loss of righting reflex, epilation, respiratory rate, food and water intake, state of fecal pellets and mortality. The animals were observed for post treatment toxic symptoms daily for 7 days after treatment

Statistical analysis

The statistical analysis was carried out using the Students't'-test [38]. Results are reported as mean $\pm$ SD and groups with $P \leq 0.01$ were considered as significant.

RESULTS

Phytochemical screening

The ethanolic extracts of roots and leaves were analyzed for the presence of various phytochemicals. Qualitative chemical tests were performed to identify the bioactive compounds. The results are summarized in Table1.

Anti–inflammatory activity

C. curviflora extracts administered orally significantly inhibited Carrageenan induced paw oedema at three doses (50,100 and 200 mg/kg).

Table 1. Result of phytochemical analysis of root and leaf extracts of *Chassalia curviflora* (Wall.) Thwaites

Phytochemicals	Plant extracts	
	Chr	Chl
Alkaloids	+	+
Flavonoids	+	–
Sterols	+	+
Saponins	+	+
Tannins	+	+
Terpenoids	+	+
Resins	+	–
Phenols	+	–
Cardiac glycosides	+	+
Reducing Sugar	+	+
Quinines	+	–
Steroids	+	+
Amino acids	+	+

At 50 and 100 mg/kg doses there was 52 and 64 % inhibition for Chr and 45 and 57 % reduction for Chl. At 200mg/kg dose Chr gave 70.67% and Chl gave 65% inhibition at 3 hr after carrageenan injection. Indomethacin (10mg/kg) produced 84 % inhibition of oedema (Table 2).

Table 2: Effect of ethanolic extracts (Chr,Chl) of *Chassalia curviflora* root and leaf and indomethacin on Carrageenan induced paw oedema in rats.

Treatment	Oral Dose (mg/kg)	Differences in paw volume at 3 h. (ml)	Percentage inhibition of oedema
Carrageenan control (CG)	—	0.75±0.05	—
CG + Indomethacin	10	0.12±0.01**	84.00
CG+Chr	50	0.36±0.06	52.00
CG+Chr	100	0.27±0.04**	64.00
CG+Chr	200	0.22±0.02**	70.67
CG+Chl	50	0.41±0.03	45.33
CG+Chl	100	0.32±0.04	57.33
CG+Chl	200	0.26±0.02**	65.33

Values are the mean ± S.D, n = 6 Students 't' test **P ≤ 0.01, compared to carrageenan control

Analgesic activity

Intraperitonial injection of acetic acid produced 55.83±1.35 writhes in control group. Chr and Chl significantly inhibited acetic acid induced writhing in a dose-dependent manner. Chr at 50 and 100 mg/kg doses showed 51.9% and 71.65% inhibition of writhing whereas Chl showed 34.9 and 58.8% inhibition of writhing at these doses. At 200mg/kg dose Chr gave 86.87% and Chl gave 62.99% inhibition of writhing. Aspirin produced 92% inhibition of writhing (Table 3).

Table 3: Effect of *Chassalia curviflora* root and leaf ethanolic extracts (Chr, Chl) and aspirin on acetic acid induced writhing response in mice.

Treatment	Oral dose (mg/kg)	Mean number of writhes in 30 min	Percentage inhibition of writhing
Acetic acid control (AA)	–	55.83±1.35	–
AA+Aspirin	25	4.33±0.47**	92.24
AA+Chr	50	25.17±0.60	54.92
AA+Chr	100	15.83±1.77**	71.65
AA+Chr	200	7.33±1.11**	86.87
AA+Chl	50	36.33±1.25	34.93
AA+Chl	100	23.00±2.16	58.80
AA+Chl	200	20.66±0.75**	62.99

Values are the mean ± S.D, n = 6 Students 't' test **P ≤ 0.01, compared to acetic acid control

In vitro anti lipid peroxidation studies

Chr at 100 mg/kg dose showed the maximum percentage inhibition of lipid peroxidation *in vitro.* A considerable increase in malondialdehyde (MDA) was seen in $FeCl_2$–AA treated rat liver homogenate compared to the control group. Chr at 100mg/kg dose showed 54.85% inhibition of MDA whereas Chl at 100mg/kg dose produced 46.85% inhibition. Chr and Chl inhibited MDA production in $FeCl_2$ –-AA treated rat liver *in vitro*, exhibiting significant anti-lipid peroxidative efficacy (Table 4).

