



Study of Anti-psoriatic activity and Evaluation of E2A Gene Expression for Psoriasis Levels by RT-PCR of *Thespesia populnea* L. Methanolic Leaf Extract

Narendar Vankudothu^{1*}, Chandrashekhar R², Sudhakar Chekuri¹, Lakshmi Bhavani N² and Anwar SY¹

¹Department of Genetics, University College of Science, Osmania University, Hyderabad, Telangana-500007, India.

²Pharmacognosy and Plant Molecular genetics Lab, Department of Botany, University College of Science, Saifabad, Osmania University, Hyderabad, Telangana-500004, India.

*Corresponding Author Email: naren.genes@gmail.com, rupavathchandrashekhar@gmail.com

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ABSTRACT

Thespesia populnea L. (*Malvaceae*) traditionally claimed to be constructive in the cure of cutaneous infections such as scabies, psoriasis, ringworm, guinea worm, eczema and herpetic diseases. Oil prepared by boiling the ground bark and leaves in coconut oil is applied externally in psoriasis and scabies. Conversely there are no customary scientific reports for its anti-psoriatic activity. Hence the plant *Thespesia populnea* has been chosen to establish scientific data for its traditional claim as anti-psoriatic. In the present study a chemical test performed against all the phytochemical constituents for their preliminary screening of each solvent extract of leaf like water, methanol, chloroform, ethyl acetate, pet ether and acetone. From the phytochemical analysis of the leaf extracts; the result infers the presence of glycosides, tannins, flavonoids, triterpenoids and phytosterols in all the solvent extracts. But phenols are more retained in methanolic leaf extract compared to all the phytochemical constituents. Screening for anti-psoriatic activity was carried out by topical application of methanolic leaf extract of *Thespesia populnea* in the form of a cream using the Perry's scientific mouse tail model. Successive methanolic leaf extract showed paramount anti-psoriatic activity than the standard drug Retion-A (0.05%). Potential function of E2A as a biomarker in psoriasis, is applied in the current investigation to study whether the methanolic leaf extracts of *Thespesia populnea* L. showed antipsoriatic effect targeting E2A gene expression for the psoriasis levels. Results indicated that methanolic leaf extract of *Thespesia populnea* can preferably be used as anti-psoriatic drug and the plant is promising for further investigations to prove its anti-psoriatic activity with no side effects.

Keywords: *Thespesia populnea* L. Anti-psoriatic activity, Phytoconstituents, Retion-A (0.05%)

INTRODUCTION

Since primeval epoch, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases. Nearly 80% of the world's population relies on conventional medicines for their primary health care, the majority of which involves the use of plant extracts (Pathare and Wagh, 2012; Sandhya *et al.*, 2006). In India, almost 95% of prescriptions are plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha (Satyavathi *et al.*, 1987). The study of the plants continues principally for the invention of novel secondary metabolites (Patwardhan *et al.*, 2004). Phytochemical screening is very essential in identifying novel source of therapeutically and industrially precious compounds like steroids, alkaloids, flavonoids, phenols, tannins and terpenoids (Akindele and Adeyemi, 2007).

Thespesia populnea is a reputed ever green tree belonging to the family *Malvaceae*; commonly known as Indian tulip tree. The plant is distributed throughout tropical regions and coastal forests of India. It is well known and all the parts are used in traditional system of medicine (Parthasarathy *et al.*, 2009). The root is acrid, the fruit is sour, acrid and sweet, difficult to digest and produce worms in the intestine and increase "kapha"; aphrodisiac; remove "vata" and "pitta"; and burning of the body. It is good for heart disease and throat troubles. In the central India, the roots are taken as a tonic. In the Konkan, the flowers are employed in the cure of itch; and the leaves are employed as a local application to inflamed and swollen joints. The fruits yield a yellow, viscid juice, which forms a valuable local application in scabies and other cutaneous diseases. The affected parts of the body are daily washed with a decoction of the bark. The leaves are ground into a paste and applied externally in children's eczema; so also oil prepared by boiling the ground bark and leaves in coconut oil is applied externally in psoriasis and scabies. The contents of the fruit which is a capsule are applied externally to ringworm. A decoction of the bark is given internally in skin diseases. The ground leaves and content of the capsules applied externally in eczema and ringworm respectively were found to be useful. A compound oil of the bark and capsules was given in cases of urethritis and gonorrhoea with beneficial results. The young buds and leaves have a pleasant taste and along with the flowers are eaten raw, cooked

or fried in butter. The leaves are lopped for fodder and manure. They are reported to be employed as a local application to inflamed and swollen joints.

