The Pharma Innovation Journal 2021; 10(9): 01-04

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(9): 01-04

www.thepharmajournal.com
Received: 02-07-2021
Accepted: 09-08-2021

RP Limsay
Nagpur Veterinary College, Seminary Hills, Nagpur, Maharashtra, India

AP Somkuwar
Nagpur Veterinary College, Seminary Hills, Nagpur, Maharashtra, India

SW Bonde
Nagpur Veterinary College, Seminary Hills, Nagpur, Maharashtra, India

VK Kakde
Nagpur Veterinary College, Seminary Hills, Nagpur, Maharashtra, India

SS Shirankar
Nagpur Veterinary College, Seminary Hills, Nagpur, Maharashtra, India

VI Borekar
Nagpur Veterinary College, Seminary Hills, Nagpur, Maharashtra, India

RA Jagdale
Nagpur Veterinary College, Seminary Hills, Nagpur, Maharashtra, India

Corresponding Author:
RP Limsay
Nagpur Veterinary College, Seminary Hills, Nagpur, Maharashtra, India

In vitro anti-inflammatory activity of *Clerodendrum infortunatum* L. leaves

RP Limsay, AP Somkuwar, SW Bonde, VK Kakde, SS Shirankar, VI Borekar and RA Jagdale

Abstract
The present study was carried out to evaluate the *in vitro* anti-inflammatory activity of hydroethanolic extract of leaves of *Clerodendrum infortunatum* L. plant. The HRBC membrane stabilizing activity and inhibition of protein denaturation (anti-arthritic activity) activity was undertaken using freshly drawn human blood and bovine serum albumin, respectively.

Preliminary phytochemical analysis showed presence of Carbohydrates, Glycosides, Tannins, Phenolic compounds and Flavonoids in 70% hydroethanolic extract of leaves of *C. infortunatum*. The extract at 10, 20, 40, 80 and 100 µg/ml concentrations showed 46.67, 49.67, 48.33, 56.00 and 62.67 percent of membrane stabilization, respectively. At the same concentrations of extract the percent inhibition of protein denaturation was 25.71, 32.5, 46.07, 57.5 and 65.36, respectively.

Many biologically active phytoconstituents including flavonoids plays a major role in neutralizing the oxidative damage and reduces inflammatory responses. The presence of such pharmacologically active principles including flavonoids in *Clerodendrum infortunatum* plant may be responsible for demonstration of *in vitro* anti-inflammatory activity which supports the use of this plant in traditional system of medicine in inflammatory conditions.

Keywords: *Clerodendrum infortunatum, in vitro* anti-inflammatory activity, HRBC membrane stabilization, inhibition of protein denaturation

Introduction
Although inflammation helps to clear infection and other noxious stimuli and initiates repair, the inflammatory reaction and the subsequent repair process can themselves cause considerable harm. There are strong associations between chronic inflammation and cardiovascular diseases, diabetes, cancer, arthritis, dementia and many other chronic diseases of ageing (Vegad, 2007; Lacopino, 2008 and Kumar *et al.*, 2013) [20, 10, 9].

In inflammation lysosomal membranes releases enzymes into cytosol which causes damage to surrounding tissues and produces various disorders (Bag *et al.*, 2013) [2]. Inflammation is a complex process involving pain along with increased vascular permeability, increased protein denaturation and membrane alteration. Denaturation of protein is caused by inflammatory conditions like arthritis (Umapathy *et al.*, 2010) [19].

Protein denaturation assays and membrane stabilization assays are frequently used to evaluate *in vitro* anti- inflammatory activities. These *in vitro* studies are helpful in developing an understanding of the mechanism of anti-inflammatory activity of herbal constituents (Sarveswaran *et al.*, 2017) [17].

Among the natural products found in plants, flavonoids and their glycosides constitute one of the largest classes of natural compounds known. Flavonoids are very common and widespread secondary plant metabolites. They have a wide range of biological and physiological activities and serve as chemotaxonomic marker compounds (Keskes *et al.*, 2017) [8].

Clerodendrum infortunatum is a gregarious perennial shrub belongs to a very large and diverse *Clerodendrum* genus. Its medical applications are described in Ayurveda, Unani and even in Homeopathic system of medicine. The plant have been reported to be used by tribes in colic, scorpion sting, snake bite, tumour and certain skin diseases, also used in Indian folk medicine as in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy (Ghosh, 2012) [5].

Therefore, the present study was planned to evaluate membrane stabilizing and anti-arthritic activity of *Clerodendrum infortunatum* leaves extract with *in vitro* assays.

