RESEARCH ARTICLE JPAR 2019

Journal of Pharmaceutical Advanced Research

(An International Multidisciplinary Peer Review Open Access monthly Journal)

Available online at: www.jparonline.com

Phytochemical analysis and *In-Vitro* anti-Inflammatory activity of *Vitex negundo* against denaturtion of protein

Bimala Tripathy*, V. Krishna Raju, Juveria Noorain, Mahreen Fatima, Bashir Saeed, Qurashe Abedulla

Dept. of Pharmacology, St. Mary's Pharmacy College, Deshmukhi (V), Hyderabad, Telengana, India.

Received: 15.04.2019 Revised: 22.04.2019 Accepted: 25.04.2019 Published: 30.04.2019

ABSTRACT: Background: Inflammation is a localized reaction that produces redness, warmth, swelling and pain as a result of infection, irritation or injury. The herbal plant extracts can represent an innovative source of effective newer compounds with significant anti-inflammatory activity. **Aim:** The present study was conducted to identify the phytoconstituents and evaluate *in-vitro* anti inflammatory effect of aqueous leaves extract of *Vitex negundo* (AQVN) against the denaturation of protein. **Method:** The extract at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. **Result:** The extract contains many important phytoconstituents which are useful to manage or cure many diseases especially inflammatory disease. Soxhlet aqueous extraction of *Vitex negundo* also contain many important bioactive constituents such as alkaloids, flavonoids, tannins and a phenolic acid are known to promote antiinflammatory activity. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the selected plant extract. The effect of plant extract was found to be significantly effective when compared with the standard drug. **Conclusion:** From the present study it can be concluded that the *Vitex negundo* aqueous leaves extract possessed marked *in vitro* anti-inflammatory effect against the denaturation of protein.

Corresponding author*
Miss. Bimala Tripathy
Associate Professor,
St. Mary's Pharmacy College, Deshmukhi (V),
Ramoji Film City, Hyderabad, Telengana, India.
Tel: +91-9848245865.
Mail ID: bimalatripathy09@gmail.com

Keywords: *In-vitro* Anti-inflammatory, Protein denaturation, *Vitex negundo*, Viscosity, Diclofenac sodium.

INTRODUCTIONS:

Inflammation is a condition in which a part of the body becomes red, sore and swollen because of infection or injury. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. Inflammation of tissue is due to response that is characterized by redness, pain, heat, swelling and loss of function occur depends on the site and extent of injury [1,2] (Fig 1). Inflammation also divided into two types such as acute and chronic which

are responsible for many other complicated disorders (Fig 2). NSAID'S are cause severe life threatening gastrointestinal (GI) bleeding and ulcers in some people. They have high risk of strokes, heart attacks, heart related deaths when used for long duration [3,4]. It also causes high BP, kidney damage in people over 60 years of age. Common adverse effects are nausea, vomiting, diarrhoea, constipation, mild head ache, dizziness, gas, bloating, and heart burn [5]. In natural products such as terpenes, triterpenes, betulinic acid, alpha-amyrin acetate, lupeol acetate, oleanolic acid, ursolic acid and their derivatives showed interesting potential anti inflammatory activity [6,7].

Fig 1. Picture of Inflammation.

Vitex negundo (*Verbenaceae*) is a large aromatic shrub distributed throughout India. Traditionally leaves of *V. negundo* are documented to possess the antibacterial, anti tumor, astringent, febrifuge, sedative, tonic and vermifuge [8]. It also possess the pharmacological properties like anti inflammatory, anti rheumatic, antibiotics, antioxidant, anti convulsant, oxidative stress, anti androgen, snake venom neutralization and anti allergic activities [9,10]. The present study was conducted to evaluate the possible *in vitro* anti-inflammatory effect of coffee extract against the denaturation of protein.

MATERIALS AND METHODS:

Drugs and Chemicals:

Diclofenac sodium was taken from Sris Pharmaceuticals**,** Hyderabad, Telengana India. Double distilled water from all glass still was used throughout the study. All other chemicals and reagents used in present work were of analytical grade and procured from authorized dealer.