Psoriasis is a familiar chronic inflammatory dermatitis. Person of all ages may develop the disease. Psoriasis is sometime allied with arthritis, myopathy, and enteropathy, spondylitic and heart disease or the AIDS. Psoriatic arthritis may be meek or may produce severe deformities resembling the joint changes seen in rheumatoid arthritis. Clinically, psoriasis most consistently affects the skin of the elbow, knees, scalp, lumbosacral areas, intergluteal cleft and glans penis (Walter and Gundula, 1981). The most characteristic lesion is a well demarcated, pink to salmon colored plaque covered by loosely adherent scales that are typically silver white in color. Psoriasis can be one cause of whole body erythema and scaling notorious as erythroderma. Nail changes occur in 30% of cases of psoriasis and consist of yellow brown discoloration (often linked to an oil slick), with pitting, dimpling, separation of the nail plate from the underlying bed, thickening and crumbling (Panda *et al.*, 2014). Psoriasis is either benign or localized or generalized or lives threatening, with associated fever, leukocytosis, arthralgias, diffuse cutaneous and mucosal pustules, secondary infection and electrolyte disturbances (Samuel *et al.*, 1986). Psoriasis may begin at any age, but in most cases it begins between the ages of 10 and 20. Psoriasis affecting the body folds is common in individuals and it is an autosomal, dominantly inherited dermatitis.

Thespesia populnea customarily claimed to be valuable in the treatment of cutaneous infections such as scabies, psoriasis, ringworm, guinea worm, eczema and herpetic diseases (Srivastav *et al.*, 2009). Psoriasis may flare up as a consequence infection of the upper respiratory tract, bladder, teeth or kidneys. It may also be triggered by physical trauma. Currently available allopathic drugs have been associated with a number of side effects. Some drugs such as lithium, β -blockers and chloroquine are also provocative factors. Conventional Chinese medication is an option method of therapy that can be administered in oral, topical, or injectable forms. It emphasizes the significance of using many herbs that are combined in diverse formulations for each entity patient. Herbal medicine uses any plant part such as the root, bark, stem, seed, flowers, or leaves as a means for treatment. Herbal medical practitioners can create many different formulas for

different types of applications (Pan *et al.*, 2014). However, there are no established scientific reports for its antipsoriatic activity. Hence, the present study plant *Thespesia populnea* has been chosen to establish scientific data for its traditional claim as antipsoriatic.

MATERIAL AND METHODS

The plant material was taxonomically identified and authenticated by Dr.B. Prathibha Devi, Professor and Head, Department of Botany, University College of Science, Osmania University, Hyderabad, Telangana-50007. The voucher specimen number is 069. (Illustrations on the flora of the Tamil Nadu Carnatic Volume II published on 28-12-1982).

Preparation of the extract and preliminary phytochemical screening

The healthy and disease free mature leaves of plant of *Thespesia populnea* L. material were collected and washed thoroughly under running tap water, shade dried in open air separately. Powder of the leaf is obtained by grinding them mechanically. About 20g dried powder of the leaf were soaked separately in 200 ml of different solvents like water, methanol, chloroform, acetone, ethyl acetate, and petroleum ether in conical flasks and then subjected to agitation on a rotary magnetic shaker for about 72 hours. After three days the leaf extracts were filtered with No 42 whatman filter paper separately.

Concentrated extracts were preserved in sterilized air tight labeled bottles and preserved in refrigerator at 4°C until required for further use. The extract was filtered under reduced pressure using rotary flash evaporator and for further preliminary phytochemical tests. Different tests were conducted for the saponins, steroids, phenols, flavonoids, terpenoids, alkaloids, cardiac glycosides: reducing sugars and tannins identification of each phytochemical constituent by using the methods adopted by Edeogal and selvi .

