European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 10, Issue 01, 2023

Original Research Article

In Vitro And In Vivo Anti-Inflammatory Studies Of Stachytarpheta Mutabilis Methanolic Extract

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ABSTRACT

Preliminary phytochemical screening of *Stachytarpheta mutabilis* with methanol revealed the presence of various phytocomponents such as phenols, flavonoids, steroids, saponins, alkaloids, tannins, terpenoids, etc. In vitro anti-inflammatory activity was assessed using a nitric oxide scavenging test at various concentrations. Indomethacin was used as the standard drug. The results showed that *Stachytarphea mutabilis* Methanol Extract (SMME) exhibited significant anti-inflammatory activity in vitro over a concentration range of 100-500 g/mL. The study aimed to evaluate anti-inflammatory activity in vivo in rats. The results obtained in the present study indicate that methanol extracts from *Stachytarphea mutabilis* can be a potential source of anti-inflammatory agent. In vitro anti-inflammatory study showed 44.19 % and in vivo studies showed that 42 % activity.

1. INTRODUCTION

Ayurveda has been practised in India for thousands of years. The traditional use of medicinal plants is widespread all over the globe, and it predates the discovery of phytochemical screening, extraction technologies, and isolation of bioactive molecules for use as anti-inflammatory, antibacterial, antipyretic, anticancer, and antiallergic agents. Plants have been revered for their generalised healing abilities since antiquity. Herbal medications, in all its guises, are becoming more popular due to their lack of negative health consequences and growing popularity in countries like India. Drugs or formulations derived from plants may have less immediate impact on the body, but their cumulative benefits are greater (Okoye et al 2014). Herbs, shrubs, trees, vines, and bushes of various shapes and sizes are being analysed for the presence of bioactive compounds such as phenolics, flavonoids, steroids, alkaloids, etc., and are increasingly being made accessible for sale and use. Antioxidant activity is influenced either directly or indirectly by the phytonutrients contained in a plant, therefore the ratio of phytochemicals to other plant compounds differs from one plant to the next. This mechanism, known as inflammation, prevents the spread of any kind of foreign particle. The purpose of anti-inflammatory medication is to lessen or get rid of painful swelling and redness.

Researchers have a persistent difficulty in coming up with safer and more effective medications to treat these illnesses. Both steroidal and non-steroidal anti-inflammatory medications are now in use to treat inflammatory diseases (NSAIDs). When it comes to alleviating the pain and discomfort caused by inflammation, nonsteroidal anti-inflammatory drugs (NSAIDs) are still the gold standard of treatment. These medications do not treat the underlying cause of the illness but rather alter the body's inflammatory reaction to it (Lawrence, 2020). Swelling and redness are symptoms of the

inflammatory cascade. It's a natural defence mechanism that helps keep out allergies, poisons, and other potentially harmful things. The *Stachytarphea mutabilis* plant is a member of the verbanaceae family and is grown as a herb. The SMME contains phytoconstituents such alkaloids, saponins, steroids, terpenoids, tannins, etc., as shown by phytochemical analysis. The current research aims to investigate the anti-inflammatory potential of *Stachytarpheta mutabilis* by the nitric oxide test due to its ethanopharmacological significance. As an in vivo model of acute inflammation, carrageenan-induced paw edoema has been widely utilised to assess the anti-edematous properties of various natural compounds. The Wistar rats utilised were both male and female. As well as the antioxidant and free radical scavenging activities, the anti-inflammatory activity of extracts has been investigated.

MATERIALS AND METHODS

1.1 Sample collection and authentication

Plant materials used were leaves of *Stachytarpheta mutabilis* harvested in January 2021 from More Nursery Pune Maharashtra. The plant identification (Voucher Number 15) was carried out in Department of Botany, Gulbarga University, GUK, Kalaburagi.

