Available online at www.ijpsdronline.com

International Journal of Pharmaceutical Sciences and Drug Research 2015; 7(1): 63-67

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

Analgesic and Anti-inflammatory Evaluation of Ethanolic Extract of *Seenthil churanam*

V. Rajalakshimi[1*], K. Gauthaman[1], Adel Ali Alhazzani[2], R. Kamalraj[3], Nazima Nisar[4], G. Devdass[5]

[1]*College of Pharmacy, King Khalid University, Abha-61431, KSA*
[2]*College of Medicine, King Khalid University, Abha-61431, KSA*
[3]*Department of Compliance, Research and development, Hospira Health Care India Pvt Ltd., Chennai-602117, India*
[4]*College of Applied Medical Sciences, King Khalid University, Al-sameer Campus, Abha-61431, KSA*
[5]*Department of Chemistry, Saastra College of Pharmaceutical Research and Education, Nellore-524311, India*

ABSTRACT

The polyherbal formulation of *Seenthil churanam* is composition of whole plant extracts of *Eclipta prostata*, *Tinospora cordifolia* and the dried powder form of Earthworm used in folk medicine. The study was conducted to evaluate the scientific figures for the treatment of anti-inflammatory and analgesic activity of ethanolic extract of *Seenthil churanam* by acetic acid induced writhing test and eddy's hot plate method, and carrageenan induced paw edema method. There was significant response in analgesic and inflammatory activity at high dose (400 mg/kg) compared to low dose 200 mg/kg against the standards Analgin (500 mg/kg), Aspirin (100 mg/kg) and Diclofenac sodium (100 mg/kg) body weight of mice and rats. The results of this study show that the chronic oral administration of an ethanolic extract of *Seenthil churanam* at a 400 mg/kg body weight dosage be a good alternative natural medicine for analgesics and anti-inflammatory drug without side effects.

Keywords: *Eclipta prostata*, *Tinospora cordifolia*, analgesic and anti-inflammatory.

INTRODUCTION

The therapeutic treatments available today to treat painful diseases usually have limited effectiveness and safety, particularly to treat pain. In fact, the repeated use of the non-steroid anti-inflammatory drugs by arthritic patients may induce several adverse effects, such as gastro intestinal lesions or renal and liver failure. [1-2] So replacement were searched in worldwide and as a result, the last decade many developed countries has growing interest in herbal medicine, acupuncture and alternative systems of medicine. Consequently, an increase in international trade in herbal medicines and other types of traditional medicines has occurred. Indian traditional medicine is based on various systems including Ayurveda, Siddha and Unani. The evaluation of these drugs is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and others. [3] Ayurvedic medicines are largely based upon herbal and herbomineral preparations and have specific diagnostic and therapeutic principles. [4] Inflammation is a disorder involving localized increases in the

***Corresponding author: Mrs. V. Rajalakshimi,**
Department of pharmacology, College of Pharmacy, King Khalid University, Abha, KSA, Pincode- 61431;
Tel.: +966508426425;
E-mail: rajii.vasudevan86@gmail.com
Received: 11 November, 2014; **Accepted:** 19 November, 2014

number of leukocytes and a variety of complex mediator molecules. [5] Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's diseases. [6]

The polyherbal formulation of *Seenthil churanam* is composition of whole plant extracts of *Eclipta prostata*, *Tinospora cordifolia* and the dried powder form of Earthworm used in folk medicine as a anti-inflammatory agent, used in hepatotoxicity, abortion and miscarriage, uterine hemorrhage, piles, insect bites, stings, swellings and other skin diseases. [7] Hence, the study was designed to investigate the anti-inflammatory and analgesic activities of ethanol extract of *Seenthil churanam* on various experimental models and evaluates the scientific figures.

MATERIALS AND METHODS

Herbal Formulation and Extraction

The polyherbal formulation, Seenthil churanam was purchased from IMPCOPS, Chennai. The powder was extracted by cold maceration process using absolute ethanol (99 % v/v). 1000 g of the churanam is macerated in a glass jar with 2000 ml of ethanol (99 % v/v) for 7 days with stirring twice a day and change of solvent every 3 days.