Evaluation for anti-psoriatic activity

Psoriatic activity is carried out by Perry scientific mouse tail model method and it is accepted as screening method for measuring anti-psoriatic activity of studied drugs. Male Wistar rats weighing around 150-250g are used for the experiment. Animals were kept in quarantine and after two days experiment was started. All animals were induced with ketamine and xylazine for anesthesia and hair removing. Half of the body of

animals is shaved (lower region) using fem hair removal. Proximal end of tail, an area on one side of the flank, Imiquad cream is applied directly to skin. The process was repeated for 5 days continuously. On the 6th day psoriasis was observed on skin where cream was applied.

A biphasic erythema is observed. After 6th day of topical application of imiquad, initial faint erythema appears. The second phase of erythema starts with the appearance of brownish-red color and the reaction is confined to the applied area with a sharp boundary. By 72-140hrs after topical application, dark brown scale is formed on the erythematous lesion. Pieces of the scale are relatively thick. The methanolic leaf extract of *Thespesia populnea* were screened for anti-psoriatic activity. Leaf extract (20µl) were formulated in the form of a cream, using liquid paraffin (2.9µl) and bees wax (0.9 µl) and applied topically. Retino-A 0.05% (Tretinoin cream U.S.P.) Janssen - Cilag Pharmaceuticals (Trademark of Johnson & Johnson, U.S.A.) in cream form was used as a standard. For the purpose of screening animals were segregated into 4 groups (n=5) namely Group-1: Normal rats; Group -2: Treated with Imiquad cream; Group-3: Treated with standard drug Retino-A 0.05% (Tretinoin cream U.S.P.); Group-4: *Thespesia populnea* in gel form. Screening of methanolic leaf extract (in the form of cream) was carried out with reference to the standard drug Retino-A 0.05% (Tretinoin cream U.S.P.). Extract (in the form of cream) and standard were applied topically once daily for one week. From the day four, *Thespesia populnea* leaf extract showed its effect in curing psoriasis.

On the period of 7th day *Thespesia populnea* leaf extract has proven much better than standard for antipsoriatic effect. After the last treatment animals were sacrificed, longitudinal sections of the tail skin were made and prepared for histological examination (hematoxylin - eosin staining) as an indicator of orthokeratosis the number of scale regions with a continuous granular layer is counted and expressed as a percentage of the total number of scale regions per section. Drug activity is defined by the increase in percentage of orthokeratotic regions.

Evaluation of E2A gene expression

Total RNA extraction and cDNA synthesis

To study the gene (E2A) expression by RT-PCR, Male wistar rats were selected and psoriasis was induced in them. After induction of psoriasis, the methanolic leaf

extract of *Thespesia populnea* was applied in the form of ointment to the rats at the regions of psoriasis. At the end of the experiment, the rats were sacrificed and skin samples were collected. Total cellular RNA was isolated from the skin by TRIZOL method. The amount of RNA was determined by absorbance at 260nm. First-strand cDNA was synthesized from about 500ug DNase I-treated RNA using Takara kit in a total reaction volume of 20ul according to manual instructions. Reverse transcription was carried out at 42°C for 60 min. and converted to cDNA by using Biorad kit according to manual instructions.

Quantitative real-time PCR

After cDNA synthesis, real-time PCR was performed on the Rotor gene Q (Qiagen, Germany). The mRNA of E2A was tested using reference gene GAPDH to normalize the gene expression. Quantitative PCR was performed in a reaction volume of 20 ul, containing 10 µl SYBR Green master mix, 1 µl Reverse primer and 1 µl Forward primer, 6 µl dH₂O (RNase free) and 2 µl cDNA. The thermal cycler conditions were as follows: 1 denaturation cycle of 95 °C for 3 min and 40 cycles of 95°C for 30s 55.4°C for 40 s and 72°C for 1min 30s (annealing and extension temperature respectively) and final extension of 72 °C for 10 min.

Amplification reactions in triplicate for each sample were performed and the results were normalized to the GAPDH gene expression level. An analysis of relative gene expression data was performed using Rotor gene Q software with regard to the fold change in studied gene expression normalized to endogenous control. The relative expression in fold change was arbitrarily set at 1. The primer sequences of E2A and GAPDH are.