1.2 Solvent Extraction and Phytochemical analysis

The leaves were cleaned with water and then dried at room temperature away from direct sunlight and finely grounded with a grinder Soxhlet extractor available in Biopharmaceutical and Nano biotechnology Laboratory, Department of Biotechnology, Gulbarga University, Kalaburagi. Methanol (0.762) is used as solvents. The Soxhlet extraction process is known as a hot continuous extraction process. The main advantage of this method is that it ensures maximum extraction with minimum quantity of solvent. (Harborne et al 1998). 90 grams of the powdered leaf of *Stachytarpheta mutabilis (*with grades between moderately coarse) was taken into the main chamber of the Soxhlet extractor. Extraction process was carried out. Around 900 ml of each solvent was used for 90 grams of the plant material. (1:10) Leaf powder to solvent ratio is maintained. The phytochemical constituents in the extracts of *Stachytarpheta mutabilis* were determined qualitatively according to standard procedures described elsewhere (Harborne, 1998). Phytochemicals tested included alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids.

2.3 In vitro anti-inflammatory assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH was measured by Griess reaction (Marcocci et al., 1994). The reaction mixture (3ml) containing sodium nitropruside (10mm) in phosphate buffer saline and the test extract (10, 25, 50 and 100µg/ml) was incubated at 25^{o}C for 150min, after incubation 1.5ml of the reaction mixture was removed and 1.5ml of the Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% Napthylethyline diamine hydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. Percent inhibition of nitric oxide scavenging was calculated using the formula.
Percentage Inhibition = (A of Control – A of Sample) /A of Control× 100. A- absorbance.

1.3 In Vivo anti-inflammatory assay

In Vivo anti-inflammatory potential of methanolic extract was assessed against using carrageenan-induced paw edema model. The experimental protocol was approved by the AEC (Animal Ethical Committee) RP 31/2122 Total 36 rats (Both male and Female) were divided into 6 groups (n=6). Paw volume of all rats was measured before administration of 1 % Carrageenan. Animals were given 0.1 ml of 1% of Carrageenan into the subplantar tissue of rat paw after 30 min of oral administration of Vehicle, Standard and Test drug. The alcoholic and n-butanolic extracts of dried leaves of Stachytarpheta cayennensis (L.C. Rich) Vahl (Verbenaceae) was assessed in antiinflammatory and antinociceptive models. Intraperitoneal pretreatment with the dried extracts at

doses ranging from 100 to 200 mg/kg, significantly inhibited carrageenin inducing edema formation.

Group I was administered 0.1 ml of 1% of Carrageenan as diseased control; Group II was administered with Lukewarm water 10ml / Kg. + 0.1 ml of 1% of Carrageenan as vehicle control; Group III was given standard drug Indomethacin 10 mg/kg + 0.1 ml of 1% of Carrageenan . After 1 h of intra-peritoneal (i.p.) administration of standard drug and plant extract, 1% carrageenan solution approximately 100 µl was injected into the left hind paw of each ER as an inflammatory mediator. To measure the volume of the paw edema, a glass beaker was filled with distilled water and positioned on a weighing balance; the weight of the beaker was tared. The paw volume of each ER was measured by immersion of the inflamed paw into water. A force *F* is applied to the balance against the movement of liquid inside the beaker and that was equal to weight of the paw.

Group (n=6)	Specifications
GROUP 1- Diseased Control	0.1 ml of 1% of Carrageenan
GROUP 2- Vehicle Control	Lukewarm water 10ml / Kg. + 0.1 ml of 1% of Carrageenan
GROUP 3- Standard drug	Indomethacin 10 mg/kg + 0.1 ml of 1% of Carrageenan
GROUP 4- Test drug 1	Methanolic extract of *Stachytarpheta mutabilis* 200 mg/kg + 0.1 ml of 1% of Carrageenan
GROUP 5- Test drug 2	Methanolic extract of *Stachytarpheta mutabilis* 400 mg/kg + 0.1 ml of 1% of Carrageenan
GROUP 6- Test drug 3	Methanolic extract of *Stachytarpheta mutabilis* 800 mg/kg + 0.1 ml of 1% of Carrageenan