The filtrate obtained is concentrated to a semisolid mass. This extract was kept in the desiccators for further solidification. The yield was found to be 10 %. The solidified extract was stored in air tight container in refrigerator. The extract was administered to animals as suspension in Tween 80 (20 % v/v) throughout the experiment. [8]

Drugs and Reagents

Aspirin, Analgin, Diclofenac sodium and carrageenan (Sigma Aldrich, USA) were used in the study. The ethanol extract of *Seenthil churanam* (200 and 400 mg/kg), carrageenan and Aspirin, Analgin and Diclofenac sodium were prepared as suspension in Tween 80 (20 % v/v) before administration. All other reagents used were of analytical grade.

Animals

Male Wistar albino rats (150-200 g) and Swiss albino mice (20-25 g) were, used throughout the study. They were housed in microloan cages in a controlled environment (temperature 25 ± 2°C and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum*. The experiments were performed according to the guidelines of the Institutional Animal Ethical Committee (IAEC).

Anti-inflammatory activity

Carrageenan an-induced rat paw edema

The rats were divided into four groups (n = 6). The different groups were treated orally with ethanol extract of *Seenthil Churanam* (200 & 400 mg/kg), Diclofenac (100 mg/kg), and vehicle control (Tween 80 (20% v/v), 1 ml/100 g of body weight). The administration of extract and drugs was 1 h prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw sub plantar of each rat. The paw volume was measured initially and then at 1, 2 and 3 h after the carrageenan injection by using plethysmometer (IITC Paw Edema Meter, UK). The anti-inflammatory effect of EIT was calculated by the following equation: -

$$\text{Anti-inflammatory activity (\%)} = (1-V_t/V_c) \times 100$$

Where V_t represents the paw volume in drug treated animals and V_c represents the paw volume of control group's animals. [9]

Analgesic activity

Acetic Acid Induced Writhing Test

The writhing test in mice was carried out using the method of Koster *et al* [10] the writhes were induced by intraperitoneal injection of 0.6 %v/v acetic acid (80 mg/kg). Two different doses of ethanol extract of *Seenthil churanam* (200 & 400 mg/kg) were administered orally to the group II and group III of six animals each. Group I served as control (Tween 80 (20 % v/v), 1 ml/100 g of body weight) and group IV animals received Aspirin at a dose of 500 mg/kg. The extract and standard drug was administered 30 min before chemical stimulus. The number of muscular contractions was counted over a period of 20 min and is expressed as writhing numbers.

Hot Plate Method

The hot plate method in rats was performed by the method of Eddy and Leimbach [11] the evaluated parameters were the latency time for paw licking and jumping responses on exposure to the hot plate surface, kept at 55 ± 1°C. The whole experiment was carried out in Hot Plate Analgesiometer (Cold & Heat chamber Ugo basile Italy). The animal was kept in the hot plate until it lifted one of its hind paws. For this method, the animals were divided into four groups of six animals each. Group I served as control (Tween 80 (20 % v/v), 1 ml/100 g of body weight), group II and group III received ethanol extract of *Seenthil churanam* at a dose of 200 & 400 mg/kg orally. Group IV received Analgin at a dose of 100 mg/kg. All the treatments were given 30 min before the thermal stimulus and the response was determined at 60, 120 and 180 min.

Statistical Analysis

All values were expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett test. *P* values < 0.05 were considered to be statistically significant when compared to control

RESULTS

Carrageenan an induced rat paws edema

The result of ethanol extract of *Seenthil churanam* against carrageenan an-induced paw edema is shown in Table 1 and Table 2. Extract of *Seenthil churanam* (200 & 400 mg/kg) gave significant ($P<0.001$) reduction of rat paw edema at all assessment times in dose dependent manner. The extract showed maximum

inhibition of 88.29 % at the dose of 400 mg/kg after 3 h of drug treatment in carrageenan-induced paw edema whereas the standard drug showed 94.68 % of inhibition (Fig. 1 and 2).

