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Anti-inflammatory Activity of Characterized Compound Diosgenin Isolated from *Tinospora malabarica* Miers in Ann. (Menispermaceae) in Animal Model

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ABSTRACT

At concentrations of diosgenin isolated from *Tinospora malabarica* was investigated in chemically-induced inflammation rodents' model. It showed a significantly ($P < 0.01$) reduced mean paw edema volume at 3h after carrageenan injection. These extracts have exhibited anti-inflammatory activity in a dose-dependent manner with the per cent inhibition of paw edema, as compared with the control group. This result indicated that the characterized compound, diosgenin at the dose of 400µg/kg.b.wt showed a maximum anti-inflammatory activity of 82.25% as compared to the reference drug, indomethacin (82.01%). Therefore, our studies support the use of active constituents from *T. malabarica* in treating inflammations.

Keywords: *Tinospora malabarica,* Diosgenin and Anti-Inflammatory Activity.

1. Introduction

Many herbal preparations are being prescribed as anti-inflammatory drugs in the traditional literature. The search for new anti-inflammatory agents from the huge array of medicinal plant resources is intensified in recent time [1]. In India, many Ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritic conditions. *Tinospora malabarica* Miers in Ann. is a tall woody climber in the Menispermaceae member. It contains a bitter substance, Berberine, Giloin, Giloinin, Fisetin, Diosgenin and Eudesmol. It is widely used in veterinary folk medicine/ ayurvedic system of medicine for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic properties [2] [3]. The plant is used in ayurvedic, "Rasayanas" to improve the immune system and the body resistance against infections. The root of this plant is known for its anti-stress, anti-leprotic and anti-malarial activities [4]. Hence, the present study was undertaken on the characterized compound diosgenin isolated from *Tinospora malabarica* to assess the anti-inflammatory activity.

2. Materials and methods

2.1 Plant collection

Tinospora malabarica Miers in Ann. (Menispermaceae) was collected during the month of January 2012 from Walayar, a part of Western Ghats, Kerala. The plant was identified and authenticated by a plant taxonomist.

2.2 Plant extraction and isolation of compound

50g of the dried whole plant of *T. malabarica* was soaked in 250ml ethyl acetate and allowed to stand for 3-4 days. The filtered extract was collected and concentrated using rotary vacuum to get crude extract. The crude extract was re-dissolved in minimum quantity of ethyl acetate. To this 60g of silica gel (60:120 Mesh) was added and allowed to dry to get free flow of mixture

This admixture was packed in column chromatography and it was eluted with the mixture of Toiluene: Ethyl acetate in different ratios starting from 8:2 to 4:6. (8:2, 6:4 & 4:6)respectively. Every 30 ml fraction was collected in different boiling tubes and the first 50 ml of the fractions were discarded because there may not be any compound in the starting of process. Fraction 48th was further treated with charcoal to get the pure compound. The fraction was confirmed as a steroidal saponin diosgenin from various spectral studies like UV spectrophotometer, ^{1}H NMR, ^{13}C NMR and Mass spectrum.

2.3 Carrageenan-induced paw oedema in adult albino male rats

All the animals were divided into 5 groups comprising five animals in each group. In all groups acute inflammation was produced by sub-plantar injection of 0.1ml freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats and paw volume was measured plethysommetrically at 0 to 180 mins after carrageenan injection. All the animals were premedicated with indomethacin (10mg/kg b.wt.) orally two hour before infection. Mean increase in paw volume was measured and percentage was calculated. All the extracts were subjected for acute toxicity studies and 1/10th of the LD_{50} dose was selected for pharmacological activity. Percentage inhibition of paw volume was calculated by the following formula [5].

$$\% \text{ inhibition} = \frac{Vc-Vt}{Vc} \times 100$$

Where,

Vt- means increase in paw volume in mice treated with test compounds

Vc- means increase in paw volume in control group of mice.

