Original Article

Effect of Leaves of *Vitex. trifolia* Linn on Different Stages of Inflammation

Aryaa Ankalikar, Agadi Hiremath Viswanathswamy

Department of Pharmaceutical Chemistry, K.L.E. University's College of Pharmacy, Vidyanagar, Hubballi 580 032, Karnataka, INDIA.

ABSTRACT

Objective: The objective of the study was to evaluate effect of hydroalcoholic extract of *V.trifolia* Linn for anti-inflammatory activity. **Methodology:** Wistar rats weighing 150-200 g were used divided into four groups which were treated with the plant extracts and with indomethacin as standard drug. Acute inflammation was produced by injecting 0.1 mL of 1% homogenized carrageenan suspension in normal saline to the left hind paw of the rats. Paw volume measured using a digital plethysmometer. Sub-acute anti-inflammatory studies were carried out by subcutaneous implantation of weighed pellets of compressed cotton and gross pith. Hematological and histological studies were also conducted. **Results:** The extract at both the doses has effectively suppressed carrageenan induced inflammation. Similarly less wet exudate formation is seen at higher dose suggesting inhibitory effect of the compounds on vascular permeability and controlled formation of dry exudate indicative of the effect on proliferative phase. Hematological parameters, lymphocyte count at both the doses and histological studies of the granuloma tissue were also performed revealing decrease in levels of macrophages, mast cells and other inflammatory mediators in groups treated with the extract as compared to animals treated in control group. **Conclusion:** The results suggest that the ethanolic extract of *Vitex trifolia* shows anti- inflammatory activity on both acute and sub-acute stages of inflammation.

Key words: *V.Trifolia*; Acute Inflammation; Sub-Acute Inflammation; Anti-Inflammatory Activity.

INTRODUCTION

Inflammation has become the major focus of global scientific research since it is suggestive of presence of disease conditions in animals as well as in humans.[1] It is a nonspecific transitory biological response of microcirculation to tissue damage or pathogen infection. A defensive mechanism which in an attempt to limit tissue damage and remove pathogenic insult induces profound physiologic adaptations.[2]

It is mainly a complex host response to injury which involves recruitment of leukocytes at the site and this, is coordinated with a range of chemical mediators such as arachidonic acid metabolites cytokines and nitric oxide.[3] Along with this, there is production of inflammatory mediators like prostaglandin (PGE_2) and Tumor Necrosis Factor (TNF-α) which cause oedema formation. Prostaglandins (PGs) induce hyperalgia and effect the transducing property of free nerve endings, bradykinins, TNF-α, interferons and this induces production of PGE_2.[4]

Currently available anti-inflammatory drugs like Aspirin, Ibuprofen and Diclofenac present some side-effects like nausea, vomiting, epigastric distress. These drugs have limited potency and on prolonged consumption can cause damage to the body. Thus search for alternatives becomes imperative.[5,6]

The genus *Vitex* includes about 270 known species of trees and shrubs. *V.trifolia* is a tropical shrub or a shrubby tree which grows up to 6m in height. Traditionally roots of *V.trifolia* have thermogenic, astringent, expectorant, carminative, anthelmen-

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Correspondence:
Dr. A.H.M.Viswanathswamy
Professor & HOD
Pharmacy Practice
K.L.E. University's College of Pharmacy, Vidyanagar, Hubballi 580 032, Karnataka, INDIA.
Phone no: 09448667355
E mail: vmhiremath2004@yahoo.com

www.ijper.org

tic and anti-inflammaotry effects. It is reported to be useful in painful inflammations, cough, bronchitis, leprosy, skin diseases, dyspepsia and fever. The leaves are useful in inflammations, hair loss, lecoderma, cough bronchitis, fever, splenomegaly, tuberculosis and it is also mentioned to be having anti-cancerous activity. The flowers are useful in fevers and the fruits are good in amenorrhoea, similarly the plant also has anti-bacterial, anti amnesic, anticonvulsant and hepatoprotective activity.[7,8]

It has been reported in literature to be useful in treatment of fever, inflammation, nematicidal and anti-tumor activity. Its organic extracts have been proven to be toxic against several cell lines and diterepenes like vitretifolin D, vitretifolins E have been isolated from different parts of the plant using various organic solvent extracts.[9,10]

The aqueous extract of leaves of *V.trifolia* has been evaluated for its effect on lipopolysaccarhide induced inflammatory genes. The extract has exhibited regulatory effects on Nuclear Factor kappa B and consequently on inflammation mediators. *In-vitro* cell line studies of aqueous extract of leaves of *V.trifolia* has exhibited significant dose and time-dependent inhibitory activity on IL-6, IL-10 synthesis but slight effect on TNF-α, all of which play a significant role in inflammation.[11]

Literature review shows the ethanolic extract of leaves of *Vitex trifolia* have been evaluated for its activity on carrageenan induced paw edema and the effect also has been compared to the anti-inflammatory potential of ethanolic extract of leaves of *Vitex negundo*.[12] Similarly aqueous extract of *Vitex trifolia* leaves has shown significant dose and time dependent inhibitory activity on interleukins and TNF-α[11]. Sub-acute inflammatory studies were carried out using air pouch and turpentine method for a period of six days at higher doses (1000 mg/kg and 2000mg/kg). *In-vitro* studies also have been done on cell lines evaluating different parameters related to inflammation.

The present study was focused on the *in-vivo* effect of hydroalcoholic extract of *V.trifolia* on different stages of inflammation. Both the stages of inflammation have been treated with 100 mg/kg and 200 mg/kg body weight of extract.