Table 1. Study design for animal study

After administration of Carrageenan, the paw volume was measured in each group at 0, ½, 1, 2, 3, 4 and 24 hr by using a plethysmometer. The inhibition of paw volume in drug treated group were compared with the diseased control group. Histopathology of the paw were done by sacrificing the rat. The Formulation of the test material was prepared in the dose levels of Mild- 200 mg/kg body wt., Moderate-400 mg/kg body wt. and Maximum-800 mg/kg body wt. in lukewarm water. While the standard drug was prepared10 mg/kg body weight. in distilled water. For histopathology examination, rats were sacrificed under anesthesia conditions. Paw tissue of all groups was taken and then fixed in 10% buffered formalin and stained with hematoxylin and eosin to the evaluation of inflammatory change in the rat's paws.

2. RESULTS AND DISCUSSION

This study's findings show that *S.mutabilis* methanol extracts have powerful anti-inflammatory properties in vitro, including the capacity to inhibit protein denaturation. Neutrophil-derived NOs, reactive oxygen species, cytokines, and prostaglandins are only some of the free radicals that are produced during the complex physiological pathological response known as inflammation (Vyaksh et al 2018). Denaturation occurs when a protein loses its third and second structures. Degradation of proteins has been linked to inflammation, as is now well accepted. The metabolic conversion of arachidonic acid is a pivotal step in the chain of events that constitutes inflammation (Mannan et al, 2008). The samples gathered for the phytochemical analysis included phenols, flavonoids, steroids, adenoids, alkaloids, tannins, and terpenoids, among other compounds. Carrageenan-induced acute inflammation is a useful model for testing anti-inflammatory medicines (Oguntibeju et al 2018). It is common practise to depict the progression of edoema in the rat model of carrageenan-induced paw edoema as a biphasic curve, which describes two distinct phases of oedema development(Azam et al 2015). After getting an injection of carrageenan, the body goes through an initial phase of inflammation within an hour. This is due in part to the traumatic impact of the injection and in part to the production of histamine and serotonin (Anoop et al 2015). The flavanoids in porterweed

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(*Stachytarpheta jamaicensis (L.)* Vahl) leaf extract have been shown to diminish the level of TNF-alpha as inflammation agent by inhibiting the metabolism of prostaglandins in the cyclooxygenase pathway, and the optimal dose for this effect is 150 mg/kg bw (body weight) (Penido et al 2006).
Due of the model's susceptibility to cyclo-oxygenase inhibitors, non-steroidal anti-inflammatory medications (NSAIDs) have been investigated in a rat model of carrageenan-induced paw oedema(Bawazeer et al 2021). NSAIDs primarily block the cyclo-oxygenase implicated in prostaglandin generation. A critical element throughout the 3 hour period of the inflammatory response's development. After 3 hours, paw edoema is reduced by 31% at 200mg/kg and by 42% at 400mg/kg, according to findings provided in. This reduction is statistically significant (P <0.01). Since methanolic extract blocks the formation of prostaglandins by inhibiting the enzyme cyclo-oxygenase, this may account for its anti-inflammatory action against carrageenan-induced inflammation (Schapoval et al 1998).