Behavioral and toxicity studies

In the behavioral and toxicity studies, the mice treated with Chr and Chl did not show any gross

Table 4: Inhibitory effect of *Chassalia curviflora* root and leaf ethanolic extracts (Chr, Chl) on $FeCl_2$–ascorbic acid (AA) induced lipid peroxidation in rat liver homogenate *in vitro*

Groups	Concentration (µg/ml)	MDA (nmoles/mg protein)	MDA inhibition%
Normal control	-	2.60±0.03	-
$FeCl_2$-AA control	-	6.29±0.08	-
$FeCl_2$-AA + Chr	25	3.47±0.08**	44.83
$FeCl_2$-AA + Chr	50	3.16±0.02**	49.76
$FeCl_2$-AA + Chr	100	2.84±0.02**	54.85
$FeCl_2$-AA + Chl	25	3.88±0.05	38.31
$FeCl_2$-AA + Chl	50	3.74±0.04	40.54
$FeCl_2$-AA + Chl	100	3.61±0.02**	42.61

Values are the mean ± S.D n = 3, Students't' test **P $\leq$ 0.01, compared to $FeCl_2$-AA control

behavioral changes and no mortality occurred with the seven doses of Chr and Chl tested. Therefore the LD_{50} value for Chr and Chl could be greater than 2000 mg/kg.

DISCUSSION

The Greek word for genus *Psychotria* is *Psyche* that means life, referring to the powerful medicinal properties or qualities possessed by its species. Several species of this genus are known to synthesize cyclotides, macrocyclic and cystine knotted peptides [22]. In several Mauritian endemic *Chassalia* species, flavanoids have been identified [40]. Benzoic acid and benzoquinone derivatives have also been isolated from *C. curviflora* [45]. Typtamineindole alkaloids, classified as monoterpineindole alkaloids (MIA) are mainly distributed in Rubiaceae. Alostrosines - A was isolated from *Chassalia curviflora* var. ophioxyloides [27]. Psychotria species also yielded bioactive extracts [14]. Antibiotic activity from *P. microlabastra* [26], antiviral activity in *P. serpens* [30] and antiviral /antifungal and anti inflammatory activities in *P. hawaiiensis* [31] have also been reported and specifies the important bioactivities of this genus. Some species of Psychotria often accumulate monoterpene indole alkaloids [28], pyrroloindoline alkaloids and emetine [17]. All these alkaloids possess significant therapeutic effects.

In the present study it was found that ethanolic extracts of root and leaves of *Chassalia curviflora* possess significant anti-inflammatory and analgesic activity. Inhibition of Carrageenan is the most feasible method to screen anti-inflammation of drug and the oedema induced is biphasic [43]. The Carrageenan paw oedema produces a non specific inflammation that results from the sequential action of several mediators. The initial phase is attributed to release of histamine, serotonin and the oedema is maintained during the plateau phase by the kinin like substances. The second phase of oedema is due to the increase of release of prostaglandin- like substances [12, 13]. Prostaglandin is the mediator of the last phase of inflammation induced by Carrageenan. Development of oedema induced by carrageenan is commonly correlated with the early exudative stage of inflammation. That is one of the most important processes of inflammatory pathology [33]. Chr and Chl significantly reduced the inflammation at 200 mg/kg dose in the anti-inflammatory assay in this study in a dose-dependent manner. Chr showed 70.6% and Chl 65% inhibition of inflammation. The significant inhibition of oedema occurred 3 hr after Carrageenan injection. Many anti inflammatory plant-derived bioactive compounds are also cyclooxygenase (COX) or lipoxygenase (LOX) inhibitors like Rutaecarpine-an indole alkaloid from *Evodiarutaecarpa* inhibits COX-2 [19]. Based on these reports it is inferred that the anti inflammatory effect of both Chr and Chl in Carrageenan induced rat paw oedema observed in the present study is by inhibiting cyclooxygenase leading to the inhibition of prostaglandin synthesis.