Acute inflammation was induced using the carrageenan whereas sub acute inflammation was induced using the cotton pellet granuloma method. Sub-acute inflammation was carried out for fourteen days. The prolonged study facilitates to observe the effect of extract on vital organs like stomach, liver and kidneys.

MATERIALS AND METHODS

Drugs and Chemicals: Standard drug indomethacin was obtained from Fabrica Italiana Sintetici, Vicenza, Italy, carrageenan was obtained from Sigma Chemical Company (St. Louis, U.S.A). All other chemicals were of Analytical grade.

Plant material and extract: The leaves of *V.trifolia* locally known as Neerlakki were collected from the herbal garden of KLE University's College of Pharmacy, Vidyanagar, Hubballi. The plant was authenticated by Dr. A.B. Sonappanavar, Dept. of Botany, P.C. Jabin Science College, Hubballi. The leaf material of *V.trifolia* was collected; shade dried and grinded in the mixer.

The powder was stored in air tight polyethylene bag. About 50g of the powdered drug was weighed using an electronic balance and blended with about 200mL 70% ethanol, refluxed for 1.5hrs at 65-70ºC. This cycle was repeated three times. The extracts freed of the solvent gave a green-semi-solid mass. Total percentage yield of the extract was found to be 22.94%.

Experimental Animals: Wistar rats weighing between 150-200g were obtained from Venkateshwara Enterprises, Bangalore. All experimental procedures were approved by the Institutional Ethics Committee (IAEC). Project Code: KLEU's010/IAEC.HBL/31st Aug 2013.

Acute Toxicity Studies: The acute toxicity studies were carried out as per OECD guidelines 423. For the toxicity studies swiss albino mice were procured from the animal house of K.L.E.University's College of Pharmacy, Vidyanagar Hubballi.

3 female mice weighing 20-30 g were selected and after acclimatisation were kept for fasting for 18h being provided only with water. An emulsion of the extract was prepared using Tween 80 and dose of 2000mg/kg b.w. was administered orally and the animals were observed under open-field conditions for behavioural, locomotion, muscle spasms, tremors, convulsions and mortality for 24 h and further for a period of 14 days for occurrence of any toxic symptoms.

Since no mortality was observed at the dose of 2000mg/kg b.w. for further experimental studies and evaluation of its anti-inflammatory activity 1/10th and 1/20th of the aforesaid dose was used.

Experimental Design and Drug Treatment

Two models were employed to evaluate the anti-inflammatory effect of the leaf extract of *V.trifolia* Linn. For acute inflammation carrageenan induced paw oedema technique was employed while for Sub-acute inflamma-

tion foreign body induced (Grass pith and cotton pellet) granuloma technique was employed.

Carrageenan Induced Paw Oedema in Rats

Wistar rats weighing between 150-200 g were obtained from Venkateshwara Enterprises, Bangalore. The rats were divided into 4 groups, each group containing 6 rats, total of 24 rats. Acute inflammation was produced by injecting 0.1 mL of 1% homogenised carrageenan suspension in normal saline to the left hind paw of the rats. One hour prior to this test drugs were administered.

Group I received 0.9% NaCl and served as control, Group II received indomethacin (10 mg/kg b.w.) Group III and IV were administered the test drug *V.trifolia* leaf extract, 100 mg/kg and 200 mg/kg body weight respectively. Administration of indomethacin and the plant extract was done by p.o. route.

A mark was made at the ankle up till which the paw was dipped and paw volume was measured at interval of 1h, 2 h, 3 h and 5 h using a plethysmometer. The mean paw volume at different intervals was measured, compared to control and percentage inhibition was calculated using:

Percentage oedema inhibition = **[(Vc- Vt) / Vc] × 100**

Where: Vt: Percentage difference in increased paw volume after the Administration of test drugs to the rats

Vc: Difference of increased volume in the control groups[13-15]

Cotton-Pellet induced Granuloma in Rats

The method is based on granuloma formation in rats over a foreign body by subcutaneous implantation of compressed cotton pellets along with grass pith.[13-16-18]

Wistar rats weighing 150-200 g were procured, housed in polypropylene cages at room temperature and acclimatised. They had free access to food and water throughout the experiment. Total of 24 animals were chosen for the studies, rats were divided into 4 groups Group I received 0.9% NaCl and served as control, and Group II received indomethacin (10 mg/kg b.w.). Group III and IV were administered the test drug *V.trifolia* leaf extract, 100mg/kg and 200mg/kg body weight respectively for a period of 14days each.

Sub-acute inflammation was produced under light ether anesthesia by shaving the ventricle aspect of the abdomen and making an incision of 3-4 cms. In this incision a subcutaneous pocket was made and cotton pellets weighing 10±1 mg each sterilised at 160°C for 30 min less than 15lbs was inserted. Along with this grass-pith measuring 1/2 mm was also inserted. Drug treatment was started 1h prior to cotton pellet implantation and the treatment was continued for the next 14 days.

On day 15, blood samples were collected through the retro-orbital route under light anesthesia using diethyl ether and subjected to differential leukocyte count. The animals were then sacrificed and cotton pellets removed by dissection, the granuloma tissue was identified as a firm vascular tissue. It was isolated and subjected to histological studies.

Cotton pellets were dried at 60°C for 24 h and net granuloma formation was calculated by subtracting initial weight.

Granuloma percentage formation was calculated as follows:

Granuloma inhibition (%) =Wc-Wt/ Wc × 100

Where: Wc and Wt represent the average weight of granuloma in the control and treated groups respectively.

Statistical Analysis: Inflammation ooedema has been expresses as mean ±SEM. The inhibition percentages are calculated from the differences between the treated groups and control groups. Statistical significance of differences between mean values was analyzed by one-way ANOVA followed by Dunnett's and Bonferroni's test using Graph pad Prism version 5.