Collection of Plant material:

V. negundo of 100 g loosely packed leaf powder was collected from local market of Hyderabad and it was manufactured by ANJANEYA HERBALS, Ibrahimpatnammandal, Krishna district.

Preparation of Plant extract:

The powder plant material (50 g) was extracted with 400 ml distilled water by boiling for 45 min. The extract was filtered and evaporated to dryness to yield the dry extract. The dry extract was kept in vacuum desiccators until use.

Preliminary phytochemical investigation:

The crude ethanolic extract of *V. negundo* was subjected to preliminary phytochemical analysis in order to detect the presence of various groups of phytoconstituents by carrying out the chemical analysis [10].

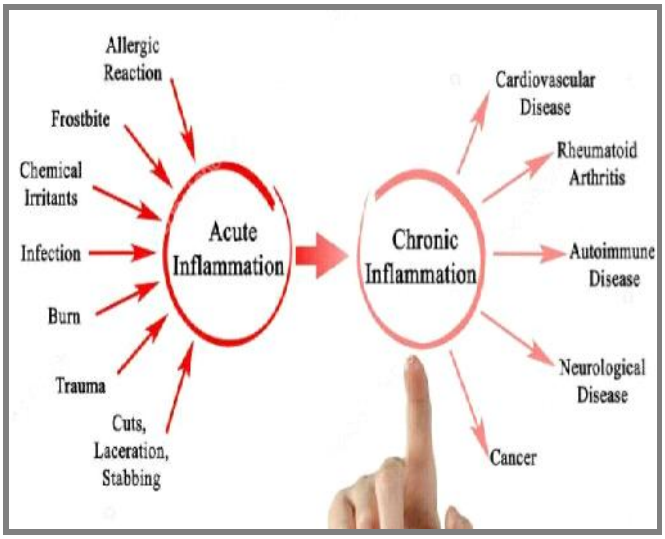

Fig 2. Acute and Chronic inflammation related complications.

Evaluation of *In vitro* anti inflammatory activity:

The reaction mixture (5 ml) was prepared, which consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of Aqueous extract of *V. negundo* (AQVN) so that final concentrations become 100, 200, 300, 400 and 500 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) ^{0}C in a BOD incubator (Labline Technologies, India) for 15 min and then heated at 70 ^{0}C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800, Japan) by using vehicle as blank and their viscosity was determined by using

Ostwald viscometer. Diclofenac sodium at the final concentration of (100 µg/ml) was used as reference drug and treated similarly for determination of absorbance and viscosity. The percentage inhibition of protein denaturation was calculated by using the following formula;

Inhibition (%) = [(Ac-As)/Ac] × 100 …….(1)

Where, Ac and As are Absorbance of control and sample. Viscosity is calculated by using following equation; η1/ η2 = t1d1/ t2d2 ……(2)

Where, η1 and η2 are viscosity of control and sample.

RESULTS AND DISCUSSIONS:

In experimental pharmacological research, animal model use was prohibited in certain areas because of some ethical issues and lack of rules and regulation for their use. In that situation other suitable methods are available to progress our research work [11]. In the present study *in-vitro* anti inflammatory activity of aqueous leaf extract of *V. negundo* was selected against protein denaturation method. Denaturation of tissue protein is one of the well-documented causes of inflammatory and arthritic diseases [11,12]. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo*. The phytochemicals present in selected extracts can prevent protein denaturation which acts as anti inflammatory activity.

Table 1. Preliminary phytochemical screening of aqueous leaf extract of *Vitex negundo*.

Sl. No.	Test	Aqueous extract
1	Alkaloids	++
2	Flavonoids	+++
3	Saponins	+
4	Steroids	+
5	Carbohydrates	-
6	Quinones	-
7	Tannins	++
8	Phenol	+
9	Terpenoids	++
10	Glycosides	++

+++ = Strong, ++ = moderately, + = poor presence and -- = absent.

According to literature review two new chromone derivatives, namely, methyl 3-(2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl) ethyl) benzoate and 3-(1-hydroxy-2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl) ethyl) benzoic acid are isolated from *V. negundo* plant which are might be responsible for anti inflammatory activity [10]. The increase in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by AQVN and reference drug diclofenac sodium. *V. negundo* aqueous extract also contain bioactive constituents such as Alkaloids, Flavonoids, Tannins and a Phenolic acid are known to promote anti inflammatory activity (Table 1) [13].