Acetic acid induced writhing attributed visceral pain in mice finds much attention in screening of analgesic drugs [24]. The ethanolic extract Chr and Chl showed significant analgesic activity. At dose of 200 mg/kg Chr showed 86.87 % inhibition of writhing while Chl showed only 62.9% inhibition. Pain sensation in acetic acid-induced writhing is elicited by triggering localized inflammatory response resulting in release of free arachidonic acid from tissue phospholipids [1] via cyclooxygenase and prostaglandin synthesis [16,18]. The significant pain

reduction by the extracts might be due to analgesic components of plant inhibiting prostaglandin synthesis. Acetic acid-induced writhing is a widely used method for the evaluation of peripheral antinociceptive activity [26]. Acetic acid is an irritant which stimulates local peritoneal receptors to induce pain with characteristic abdominal constrictions when injected into peritoneal cavity [44]. The abdominal writhing induced by acetic acid was also reported to be less selective [10] and act indirectly by releasing endogenous mediators stimulating neurons that are sensitive to other drugs such as narcotics-centrally acting agents [42]. The extract remarkably reduced the abdominal constrictions at all the three doses tested in the study.

In biological systems, lipid peroxidation generates a number of degradation products such as malondialdehyde, and is found to be an important cause of cell membrane destruction and cell damage [15]. It has become evident that non enzymatic or unspecified lipid peroxidation occurs during experimental inflammation in rats [35]. Lipid peroxides may be pro inflammatory and can damage the tissues directly [7]. Protection against free radical lipid peroxidation by plant extracts is of great significance for their traditional use against inflammatory disorders, many of which are associated with membrane damage and tissue recovery [23]. Mixture of $FeCl_2$- ascorbic acid could stimulate lipid peroxidation. Disintegration of lysosomes has been correlated with the peroxidative decomposition of lysosomal lipids [11]. In the present study both Chr and Chl significantly inhibited *in vitro* lipid peroxidation in rat liver homogenate with significant reduction in the MDA levels.

Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins,quinines, terpenoids, saponins, steroids and sterols in Chr and alkaloids,terpenoids, steroids and sterols from Chl. Active compounds reported earlier from *Psychotria species* include naphthoquinones, peptides, benzoquinones, pigments and alkaloids [20, 25, 39, 46]. The alkaloids isolated from other species of *Psychotria* are reported to have cytotoxic, anti malarial, anti inflammatory, immune modulatory, anti oxidant and psychoactivity [14]. Moreover, the anti inflammatory and analgesic activities of many other species of *Psychotria* are already reported. Herbal drugs used in various system of medicine possess action in the central nervous system and many species of *Psychotria* are used traditionally as pain killers which act on central nervous system. Thus the results show significant anti-inflammatory, analgesic activity and anti-lipid peroxidation properties of Chr and Chl which can be attributed to the presence of such bioactive compounds in the plant.

CONCLUSION

Chassalia curviflora (*Psychotria curviflora*) has been used in traditional medicine to treat various types of inflammations and injuries. The present pharmacological study confirmed that the root and leaf ethanolic extracts of the plant possess potent anti inflammatory, analgesic and anti lipid peroxidative effects. Further studies on the isolation of active principles present in this plant and understanding of their mechanism of action are now being followed to obtain more insights on the proposed therapeutic efficacy of this herb.

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