E2A Primer Sequence

Forward - CACCAGCCTCATGCACAAC

Reverse - GTGGTCGGAGTACGTGTTG

GAPDH Primer Sequence

Forward - TGATTCTACCCACGGCAAGTT

Reverse - TGATGGGTTTCCCATTTGATGA

RESULTS AND DISCUSSION

Phytochemical analysis of the leaf extracts of *Thespesia populnea*

The phytochemical constituents identified in different extracts of *Thespesia Populnea* are as follows. The phytochemicals saponins, steroids, phenols, flavonoids, alkaloids, and tannins are present poorly in the aqueous extracts of *Thespesia Populnea* leaf whereas the terpenoids, cardiac glycosides, reducing sugars, amino acids, carbohydrates and anthocyanins are absent. The methanol extract of leaf showed strong presence of the phenols whereas the saponins, flavonoids, terpenoids are moderately present, the steroids, alkaloids, cardiac glycosides, tannins, carbohydrates and anthocyanins are poorly present. In the Chloroform extract poor presence of phenols, steroids, flavonoids, carbohydrates, reducing sugars and anthocyanins is seen.

The acetone extract of leaf showed poor presence of phenols, saponins, steroids and cardiac glycosides. Saponins, steroids, phenols, cardiac glycosides and anthocyanins are poorly present in the ethyl acetate extract of leaf. In the pet ether extract saponins are present moderately whereas flavonoids and cardiac glycosides are poorly present. (Table 2).

Table 1: Qualitative chemical analysis of various leaf extracts of *Thespesia populnea*

Sr. No	Phytochemicals	Aqueous	Methanol	Chloroform	Acetone	Ethyl acetate	Pet ether
1	Saponins	+	+	-	+	+	++
2	Steroids	+	+	+	+	+	-
3	Phenols	-	+++	+	+	+	-
4	Flavonoids	+	+	+	-	-	+
5	Terpenoids	+	+	-	-	+	-
6	Alkaloids	+	+	-	-	-	-
	Cardiac glycosides						
8	Reducing sugars	+	-	+	-	-	-
9	Tannins	+	+	-	-	-	-
10	Amino acids		-	-	-	-	-
11	Carbohydrates	+	+	+	-	-	-
12	Anthocyanins	-	+	+	-	+	-

(+++)= strongly present; (+) = poorly present; (++) = moderately present; (-) = absent