Table 2. Calculation of average time in sample flow by using Ostwald's viscometer.

Sl. No.	Sample	Time of flow (s)			Average in time (t)
		T1	T2	T3	
1	DW (control)	1.08	1.10	1.04	1.08
2	Sample-1	1.03	1.05	1.02	1.03
3	Sample-2	1.04	1.07	1.05	1.06
4	Sample-3	1.08	1.07	1.05	1.07
5	Sample-4	1.03	1.02	1.07	1.04
6	Sample-5	2.02	2.0	2.04	2.02

DW - Distilled water.

Agents that can prevent protein denaturation and it might be helpful for development of anti-inflammatory drug. The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by AQVN and reference drug diclofenac sodium. This anti-denaturation effect was further supported by the change in viscosities [14,15]. It has been reported that the viscosities of protein solutions increase on denaturation. In the present study, the relatively high viscosity of control dispersion observed this fact. Presence of AQVN prevented this, implying inhibition of protein denaturation. Here, the viscosities decreased when compared with control where no test extract/drug was added. However, the viscosities were found to decrease with concomitant decrease in concentration of test extract and reference drug as well. Although, the viscosities of the test samples (extract/drug), of all concentrations were always less than that of control. This decrease in viscosities may be due to decrease in concentration of test extract/drug in reaction mixture, which resulted in decreased viscosity; and/or other uncertain physico-chemical factors (Table 3) [16]. Nevertheless, the viscosity data indicated inhibition of protein (albumin) denaturation. The major constituents of *V. negundo* are Flavonoid, Polyphenolic compounds like tannins and a Phenolic acid namely chlorogenic acid. Polyphenols are well known natural products known to possess several notable biological properties.

In the present study, the *In vitro* anti-inflammatory activity of *V. negundo* can be attributed to its polyphenols content. The effect may be due to the synergistic effect rather than single constituent.

It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH (pH: 6.2-6.5). Therefore, from the results of the present preliminary study it can be concluded that nirgundi possessed marked *In vitro* anti-inflammatory effect against the denaturation of protein (Table 2). Further definitive studies are necessary to ascertain the mechanisms of the constituents behind its anti-inflammatory actions. *V. negundo* aqueous extract also contain many important bioactive constituents such as Alkaloids, Flavonoids, Tannins and a Phenolic acid are known to promote anti inflammatory activity [19, 20].

Table 3. Percentage inhibition effect of AQVN on Protein denaturation and Viscosity.

Sl. No.	Drugs	Conc. (µg/ml)	Inhibition (%)	Viscosity (Cp)
1	Control	-	-	1.42
2	Aqueous extract	100	27.05	0.67
3		200	31.26	0.71
4		300	43.09	0.83
5		400	52.17	0.86
6		500	64.24	0.89
7	DS (S)	100	77.05	1.1

DS – Diclofenac Sodium and S - Standard.

CONCLUSION:

Now a day herbs are extensively use for the research purpose and it possess more than one chemical entity so it has widely carried out for the research investigation. On the basis of medicinal herb so many herbs has been investigated and however mechanism of action of *Vitex negundo* containing phytoconstituents has not identified so far. The *V. negundo* is an interesting example of a plant used in traditional medicine for many year and the reported phytochemical and pharmacological studies supports its traditional uses and proved to be useful for clinical studies and development of commercial drugs. Two new chromone derivatives, namely, methyl 3-(2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl)ethyl) benzoate and 3-(1-hydroxy-2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl)ethyl)benzoic acid are isolated from *V. negundo* plant which are might be responsible for anti inflammatory activity [10]. *V. negundo* aqueous extract also contain many important bioactive constituents such as Alkaloids, Flavonoids, Tannins and a Phenolic acid are known to promote anti inflammatory activity.