Effect of Seenthil churanam on acetic acid induced writhing in mice

The extract (200 & 400 mg/kg) dose dependently reduced acetic acid induced writhing in mice (Fig. 3 and 4). The reduction was statistically significant (P<0.01) when compared to Control. The results were showed in Table 3 and Table 4.

Table 1: Effect of *Seenthil churanam* on carrageenan induced paw edema in rats

Groups	Initial paw volume (ml)	Increase in paw volume (ml) Mean ± S.E			
		1st h	2nd h	3rd h	4th h
Control	1.21 ± 0.013	0.465 ± 0.03	1.00 ± 0.07	0.952 ± 0.03	0.94 ± 0.05
Diclofenac 100 mg/kg	1.207± 0.016	0.33 ± 0.03*	0.103 ± 0.013*	0.063 ± 0.011*	0.05 ± 0.02*
Seenthil churanam 200 mg/kg	1.198 ± 0.009	0.425 ± 0.04*	0.33 ± 0.04*	0.26 ± 0.04*	0.25 ± 0.05*
Seenthil churanam 400 mg/kg	1.19 ± 0.01	0.45 ± 0.01*	0.26 ± 0.02*	0.17 ± 0.02*	0.11 ± 0.03*

*p < 0.05 significant when compared to control.

Table 2: Percentage inhibition of inflammation

Treatment	% Inhibition
Diclofenac 100 mg/kg	94.68
Seenthil churanam 200 mg/kg	73.4
Seenthil churanam 400 mg/kg	88.29

Table 4: Percentage inhibition of writhing in albino mice

Treatment	% inhibition
Aspirin 100 mg/kg	80.99
Seenthil churanam 200 mg/kg	29.28
Seenthil churanam 400 mg/kg	52.09

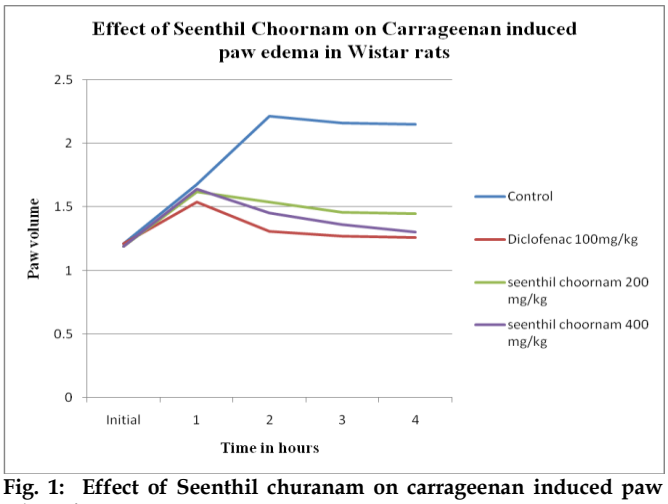

Fig. 1: Effect of Seenthil churanam on carrageenan induced paw edema in rats

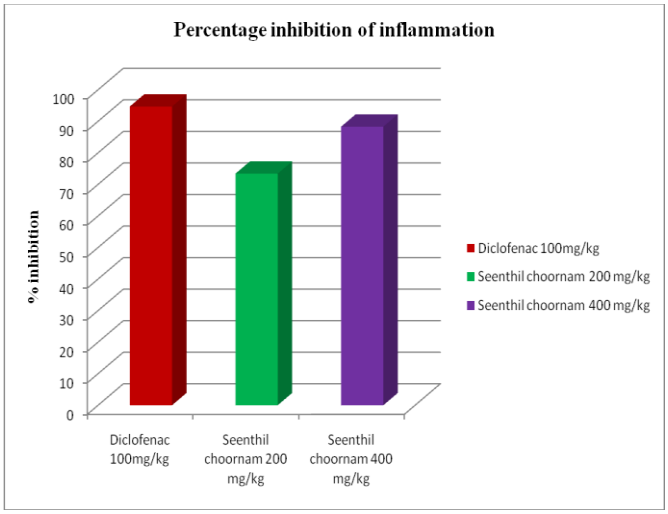

Fig. 2: Percentage inhibition of inflammation

Table 3: Effect of Seenthil churanam on acetic acid induced writhing in albino mice

Treatment	No. of writhing (Mean ± SE)
Group: 1 Control	43.83 ± 1.30
Group: 2 Aspirin 100 mg/kg	8.33 ± 2.51 *
Group: 3 Seenthil churanam 200 mg/kg	31 ± 3.61 *
Group: 4 Seenthil churanam 400 mg/kg	21 ± 1.41 *

*p < 0.001, (significant difference) when compared to control.