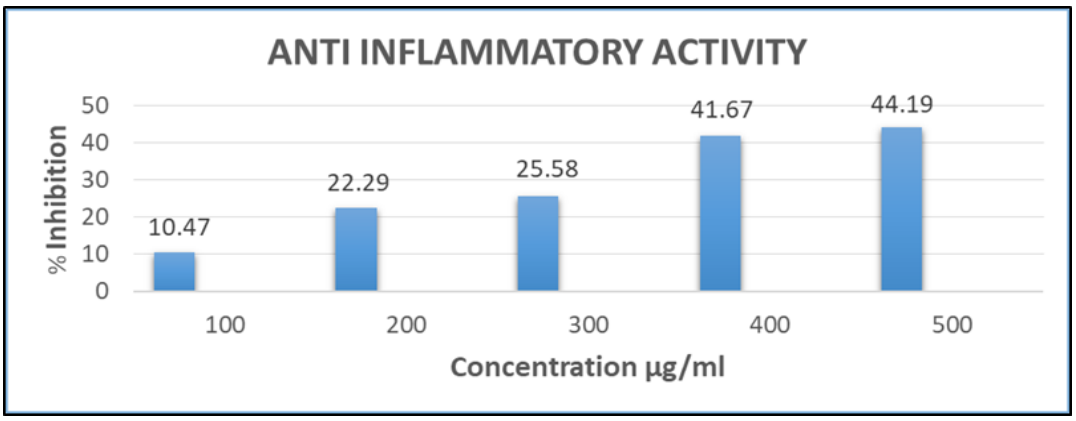

Fig.1 Graph showing percent inhibition and concentration

Groups	Initial	0 M	30 M	1H	2 H	3 H	4H	24 H
NC	0.873							
DC (1% Carrageenan)	0.932	0.995	1.148	1.188	1.385	1.445	1.540	1.328
VC (1% Carrageenan+ Lukewarm water)	1.122	1.157	1.253	1.350	1.537	1.692	1.690	1.472
STD (1% Carrageenan+ 10 mg/kg Indomethacin)	0.970	0.995	1.128	1.133	1.125	1.107	1.088	1.065
T1 (1%Carrageenan + Methanolic extract of *Stachytarpheta mutabilis* 200 mg/kg)	0.767	0.933	0.993	0.988	0.943	0.967	0.943	0.820
T2 (1%Carrageenan + Methanolic extract of *Stachytarpheta mutabilis* 400 mg/kg)	0.872	0.963	1.105	1.108	0.885	0.958	0.907	0.843
T3 (1%Carrageenan + Methanolic extract of *Stachytarpheta mutabilis* 800 mg/kg)	0.857	0.995	1.113	1.193	1.207	1.172	1.078	0.982

Table 2. Mean of Paw volume (ml)

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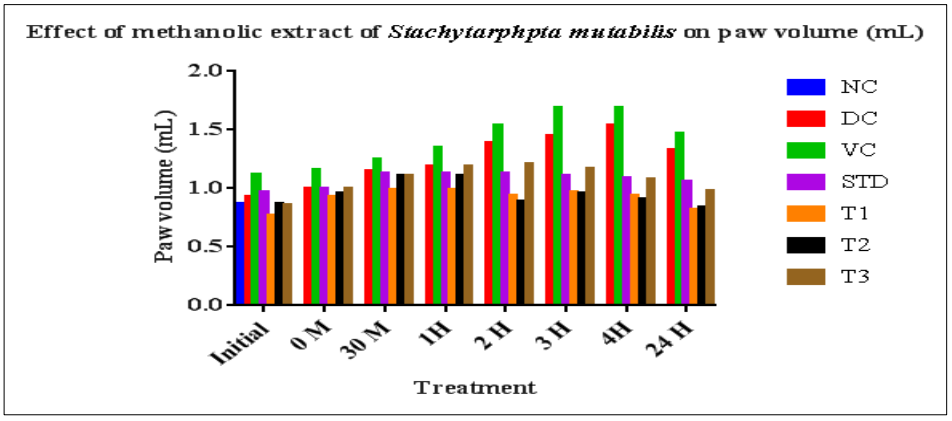

Fig.2 Graph showing effect of methanolic extract of *S.mutabilis* on paw volume

In vitro anti-inflammatory activity of extract showed that the 400µg/ml and 500µg/ml showed 41.67% and 44.19% of anti-inflammatory activity in protein denaturation assay in Fig.1. In in vivo studies paw volume was found to be decreased after 3 hours gradually as compared to other dose concentrations indicated in fig.2