RESULTS

Acute Toxicity Study

On observation the animals treated with the hydroalcoholic extract of leaves of *V.trifolia* L. showed no behavioural changes and on administration of maximum dose of 2000 mg.kg body weight there was no mortality observed even after 14 days thus it was concluded that 2000 mg/kg is a safe dose and $1/10^{th}$ and $1/20^{th}$ of it i.e. 100 and 200 mg/kg b.w. was used for further pharmacological studies.

Effect of Carrageenan induced paw oedema in Rats

On injecting carrageenan in the subplantar tissue of the left hind paw of rats oedema formation reached its maximum after 4 h of injection. As per results from Acute Toxicity Studies, the hydroalcoholic extract of leaves of *V.trifolia* were studied at two different doses *viz.* 100 mg and 200 mg /kg body weight.

Prior to injecting carrageenan at 0 min the mean paw volume for control was found to be 0.35 mL ±0.01, while for groups treated with 100 and 200 mg/kg body weight and standard drug Indomethacin the mean paw

oedema volume was found to be 0.37 mL ±0.01, 0.38 mL ±0.01 and 0.38 mL ±0.01 respectively.

On measuring the paw volume after 1hr. of carrageenan challenge for control group it was found to be 1.2 mL ±0.09 while for groups treated with 100 and 200 mg/kg body weight and standard drug Indomethacin the mean paw oedema volume was found to be 0.67 mL ±0.03, 0.58 mL ±0.05 and 0.46 mL ±0.03 respectively

The paw volume for animals belonging to control group after 2 h. of carrageenan challenge was found to be 2.0 mL ±0.11 and for group of animals treated with 100 and 200 mg/kg body weight and standard drug Indomethacin the mean paw oedema volume was found to be 0.78 mL ±0.009, 0.7 mL ±0.01 and 0.36 mL ±0.01 respectively.

At interval of 3h. carrageenan challenge the paw volume for control group was found to be 1.9 mL ±0.02 and for group of animals treated with 100 and 200 mg/kg body weight and standard drug Indomethacin the mean paw oedema volume was found to be 0.78 mL ±0.07, 0.42 mL ±0.02 and 0.29 mL ±0.01.

At the end of the study, at 5hr. the paw volume for control group was found to be 1.7 mL ±0.03 and for group of animals treated with 100 and 200 mg/kg body weight and standard drug Indomethacin the mean paw oedema volume was found to be 0.38 mL ±0.01, 0.37 mL ±0.01 and 0.16 mL ±0.01.

At 100 mg/kg body weight the extract has shown effect after 3 h of carrageenan challenge while at 200 mg/kg body weight its anti-inflammatory action is observed at 2h. ($p<0.001$)

The percentage inhibition of inflammation and oedema formation at the end of 5 hrs was 69.92% and 72.23% respectively at both 100 and 200mg/kg b.w. while indomethacin was seen to be having percentage inhibition of 90.46% (Figure 1).

The results of treatment groups were also compared with the results of the animals treated with standard drug and it comparison was done on hourly basis. At the first and fourth the results of animals belonging treated with 100 mg/ kg b.w. of the extract were seen to be statistically significant ($p< 0.01$) when compared to the group of animals treated with standard while the results of animals belonging to group treated with 200 mg/kg b.w. of the extract was not significant. At second and fifth hour however the groups treated with extract at 100 and 200 mg/kg b.w. have shown significant inhibition in edema formation when as compared to the group of animals treated with the Standard drug.

Effect of Cotton-Pellet induced Granuloma in Rats

Assessment of Wet and Dry Exudate Formation

The study shows that the extract of leaves of *V.trifolia* L. inhibits wet and dry exudate formation by 36.94% and 36.45% at 100 mg/kg b.w. respectively while at 200 mg/kg b.w. wet and dry exudate formation is inhibited at 58.22 % and 63.08% respectively.

Indomethacin shows 65.71% inhibition of wet exudate formation and 67.43% inhibition of dry exudate formation at dose of 10 mg/kg body weight. (Figures 2 & 3)

Evaluation of Hematological Parameters

The mean values for TLC (Total Leucocyte Count) for normal group was seen to be 6300 ±13 control group was seen to be 13733±260 cells/cm^3, for group treated with 100 mg/kg b.w. plant extract TLC was 7767±586 cells/cm^3 and for group treated with 200 mg/kg b.w. plant extract TLC was 9333±448 cells/ cm^3 respectively. Animals treated with indomethacin showed a count of 11717±444cells/ cm^3

In the total leucocyte count for each of the groups, the percentage of PMN(Polymorphonucleocytes) and Lymphocyte count for Normal group was 21% and 80% respectively, control group was 37.67% and 58.67% respectively while for groups treated with plant extract at 100 mg/kg b.w. it was 24.00% and 72.50% respectively. Group treated with 200mg/kg b.w. the percentage of PMN and Lymphocyte count was 23.33% and 73.67%respectively while indomethacin treated group showed a count of 23.83% and 70.50% respectively. (Table 1)

Histological Studies

Light microscopic examination of granuloma tissue was done. Similarly vital organs like Stomach and kidneys were isolated and subjected to Histological studies. The study was done at 100x.

A 5 μ section of the tissue to be subjected for histological studies was obtained with a standard microtome isolated and fixed using 10% Formalin, immersed in Paraffin. The tissue was then stained with hematoxylin and eosin and then subjected to histological studies.

DISCUSSION

The focus of the present study was to evaluate the effect of hydroalcoholic extract of leaves of *V.trifolia* L. on different stages of inflammation.

The carrageenan induced paw oedema test is a highly reproducible and well researched model for evaluating acute anti-inflammatory actions of natural products.