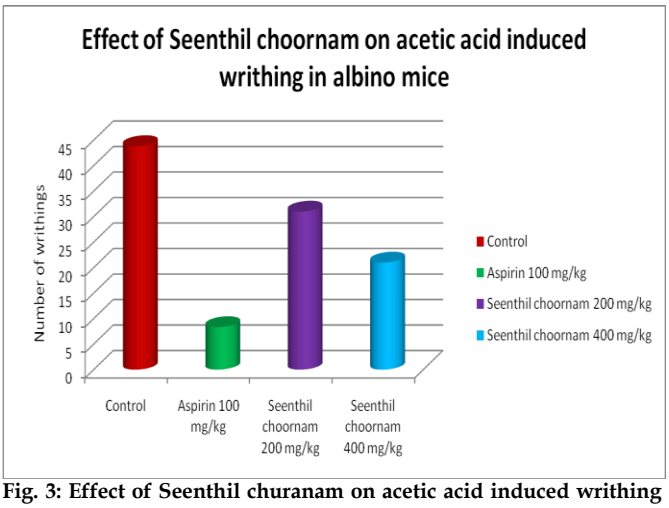

Fig. 3: Effect of Seenthil churanam on acetic acid induced writhing in albino mice

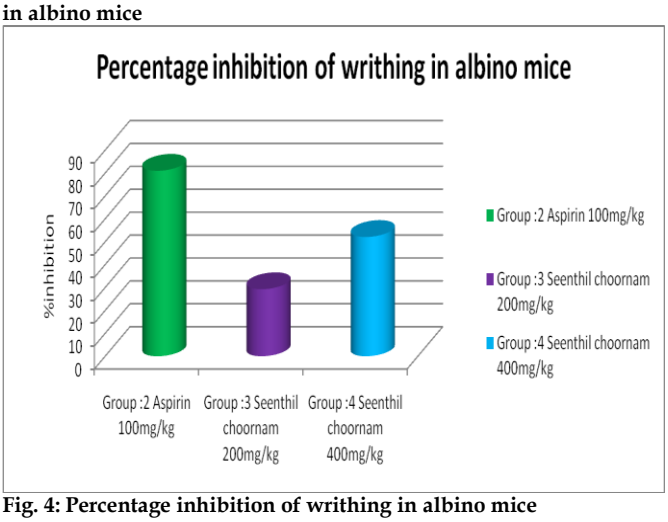

Fig. 4: Percentage inhibition of writhing in albino mice

Effect of Effect of Seenthil churanam on Hot plate method

The animals pretreated with Seenthil churanam on (200 & 400 mg/kg) showed a dose dependent Increase in latency of response in the hot plate method (Fig. 5 and 6). The increase in the latency responses were significant (P<0.01). The results were showed in Table 5 and Table 6.

Table 5: Effect of *Seenthil churanam* on Hot plate method

Treatment	Initial response Time (Sec)	Mean reaction time in seconds (mean ± SEM)					
		30 min	60 min	90 min	120 min	150 min	180 min
vehicle control	5.58 ± 0.75	5.07 ± 0.72	4.9 ± 0.69	5.27 ± 0.84	5.52 ± 0.71	5.9 ± 0.74	5.98 ± 0.72
Analgin (500 mg/kg)	4.6 ± 0.16	11.82 ± 1.41*	13.97 ± 1.11*	10.52 ± 0.88*	12.57 ± 0.78*	8.5 ± 0.67*	7.8 ± 0.50*
Seenthil churanam (200 mg/kg)	4.46 ± 0.73	12.57 ± 0.56*	10.83 ± 0.62* #	13.73 ± 0.88* #	11.58 ± 0..43*	10.7 ± 0.3* #	8.7 ± 1.47* #
Seenthil churanam (400 mg/kg)	6.13 ± 0.65	13.5 ± 1.12*	12.41 ± 0.77*	14.43 ± 0.48* #	12.66 ± 0.64*	11.65 ± 0.42* #	9.93 ± 0.21* #

*p<0.001, (significant difference) as compared with vehicle control.
#p<0.05, (significant difference) as compared with Standard (Analgin).