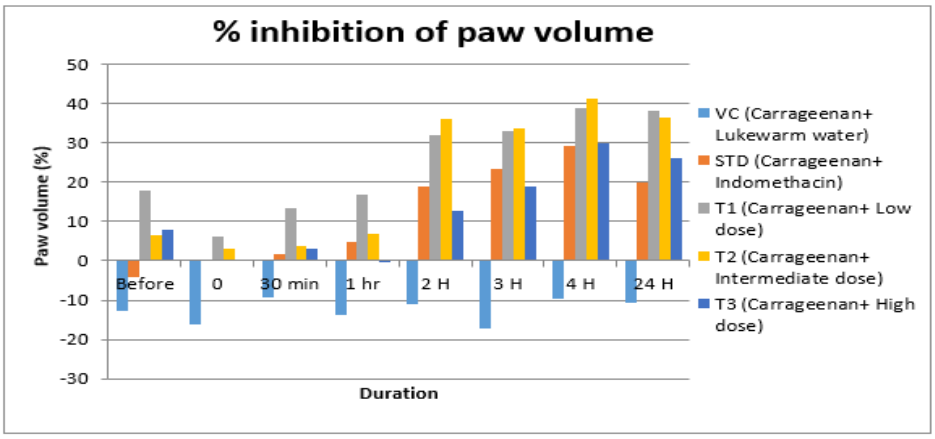

Fig.3 Inhibition of paw volume

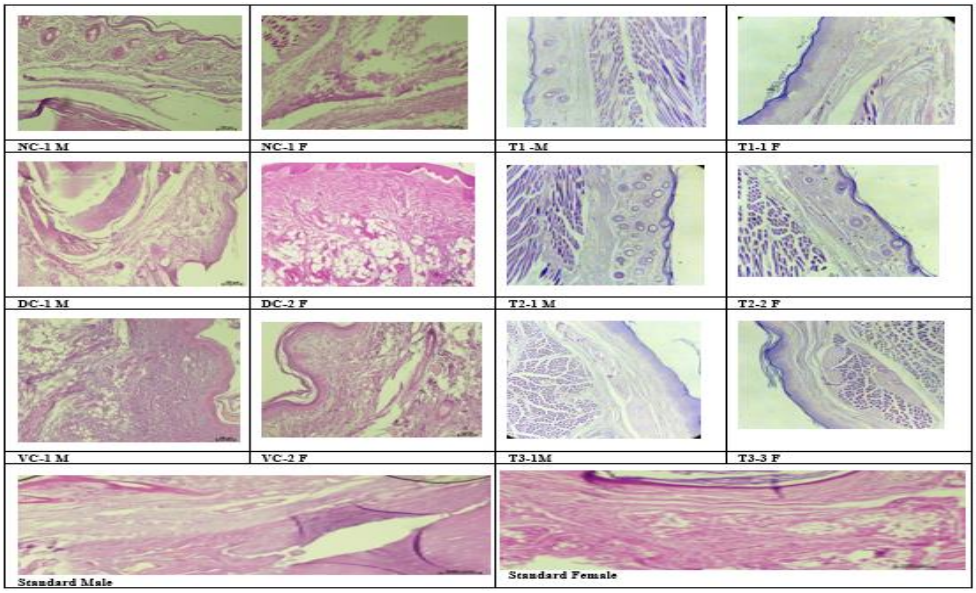

Fig. 4 . Histopathology images of paw treated with different concentrations (T1 200mg/kg, T2 400 mg/kg and T3 800 mg/kg) NC- Normal Control, VC- Vehicle Control, DC- Diseased Control, STD- Standard