ACKNOWLEDGEMENT:

Authors wish to thank the authority of St. Mary's Groups of Institution, Deshmukhi, Hyderabad for providing laboratory facility to carry out the research work

REFERENCES:

1. Jagtap VA, Agasimundim YS, Jayachandran E, Sathe BS. *In vitro* anti-inflammatory activity of 2-amino-3-(substituted benzylidinecarbohydrazide)-4,5,6,7- tetrahydrobenzothiophenes. J Pharm Res, 2011; 4: 378-379.
2. Nayeem N, Denny G, Mehta SK. Comparative phytochemical analysis, antimicrobial and antioxidant activity of the methanolic extracts of the leaves of *Coffea arabica* and *Coffea robusta*. Der Pharmacia Lettre, 2011; 3: 292-297.
3. Williams LAD, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA, *et al*. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Med J, 2008; 57: 327-331.
4. VbOpie EL. On the relation of necrosis and inflammation to denaturation of proteins. J Exp Med,1962; 115: 597-608.
5. Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN, *et al*. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plants Res, 2010; 4: 789-795.
6. Matsumoto H, Naraba H, Ueno A, *et al*. Induction of cyclooxygenase-2 causes an enhancement of writhing response in mice. Eur J Pharmacol, 1998; 352(1): 47–52.
7. Anson ML, Mirsky AE. The effect of denaturation on the viscosity of protein systems. Gen Physiol, 1932; 15: 341-350.
8. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Pune (India): Nirali Prakashan; 2010. pp. 3.70-3.71.

9. Jagtap, VA, Agasimundim YS, Jayachandran E, Sathe BS. *In vitro* anti-inflammatory activity of 2-amino-3-(substituted benzylidine carbohydrazide)-4,5,6,7- tetrahydro benzo thiophenes. J Pharm Res, 2011; 4: 378-379.
10. Liu H, Xu R, Feng L, *et al*. A novel chromone derivative with anti-inflammatory property via inhibition of ROS-dependent activation of TRAF6-ASK1-p38 pathway. PLoS One, 2012; 7(8): e37168-37174.
11. Shaveta, Singh A, Kaur M, Sharma S, Bhatti R, Singh P. Rational design, synthesis and evaluation of chromone-indole and chromone-pyrazole based conjugates: identification of a lead for anti-inflammatory drug. Eur J Med Chem, 2014; 77: 185–192.
12. Yagura T, Ito M, Kiuchi F, Honda G, Shimada Y. Four new 2-(2-phenylethyl) chromone derivatives from withered wood of *Aquilaria sinensis*. Chem Pharm Bull, 2003; 51(5): 560-564.
13. Khanna D, Sethi G, Ahn KS, Pandey MK., Kunnumakkara AB, Sung B, Aggarwal A, Aggarwal BB. Natural products as a gold mine for arthritis treatment. Curr Opin Pharmacol, 2007; 7: 344-351.
14. Kirtikar KR, Basu BD. Indian medicinal plants. Vol. 3. Dehradun: International Book Distributors; 2007. pp. 1937-1940.
15. Meena AK, Niranjan US, Rao MM, Padhi MM, Babu R. A review of the important chemical constituents and medicinal uses of *Vitex g*enus. Asian J Trad Med., 2011; 6(2): 54-60.
16. Mizushima Y, Kobayashi M. Interaction of anti inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol, 1968; 20: 169-173.
17. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Biomed, 2011; 7: S178-S180.
18. Tandon VR, Gupta RK. *Vitex negundo* Linn (VN) leaf extract as an adjuvant therapy to standard anti inflammatory drugs. Indian J Med Res, 2006; 124: 447-450.
19. Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh Chungag BN. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plants Res, 2010; 4: 789-795.
20. Chawla AS, Sharma AK, Handa SS, Dhar KL. Chemical investigation and anti-inflammatory activity of *Vitex negundo* seeds. J Nat Prod, 1992; 55(2): 163-167.

Conflict of Interest: None
Source of Funding: Nil
Paper Citation: Tripathy B*, Raju VK, Noorain J, Fatima M, Saeed B, Abedulla Q. Phytochemical analysis and *In-Vitro* anti-Inflammatory activity of *Vitex negundo* against denaturtion of protein. J Pharm Adv Res, 2019; 2(4): 535-539.