Inflammatory response is a plurifactorial, polyphasic tissue reaction, which begins with a rapid short lived increased vascular permeability to a prolonged cellular infiltration and proliferation. Thus assessing the efficiency of an anti-inflammatory agent at all stages, namely acute, sub-acute and chronic stages of inflammation becomes imperative.[17-19]

Inflammation induced by carrageenan involves three distinct phases of release of mediators, including serotonin and histamine in the first phase (0-2 h) it is also called as the exudative stage of inflammation, followed by release of kinins, leukotrienes and PMN cells, in second phase (3 h) and prostaglandins in the third phase where oedema reaches its peak volume(>4 h). The paw oedema formation due to carrageenan is due to the production of protein-rich exudates containing a large number of neutrophils. All these factors contribute to increased blood flow and redness at the site of inflammation [20].

Anti-inflammatory effect of hydroalcoholic extract of *V. trifolia* is seen at both 100 and 200 mg/kg b.w. The extract has shown inhibitory activity at different time intervals after carrageenan challenge. The reference drug indomethacin has also caused marked suppression of carrageenan-induced rat paw oedema.

The inhibitory effect of the plant extract maybe attributed to the presence of flavanoids such as persicogenin, artemetin, luteolin, penduletin, and vitexicarpin.[21]

Cotton pellet induced granuloma method

The cotton pellet granuloma technique was used to study the effect of the *V.trifolia* extract on granuloma formation provoked by subcutaneous implantation of compressed cotton pellets.

A foreign body like cotton pellet when implanted in the abdominal pouch of animal disrupts the cellular and molecular processes of the cell responsible for homeostasis[22] and leading to production of undifferentiated connective tissue around it indicating inflammation.[22] This provocation gives rise to formation of undifferentiated connective tissue along with infiltration of fluid containing neutrophils, fibroblasts and collagen which are a basic source for granuloma formation indicative of proliferative phase. Decrease in granuloma formation is indicative of suppression of proliferative phase [23]. The study is suggestive that the extract has significantly prevented wet exudate formation as compared to the control group suggesting inhibitory effect of the extract on vascular permeability. The amount of newly formed connective tissue is measured by weighing dried pellet which is a suggestive index of the severity of inflammation. The extract has also shown controlled formation of dry exudate indicating the positive effect of the drug on the proliferative phase of inflammation. Newly formed connective tissue is measured by weighing the dried pellet after removal which serves as an index of severity of inflammation.[24]

Migration of WBC at the site of inflammation and elevated levels of WBC count acts as a marker for inflammatory process. Blood samples of animals belonging to the control group showed a steep rise in WBC and PMN count demonstrating aggravated inflammation. In case of animals belonging to group treated with the extract of leaves of *V.trifolia*. L there is seen to be significant inhibition in WBC migration, count of WBC and reduced dry and wet weight of granuloma. The extract at higher dose has shown better inhibitory and dry and wet weight reduction activity, while the action on lymphocyte count is not dose dependent.

Histological Studies

Granuloma Tissue: Light microscopic examination of Granuloma tissue of animals belonging to control as well as treated groups was done. A predominant migration and deposition of Macrophages, Mast cells, neutrophils, mixed inflammatory mediators and Giant cells was seen in tissue of animals belonging to Control Group (Figure 4) while the quantity of collagen fibers and organized fibrous sheath associated with the healing process was seen to be very less depicting aggravated inflammation. On the other hand granuloma tissue obtained from animals belonging to groups treated with hydroalcoholic extract of leaves of *V.trifolia* show comparatively less deposition of macrophages and mast cells but a predominant deposition of collagen fibers signifying anti-inflammatory activity (Figure 5, 6 & 7).

The stomach, liver and kidneys are the primary organs affected by metabolic reaction caused by toxicants.[25]

Histological Analysis of Liver: Light microscopic examination of liver sections of animals belonging to Control Group exhibits acute congestion and deposition of RBCs as seen in (Figure 8). The sections of liver belonging to animals treated with ***V. trifolia* 100 mg/kg** show appearance of Acute Focal Congestion but normal appearance of hepatic lobules, sinusoids and hepatic cells (Figure 9). The sections of liver belonging to animals treated with 200 mg/kg although show normal appearance of the hepatic lobules and hepatic cells there is slight damage to hepatic arteries (Figure 10). However the sections of Liver obtained from animals treated with the standard drug depict acute congestion (Figure 11). The sections of liver belonging to animals

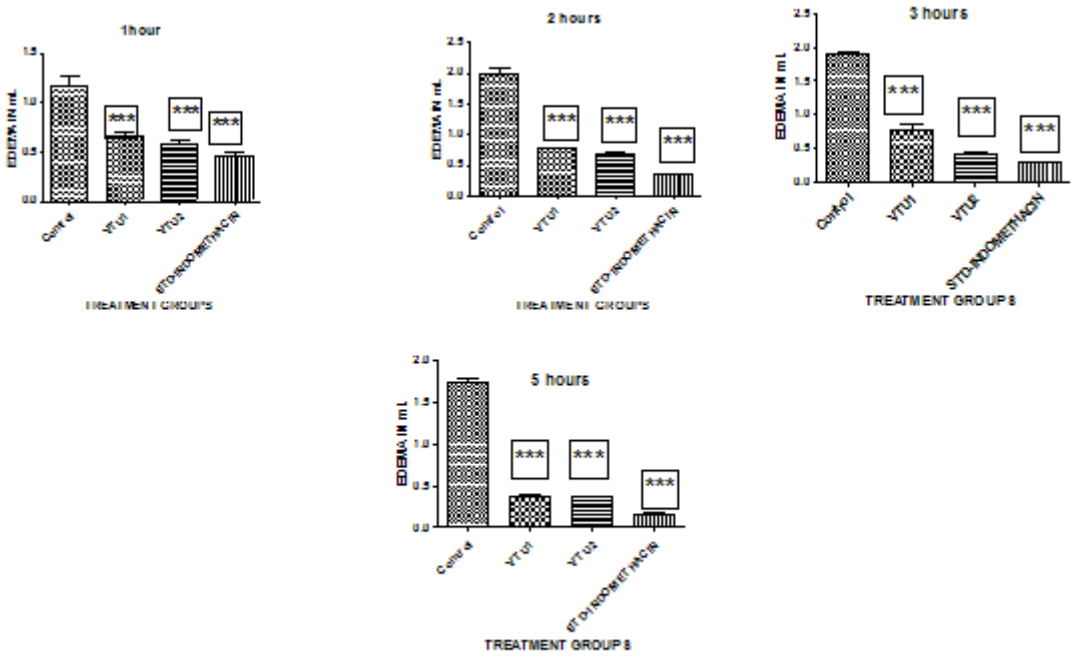
of Control group depict acute congestion, damage to hepatic arteries and deposition of RBCs (Figure 12).