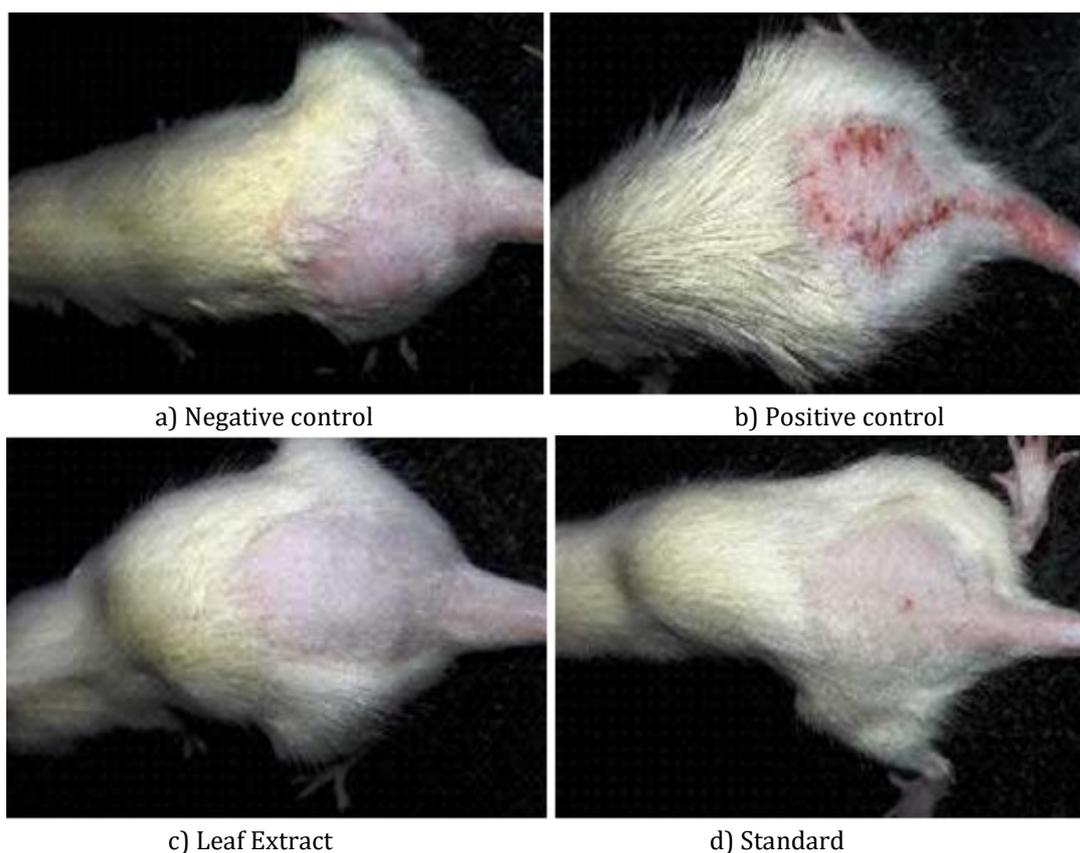


Figure 1: Physical evidence of in-vivo anti-psoriatic Activity of methanol leaf extract of *Thespesia populnea* a) Negative Control b) Positive Control, c) Leaf Extract and d) Standard.

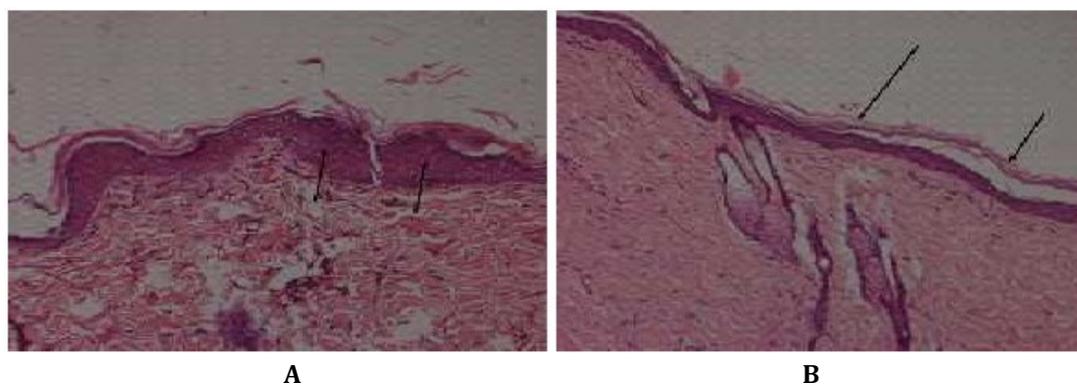


Figure 2: Histopathological screening of psoriatic induced rat skin treated with methanol leaf extract of *Thespesia populnea* and standard: Retino-A 0.05% (Tretinoin cream U.S.P.) A) Test Skin - Mild epidermal thickening noticed in the skin - black arrow. B) Standard Skin- Mild hyperkeratosis sin which keratin deposition in the stratum corneal layer of epidermis - arrow.

Table 3: Ct Values of the Psoriasis Samples. Data are Mean ± S.E. (n = 3).

S. No	Sample	Ct values	Ct values	Ct values	Mean±SD
1	Negative Control	22.77	22.67	22.58	22.17±0.109697
2	Positive Control	22.96	22.65	22.95	22.85±0.176163
3	Standard	23.33	23.85	23.66	23.61±0.263122
4	Test Samples	26.15	26.19	26.45	26.26±0.162891
5	GAPDH	22.05	22.55	23.02	22.54±0.002

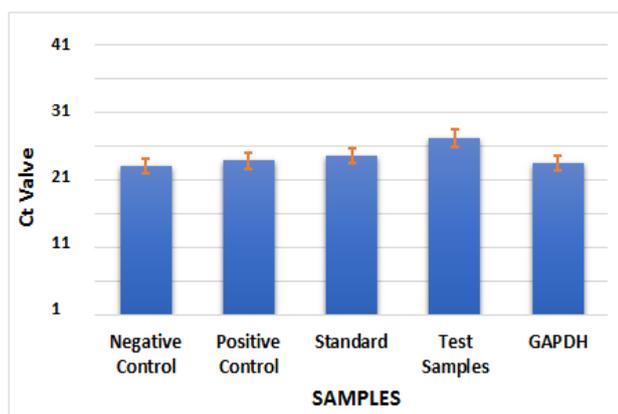


Fig. 3: Gene Expression levels of Psoriasis Samples: Test Samples showing high expression when compared to standard.