Table 6: Percentage increase of reaction time in albino mice (Hot plate method)

Group	% Increase of reaction time
Analgin (500 mg/kg)	124
Seenthil churanam (200 mg/kg)	110
Seenthil churanam (400 mg/kg)	130

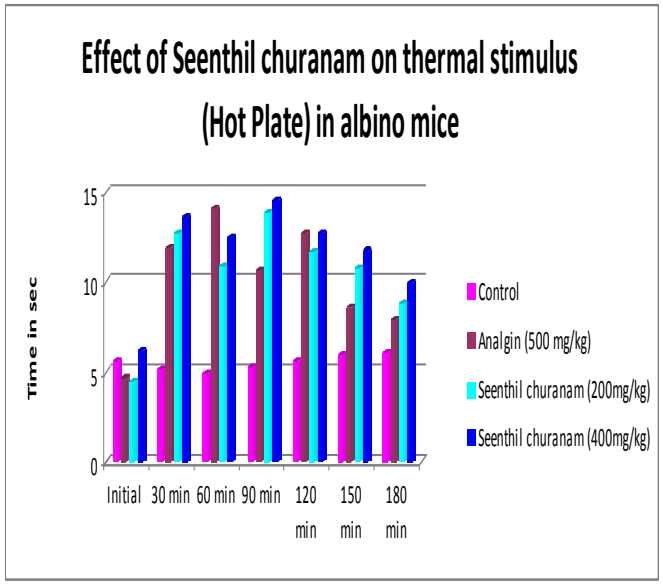

Fig. 5: Effect of *Seenthil churanam* on Hot plate method

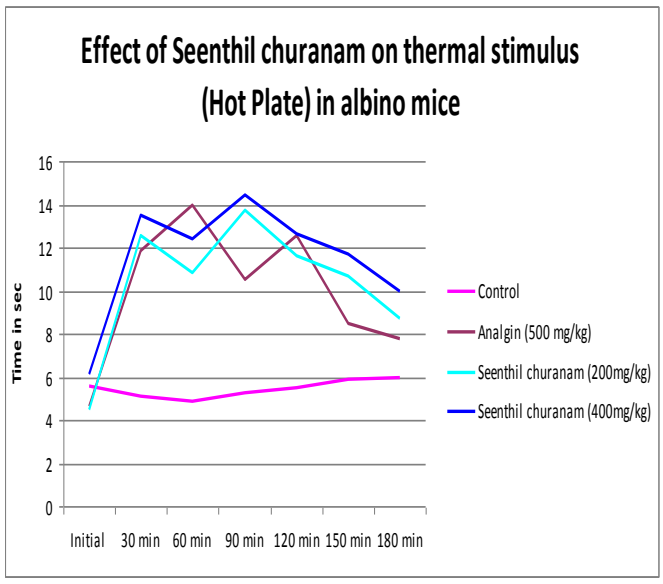

Fig. 6: Percentage increase of reaction time in albino mice (Hot plate method)

DISCUSSION

The most widely used primary test for screening of anti-inflammatory agents is carrageenan induced edema in the rat paw hind paw. [9] The development of edema in the paw of the rat after injection of Carrageenan is believed to be biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin; the second phase is due to the release of prostaglandin-like substances. [12] Based on this, it could be argued that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclooxygenase. [13] Besides, in the carrageenan induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism. Therefore, it is suggested that the mechanism of action of ethanol extract of Seenthil churanam may be related to prostaglandin synthesis inhibition.