Histological Analysis of Kidneys[26]

Light microscopic examination of kidney sections of animals belonging to Control group as well as those treated with *V.trifolia* show normal renal cytoarchitecture with normal renal corpuscles, renal tubules, glomeruli and Bowman's capsule. (Figure 12, 13 & 14) while those belonging to the Standard Group show congestion, vacuolation of cell lining and tubules and glomerular contraction. (Figure 15)

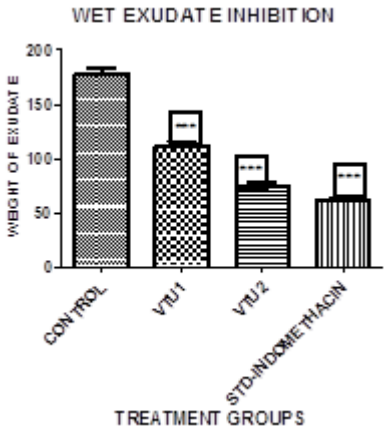
Histological Analysis of Stomach

Light microscopic examination of transverse sections of stomach was obtained from animals belonging to



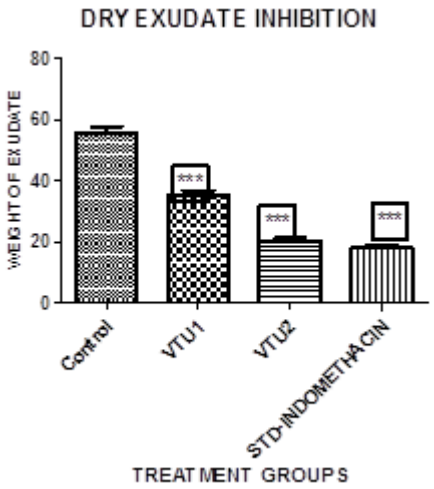
Values are expressed as mean±SE (n=6) ***Significantly different from control P<0.001

Figure 1: Oedema Formation at different time intervals on inducing Acute Inflammation using carrageenan.



Values are expressed as mean±SE (n=6) ***Significantly different from control P<0.001

Figure 2: Wet Exudate Weight and Percentage Inhibition Capacity of hydroalcoholic extracts of *V. trifolia* and Std. Drug indomethacin.



Values are expressed as mean±SE (n=6) ***Significantly different from control P<0.001

Figure 3: Dry Exudate Weight of hydroalcoholic extracts of *V. trifolia* and Std. Drug indomethacin.