The percentage of paw volume inhibition was found to be effective against mid or intermediate dose that is 400mg/kg. It was found to be 41% and 33% with low dose that is 200mg/kg.30% anti-inflammatory activity was found to be shown by high dose. Fig.3 explains the efficacy of mid dose over the other two dosage concentrations through graphical presentation. Histamine and dextran were both able to trigger inflammation, however the extract significantly mitigated the reaction. Analgesic, antimalarial, and anti-inflammatory properties have been observed in the leaves of *Stachytarpheta cayennensis* (Okokone et al 2008). It was discovered that the methanol extract included flavonoids, tannin, terpenoids, and saponins. These chemical components may account for the plant's anti-inflammatory properties (Sahoo et al 2014). Fig.4 displays histopathological scans revealing the absence of abnormalities. Table 3 shows that mononuclear cells infiltrated the dermal layer of the paw's skin under the subcutis area. Immune cells like neutrophils and macrophages release ROS, which aid in the inflammatory response. In addition, ROS increase inflammation by triggering the production of cytokines including interleukin-1, tumour necrosis factor-alpha, and interferon-alpha, which in turn push even more neutrophils and macrophages to develop. Since free radicals are the primary mediators that set off the inflammatory process, antioxidants and radical scavengers are essential.

Sr no/	Group and Animal Code No.	Histopathological Observations – Paw tissue			Overall Pathological grade / lesion score
		Congested blood vessels, Hemorrhagic foci in the dermis and sub cutis of paw.	Acute inflammatory and edematous changes in the in the sub-cutis region and connective tissue of paw below skin dermal layer	Inflammatory changes with MNC(mononuclear cell)infiltration in the sub-cutis region of paw below skin dermal layer	
1	NC-1 M	NAD	NAD	NAD	NAD
2	NC-2 F	NAD	NAD	NAD	NAD
3	DC-1 M	NAD	+1	+2	Mild (+2)
4	DC-2 F	NAD	Focal (+)	+1	Minimal (+1) to Mild (+2)
5	VC-1 M	NAD	Focal (+)	Focal (+)	Minimal (+1)
6	VC-2 F	NAD	Focal (+)	Focal (+)	Minimal (+1)
7	STD -1 M	NAD	NAD	NAD	NAD
8	STD – 2 F	NAD	NAD	NAD	NAD
9	T1- 1 M	NAD	NAD	NAD	NAD
10	T1- 2 F	NAD	NAD	NAD	NAD
11	T2 – 1 M	NAD	NAD	NAD	NAD
12	T2 – 2 F	NAD	NAD	NAD	NAD
13	T3 - 1 M	NAD	NAD	NAD	NAD
14	T3 – 2 F	NAD	NAD	NAD	NAD
Note : Overall Grade score as- NAD =No Abnormality Detected, Minimal changes (+1), Mild changes (+2), Moderate changes (+3), Severe changes (+4).					

Table 3 Histopathological observations of paw tissue

Plant extracts and isolated compounds derived from *S. indica* have the potential to inhibit bacterial growth, promote tissue regeneration and reduce inflammation, hence, potentially providing the basis for a novel therapeutic for the treatment of wounds. Schapoval et al (1998). have also isolated iridoid ipolami-ide and acetoside from the leaves of another plant of the same genus, *S. cayennensis*, and this plant was also found to exhibit anti-inflammatory and antinociceptive activities(Melita et al 1996). During the course of the investigation, no clinical complaints were detected in the animals except from paw inflammation. According to the results of the histopathological examination of the paw tissues, both the disease control group and the vehicle control group showed mild (+2) to minimal (+1) Acute inflammatory and edematous changes in the sub-cutis region and connective

tissue of the paw below the skin dermal layer. Whereas in the Normal control, Standard, and Test groups, no anomalies were found. After receiving 1% carragenan, the Paw volume rose in the Diseased Control, Vehicle Control, Standard, Test 1, Test 2, and Test 3 groups, however the severity of inflammation was decreased in the Standard Control and Test drug groups compared to the Diseased Control group. The STD group's paw volume was lower than that of the sick control group. Animals in the low-dose group in Test 1 had less paw inflammation than those in the intermediate- and high-dose groups in Test 3 and the sick control group. Test 1's low-dose group saw a greater loss in paw volume compared to Test 2's intermediate-dose group and Test 3's high-dose group. The data shown above suggest that the "Methanolic extract of *Stachytarpheta mutabilis*," at a dosage of 400 mg/kg, is most effective against inflammation generated by carrageenan. The SMME extract can be further analysed for its pharmacological properties along with its proved anti-inflammatory activity.

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