It is known that non-steroidal anti-inflammatory drugs usually do not increase the pain threshold in normal tissues, whereas local anesthetics and narcotics. [14] However, the hot plate test was undertaken to verify if ethanol extract of Seenthil churanam would have any central analgesic effect. The results for the group treated with ethanol extract of Seenthil churanam showed significant activity when compared to control group and nearly equal to the group treated with Analgin (500 mg/kg). Hence, it is assumed that ethanol extract of Seenthil churanam has significant analgesic effect on the central nervous system.

The quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid. They found high levels of prostaglandins $PGE_{2\alpha}$ and $PGF_{2\alpha}$ during the first 30 min after acetic acid injection. [15] Thus the results obtained for the writhing test using acetic acid are similar to those obtained for the edematogenic test using carrageenan, since ethanol extract of Seenthil churanam was effectively inhibiting the writhing in mice in dose dependent manner. The results were comparable with the group treated with aspirin.

In conclusion, the present findings in this study point to possibly developing the ethanol extract of Seenthil churanam as a novel and potential agent in the management of inflammation and pain which are probably mediated via inhibition of various autacoids formation and release. Further detailed investigation is underway to determine the exact phytoconstituents that are responsible for these activities.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the management of College of Pharmacy, King Khalid University, Abha, KSA and Hospira Health Care India Pvt Ltd for providing facilities.

REFERENCES

1. Rao P, Knaus EE. Evolution of non-steroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. Journal of Pharmacy & Pharmaceutical Sciences 2008; 11(2):81s-110s.
2. Francis FY Lam, Ethel SK Nget. Substance P and glutamate receptor antagonists improve the anti-arthritic actions of dexamethasone in rats. British Journal of Pharmacology 2010; 159(4): 958-969.
3. Aswal BS, Bhakuni DS, Goel AK, Kar K, Mehrotra B. Screening of Indian plants for biological activity. Indian Journal of experimental Biology 1984; 22: 487-504.
4. Patwardhan B, Hopper B. Ayurvedic and future drug development. J. Alter. Complement Med 1992; 19(12): 9-10.
5. Mantri P, Witiak DT. Inhibition of cyclooxygenase and 5-lipoxygenase. Curr. Med. Chem 1994; 1:328-355.
6. Smith WL, De Witt DL. Biochemistry of prostaglandins endoperoxide H synthase-2 and their differential susceptibility to NSAIDs. Sem. Nephrol. 1995; 15:179-194.
7. Sharma PV. Siddha medicine. In History of Medicine in India (Ed.) P. V. Sharma. New Delhi: The Indian National Science Academy1992: 445-450.
8. Ramnath S, Sarenga Rai SS. Study of Hypoglycemic principle isolated from *Tinospora cordifolia*. BJP 1971; 31(1): 201-209.
9. Winter CA, Risely EA, Nuss GW. Carrageenan in-induced edema in hind paw of rats an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol 1962; 3: 544- 547.
10. Koster R, Anderson N, Beer EJ. Acetic acid for analgesic screening. Federation proceeds 1959; 18: 412-416.
11. Eddy NB, Leimbach DJ. Synthetic analgesic dithienyl butanyl and dithinylbutylamines. J. Pharmacol. Exptl. Therap 1953; 107: 385-393
12. Brito ARMS, Antonio MA. Oral anti-inflammatory and antiulcerogenic activities of a hydro alcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae). J Ethnopharmacol 1998; 61: 215-228.
13. Olajide OA, Awe SO, Makinde JM. Effect of the aqueous extract of *Bridelia ferruginea* stem bark on carrageenan an-induced edema and granuloma tissue formation in rats and mice. J Ethnopharmacol 1999; 66: 113-117.
14. Ferreira SH. Antialergic effect of Aspirin like drugs and the inhibition of prostaglandin synthesis. The recognition of anti-rheumatic drugs. MTP Press Limited, St. Leonard House, Lancaster, 1978, pp. 25-37.
15. Hokanson GC. Acetic acid for analgesic screening. J. Nat. Prod. 1978; 41: 497-498.

Source of Support: Nil, Conflict of Interest: None declared.