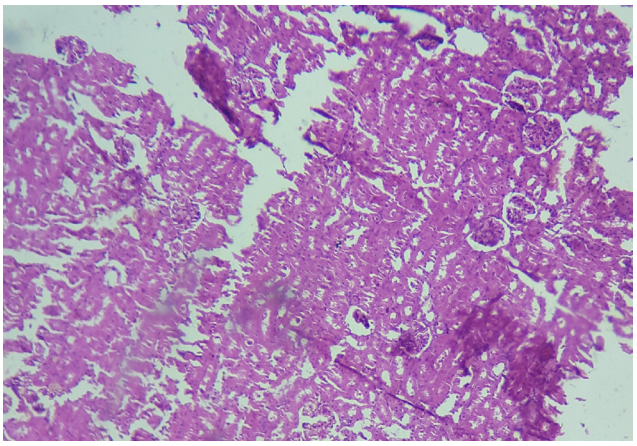


Figure 4: Granuloma Tissue of rats belonging to Control Group

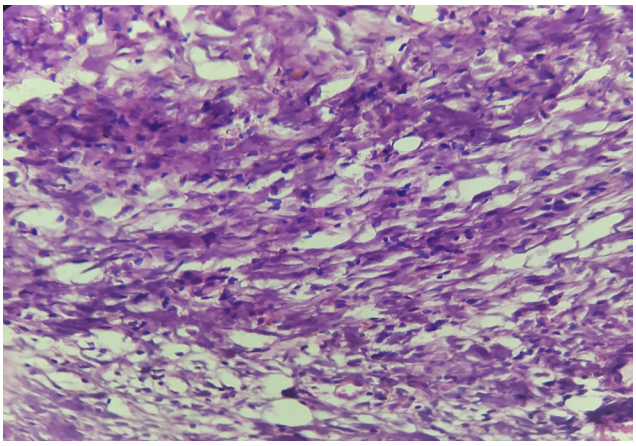


Figure 7: Granuloma Tissue of rats treated with Indomethacin

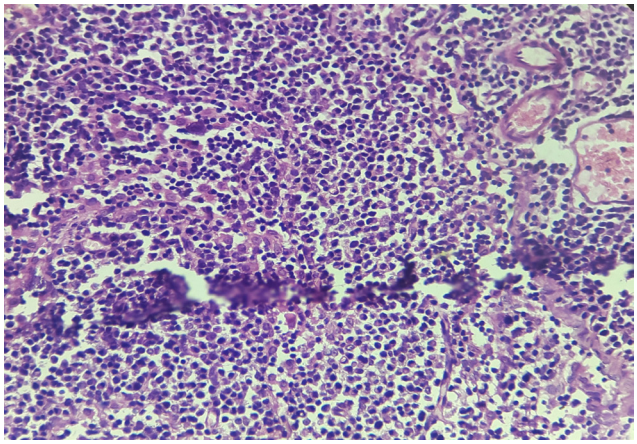


Figure 5: Granuloma Tissue of rats treated with 100mg/kg b.w. of V. trifolia

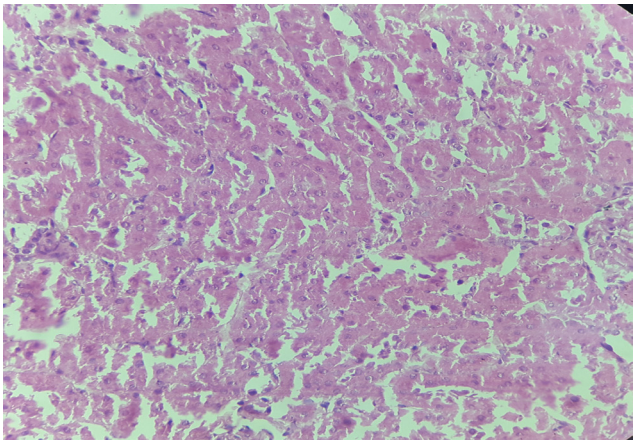


Figure 8: TS of Liver of rats belonging to Control Group

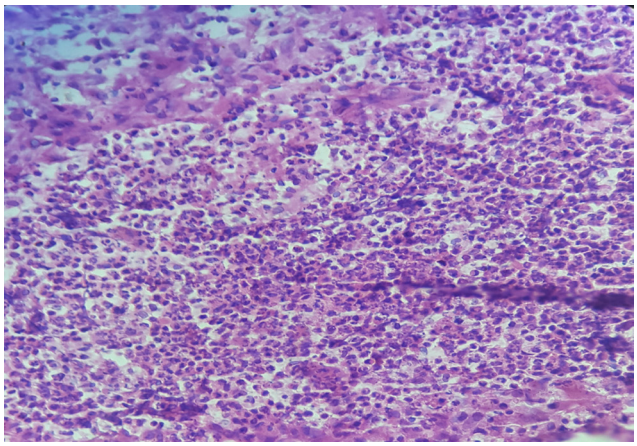


Figure 6: Granuloma Tissue of rats treated with 200mg/kg b.w. of V. trifolia

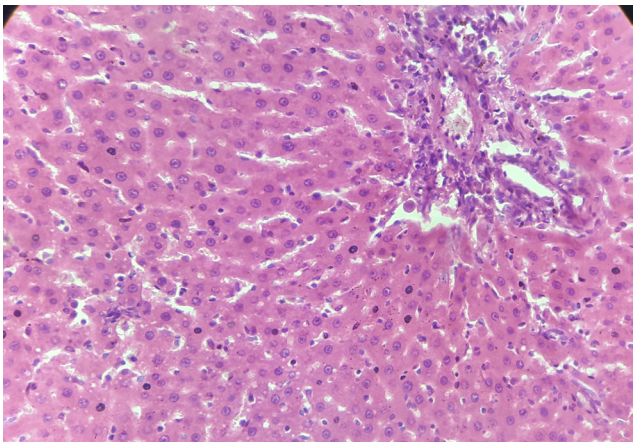


Figure 9: TS of Liver of rats treated with 100mg/kg b.w. of V. trifolia

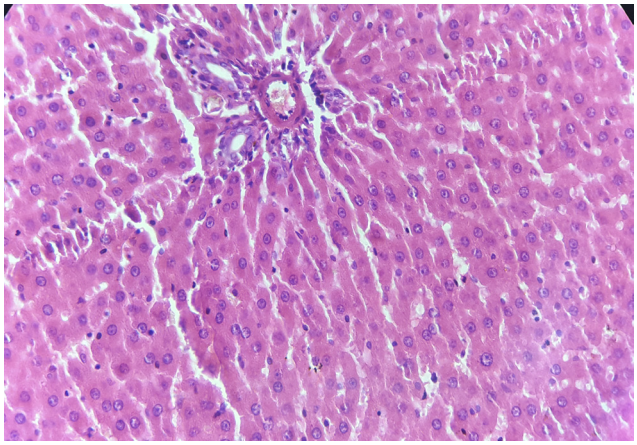


Figure 10: TS of Liver of rats treated with 200mg/kg b.w. of V. trifolia

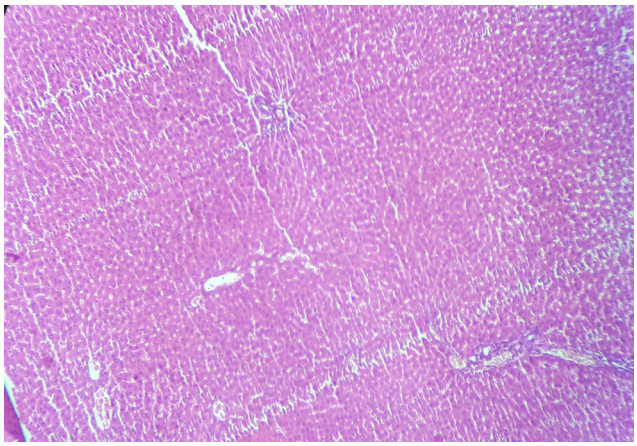


Figure 11: TS of Liver of rats treated with Indomethacin

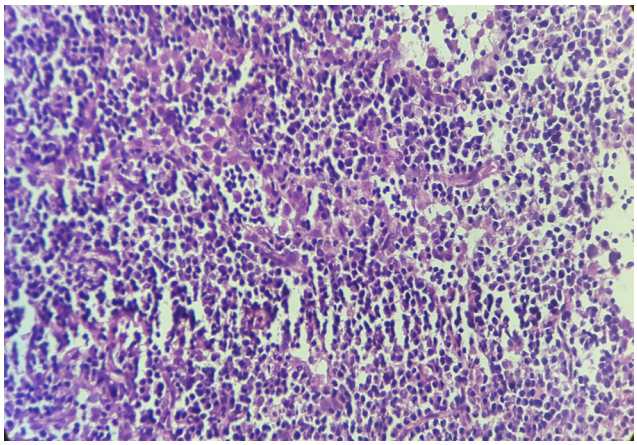


Figure 12: TS of Kidney of rats belonging to Control Group

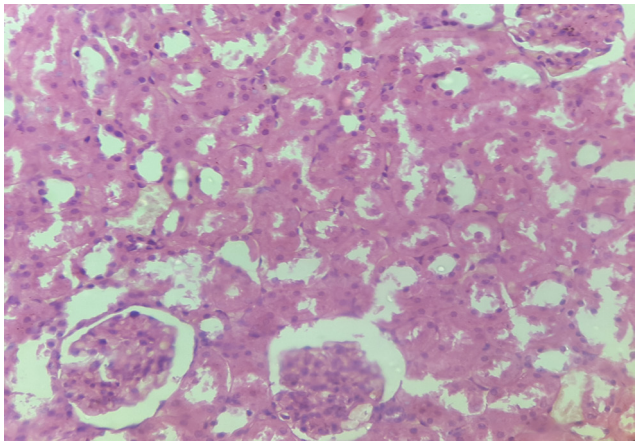


Figure 13: TS of Kidney of rats treated with 100mg/kg b.w. of V. trifolia

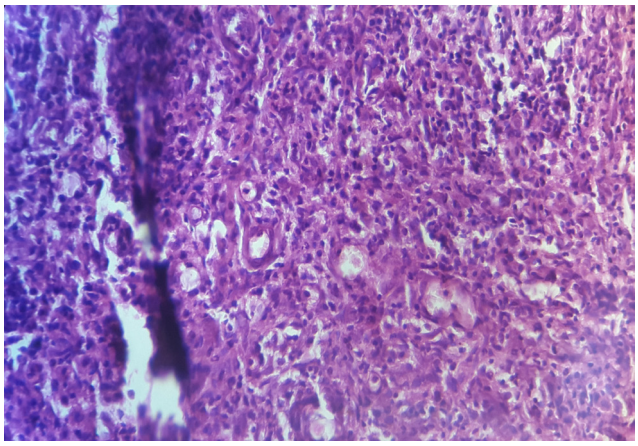


Figure 14: TS of Kidney of rats treated with 200mg/kg b.w. of V. trifolia

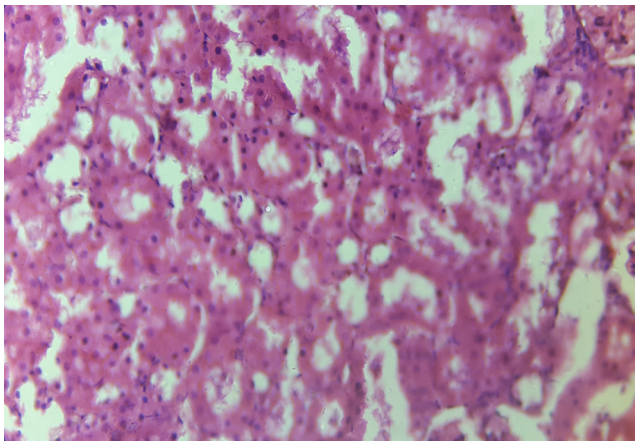


Figure 15: TS of Kidney of rats treated with Indomethacin

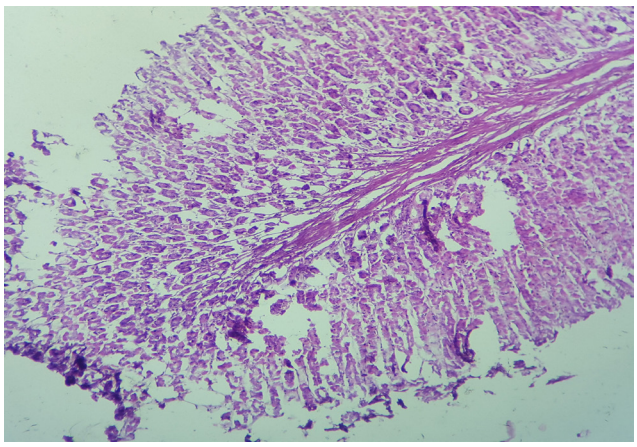


Figure 16: TS of Stomach of rats belonging to Control Group

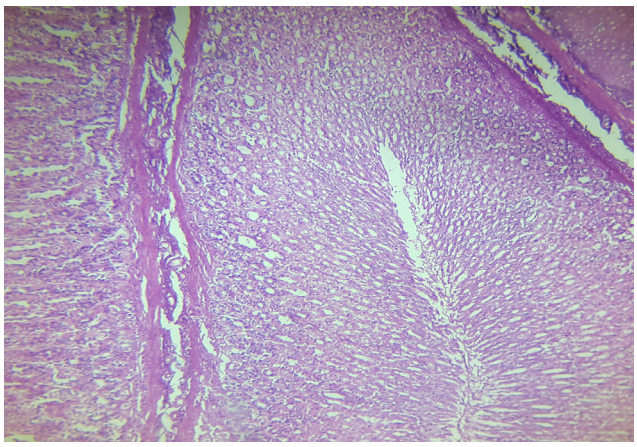


Figure 17: TS of Stomach of rats treated with 100mg/kg b.w. of V. trifolia

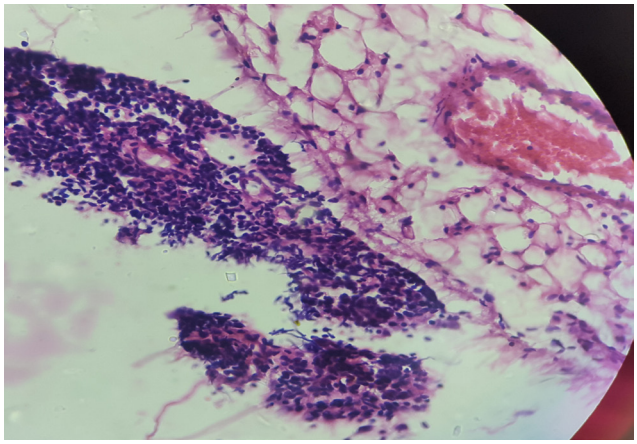


Figure 18: TS of Stomach of rats treated with 200mg/kg b.w. of V. trifolia