Based on the phytochemical screening it is reported that the *Thespesia Populnea* L leaves are rich in phenols and moderate presence of flavonoids and saponins.

Estimation of anti-psoriatic activity

Screening of anti-psoriatic activity of methanol extract of *Thespesia populnea* was carried out using Imiquad cream induced psoriasis in rat. The rat skin treated with methanolic leaf extract of *Thespesia populnea* showed a significant reduction in the total epidermal thickness, as well as a significant retention of the stratum granulosum, the absence of movement of neutrophils further substantiates that the methanol leaf extract and isolated flavonoids have been very useful in containing the changes that occur in the skin due to Imiquad cream induced psoriasis. The Epidermal layer of skin appeared normal (Fig. 1). Fig. 2 shows the histopathological screening of psoriatic induced rat skin treated with methanol leaf extract of *Thespesia populnea* and standard: Retino-A 0.05% (Tretinoin cream U.S.P.).

The histological examination (hematoxylin – eosin staining) of the tail sections were done. Drug activity is defined by the increase in percentage of orthokeratotic regions. The methanol extract increased the orthokeratotic region by 30%. The retinol A drug treated showed an increase of 70% orthokeratotic region in comparison to the normal and Imiquad cream treated samples (Fig. 2).

E2A gene expression in wistar rat skin

The effect of the methanolic leaf extracts of *Thespesia populnea* on the E2A gene expression in Wistar skin samples was analyzed. The mRNA levels of E2A were

evaluated by Real Time PCR. Table 2 and Figure 3 show that the mRNA levels of E2A increased with a Ct value of 26.02. A 1.2-fold increase in the gene expression was also noted in the treated samples in comparison to that of the control. The results indicate that the methanolic leaf extracts showed an up-regulating effect on E2A gene expression in HepG2 cell lines.

CONCLUSION

Analysis of different extracts of *Thespesia populnea* leaves is beneficial for assessment of medicinal and pharmacological efficacies of this plant. Phytochemical investigation of *Thespesia populnea* leaf extracts revealed mainly the presence of phenols, saponins, flavonoids, terpenoids, steroids, alkaloids, cardiac glycosides and tannins. Hence the phytochemical properties in leaves of *Thespesia populnea* are said to improve the health status of people and also have a use in industrial, pharmaceutical and nutraceutical products of commercial importance. Even though many compounds isolated from plants are being scrupulously tested for their anticancer properties, it is becoming progressively acknowledged that the favorable effects of plants are due to a complex interplay of the combined mixture of compounds present in the whole plant rather than constituent single agents alone.

In the current study analysis of phytochemical constituents, antipsoriatic of methanolic leaf extracts and evaluation of E2A gene expression for psoriasis levels by RT-PCR of *Thespesia populnea* was studied. The antipsoriatic effect using Perry scientific mouse tail model revealed that methanolic leaf extract increased the orthokeratotic region by 30%. Known the possible role of E2A as a biomarker in psoriasis, it was considered in the present analysis to study whether the methanolic leaf extracts of *Thespesia populnea* showed antipsoriatic effect targeting E2A gene expression. Numerous studies have revealed that E2A was enormously critical for cell proliferation and differentiation. Even though E2A binding partner, Id1, has been discovered to be important in psoriasis conferring to various reports the information concerning the molecular involvement of E2A in psoriasis still remains narrow.

The work so far achieved on *Thespesia populnea* methanolic leaf extraction also sets the source of future studies on the particular constituents for

characterization and assessed for their biological activities simultaneously. The information obtained from the antipsoriatic activity may be focused for full description of the bioactive compound, and afford easy and appropriate detection of specific targeted bioactive metabolite(s). *Thespesia populnea* plant could be one of them particularly because of its widespread use for its abundant therapeutic properties. Finally, it can conclude that methanolic leaf extract of *Thespesia populnea* can preferably be used as anti-psoriatic remedy and the plant is potential source for advance investigation on anti-psoriatic activity.

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Conflict of Interest/ Author Contributions

Prof.S.Y.Anwar designed the work, Narendar.V performed studies, Sudhakar Chekuri and Dr.R. Chandrashekhar helped the data interpretations. All the authors reviewed the manuscript.

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