3. Result and Discussion

The anti-inflammatory activity of characterized compound, Diosgenin from *T. malabarica* was evaluated by carrageenan induced rat paw oedema method. The compound was tested at various dose levels such as 100µg/kg, 200µg/kg and 400µg/kg b.wt. The highest dose level 400µg/kg b.wt, significantly reduced the carrageenan induced paw oedema inflammation as compared to the standard drug. This result indicated that the characterized compound, Diosgenin at the dose of 400µg/kg.b.wt showed a maximum anti-inflammatory activity (82.25%) and it was compared with the reference drug, indomethacin (82.01%) (Table-1).

Table 1: Effect of characterized compound Diosgenin isolated from*Tinospora malabarica* on the percent inhibition of carrageenan induced paw oedema in adult albino rats.

Treatment	Oedema volume(mm)					% Inhibition after 180 min
	Dose mg/kg	0 min	60 min	120 min	180 min	
Group I	Normal saline	24.13±1.83	59.54±1.32	94.23±1.89	139.63±1.50	_
Group II	100µg/kg	25.85±1.96	98.49±1.88	62.13±1.32*	56.24±1.82*	**59.77**
Group III	200µg/kg	28.48±1.64	87.21±1.27**	43.44±1.83**	31.30±1.66***	**77.58**
Group IV	400µg/kg	26.78±1.93	51.84±1.07**	34.22±1.28**	24.78±1.27***	**82.25**
Group V	100mg/kg	29.13±1.62	36.13±1.05**	32.27±1.22**	25.11±1.37***	**82.01**

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01;* ** P<0.001 Compared paw oedema induced control vs. Drug treated rats

Group I: Rats given normal saline

Group II: Rats given characterized compound Diosgenin isolated from *T. malabarica* extract at the dose of 100µg/kg *b.wt.*

Group III: Rats given characterized compound Diosgenin isolated from *T. malabarica* extract at the dose of 200µg/kg *b.wt.*

Group IV: Rats given characterized compound Diosgenin isolated from *T. malabarica* extract at the dose of 400µg/kg *b.wt.*

Group V: Rats given indomethacin at the dose of 100mg/kg *b.wt*

The presently obtained results showed that the characterized compound diosgenin from *T. malabarica* has anti-inflammatory activity on an acute inflammatory process like in carrageenan induced paw edema in rat paws. It is well known that leukocytes migration to the injured tissues is an important aspect of the inflammatory process. Histamine and serotonin are responsible for the immediate inflammation response, whereas Kinins and prostaglandins mediate prolonged response [6].

Almeida *et al.,* [7] have surveyed global review on plant with anti-inflammatory activity for more than 79 families. Among these, Menispermaceae family members are placing the major part. Dry barks of *Tinospora cordifolia* have anti-inflammatory [8]. The dried stem of *T. cordifolia* produced significant anti-inflammatory effect in both acute and subacute models of inflammation. *T. cordifolia* was found to be more effective than acetylsalicylic acid in acute inflammation [9]. The methanolic leaf extract of *Cocculus hirsutus* (100 and 200mg/kg) (Menispermaceae) was investigated for its anti-inflammatory effect in laboratory animals. In eddy's hot plate analgesic study, both the doses of *C. hirsutus* showed significant activity. In acetic acid induced writhing model, the onset of

writhing was delayed and duration of writhing was shortened by the methanolic extract of *C. hirsutus*. It showed significant anti-inflammatory activity on both carrgeenan as well as cotton pellet induced granuloma models in rats [10]. Udegbunam *et al.* [11] have evaluated the hexane, chloroform, ethyl acetate and methanol extracts of *Stephania dinklagei* which have potent anti-inflammatory activity by using various models. The extracts also showed dose dependent anti-inflammatory activity with 300mg/kg of the extracts being more potent.

3. Conclusion

The magnitude of activity of the compound diosgenin isolated from *T. malabarica* indicate a high potency of anti-inflammatory effect and also provide impetus to continue the search for novel anti-inflammatory constituents from this plant.

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