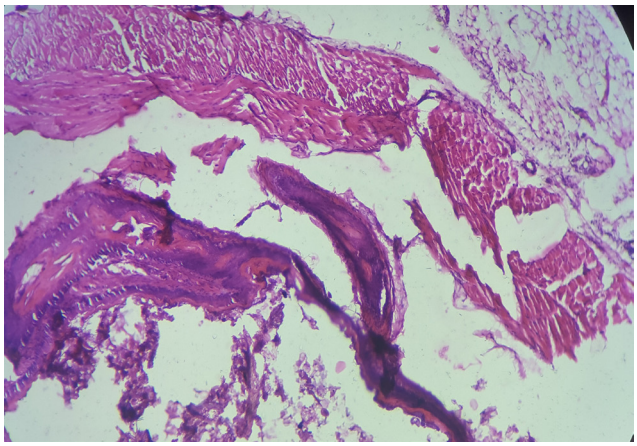


Figure 19: TS of Stomach of rats treated with Indomethacin

Table 1: Effect of hydroalcoholic extracts of *V.trifolia* on Differential Leucocyte Count, PMN count and Lymphocyte Count

	Differential Leucocyte Count (cells/cmm)	% of Polymorpholeucocytes	% of Lymphocytes
Normal	6300±13	21±0.68	80±2
Control	13733±26	37.67±2.71	58.67±3.07
V.trifolia (100mg/kg)b.w.	7767*±58	24.00*±1.00	72.50*±1.14
V. trifolia (200mg/kg)b.w.	9333*±44	23.33*±0.80	73.67*±1.17
Indomethacin	11717*±43	23.83*±0.74	70.50*±0.34

Values are expressed as mean±SE (n=6)
***Significantly different from control P<0.001

Control as well as Treatment groups. The section of stomach belonging to the animals of Control group exhibit acute mucosal necrosis, congestion, ulceration along with inflammatory process characterized by infiltration of neutrophils (Figure 16). *V.trifolia* treated group of animals at 100 mg/kg b.w. dose has exhibited unremarkable cellular structure, normal muscular layer, abundant epithelia and normal mucin producing glands (Figure 17) while animals treated with 200 mg/kg b.w. have shown slight ulceration and congestion (Figure 18).

The sections of stomach of animals treated with Std. drug indomethacin show gastric mucosa with ulceration, mixed inflammatory cells in lamina propia and necrosis of mucosa. (Figure 19).

CONCLUSION

The present experimental study has shown that the hydroalcoholic extract of leaves of *V.trifolia* has elicited significant anti-inflammatory activity against both acute and sub-acute models of inflammation. Further evaluation of the extract for its effect on chronic stage of inflammation and its effect on vital inflammatory biochemical parameters like TNF-α and IL and chemical profiling of the extracts is in progress.

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CONFLICT OF INTEREST

There are no of conflict of interest.

ABBREVIATION USED

PGE2: Prostaglandins; ***V.trifolia:*** *Vitex trifolia;* **RBC:** Red Blood Corpuscles; **WBC:** White Blood Corpuscles.

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SUMMARY

- Inflammation has become the major focus of global scientific research as it is suggestive of presence of disease condition in animals as well as humans.
- The genus Vitex includes about 270 known species and Vitex trifolia is shrub which has been traditionally used in inflammations, hair loss, fever and bronchitis.
- On conducting experimental study on the hydroalcoholic extract of its leaves it has elicited significant anti-inflammatory activity on acute as well as sub-acute stages of inflammation.
- Evaluating the plant further for its effects on biochemical biomarkers and its chemical profiling is required and is in process.

About Authors

Dr. A.H. M. Viswanathswamy: He is a Professor and Head of Department of Pharmacy Practice with more than 20 years of experience at hand in research and teaching. His area of interests mainly involve screening of plants and its isolates for their anti-cancer potential.

Mrs. Aryaa Ankalikar: She is an Assistant Professor having 10 years experience in teaching whose main interests lies in screening different plant extracts and their isolates for their potential pharmacological activity.

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