Materials and Methods

Collection, authentication and extraction of plant material

The plant material was collected from Amravati road area of Nagpur city. The sample of plant exhibiting typical characters was submitted to the Department of Botany, RTM Nagpur University, Nagpur for authentication and obtained voucher specimen number 10055.

The properly cleaned leaves free of any foreign material were shed dried in the department and were powdered. The powdered plant material was defatted with N-Hexane and then refluxed to obtain the desired 70% hydroethanolic extract (70% ethanol and 30% distilled water) (Fransworth, 1996) [3].

Qualitative phytochemical analysis

The prepared extract was subjected to preliminary qualitative phytochemical analysis to assess the presence of bioactive secondary metabolites (active principles). It was carried out using various standard sets of chemical tests widely used for preliminary phytochemical analysis (Rosenthaler, 1930 and Tiwari, 2011) [15, 18].

HRBC membrane stabilizing activity

The HRBC membrane stabilizing activity was carried out by using heparinized blood collected freshly from healthy human volunteer who has not used any NSAIDs for past 15 days. It was washed thrice with isotonic saline and later on centrifuged at 3000 rpm for 10 minutes. The packed blood cells were again washed with isotonic saline solution and a 10% v/v HRBC suspension was made by using isotonic phosphate buffer. The extract and standard drug dexamethasone were diluted and used in concentrations of 10, 20, 40, 80 and 100 µg/ ml for the experiment.

The positive control extract or dexmethasone at the volume of 0.5 ml in different concentrations of 10, 20, 40, 80 and 100 µg/ ml were mixed with 1 ml of PBS, 2 ml of 0.36% hyposaline solution and 0.5 ml of 10% HRBC suspension. Distilled water served as negative control. After an incubation period of 10 minutes it was centrifuged at 3000 rpm for 10 minutes and supernatant was observed for absorbance at 560 nm with UV spectrophotometer (Thermo Fisher) (Sadique *et al.*, 1989) [16]. Following formula was used to calculate the membrane stabilization –

% Membrane Stabilization = 100 – [(O. D. of Test/O. D. of Control) X 100]

Inhibition of protein denaturation (Anti-arthritic activity)

Bovine serum albumin as 5% aqueous solution was mixed with 0.5 ml of extract or standard drug dexamethasone at different concentrations of 10, 20, 40, 80 and 100 µg/ml. A product control was made by adding 0.45 ml of ethanol with 0.5 ml of test solution. The pH of all the solutions was adjusted to 6.3 by using 1% HCl. The samples were incubated for 20 minutes at 37^{O}C and further at 57^{O}C for 3 minutes. The absorbance was measured with UV spectrophotometer at 416 nm (Mizushima and Kobayashi, 1968 and Geetha *et al.*, 2014) [12, 4]. Following formula was used to calculate percent inhibition of protein denaturation-

Percentage inhibition = 100-[(O. D. of test solution – O. D. of product control)/(O. D. of test control) ×100]

Results and Discussion

Qualitative phytochemical analysis

The active principles like Carbohydrates, Glycosides, Tannins, Phenolic compounds and Flavonoids were observed in the 70% hydroethanolic extract of leaves of *C. infortunatum*. The details of tests carried out and its results are depicted in table 1. The active principles like alkaloids, steroids, flavonoids, reducing sugars (carbohydrates), saponins and gums were present in the extract of *C. infortunatum* (Ahmed *et al.*, 2007) [1].

Table 1: The details of tests carried out and its results are depicted

Sr. No.	Active principle investigated	Tests applied	Results
1.	Alkaloids	Hager's Test	Negative
		Mayer's Test	Negative
		Dragendroff's Test	Negative
2.	Carbohydrates	Fehling's Test	Positive
		Benedict's Test	Positive
3.	Glycosides	Borntrager's Test	Positive
		Legal's Test	Negative
		Keller-Killiani Test	Negative
4.	Saponins	Foam Test	Negative
		Test for Triterpenes	Negative
5.	Sterols	Salkowski Test	Negative
		Leibermann's Reaction	Negative
6.	Tannins and Phenolic compounds	Ferric chloride solution Test	Positive
		Lead Acetate Test	Positive
		Dil. HNO_3 Test	Positive
7.	Flavonoids	Shinoda Test	Positive
		Lead Acetate Test	Positive
		Sodium Hydroxide Test	Positive
8.	Proteins	Xanthoprotein Test	Negative
		Biuret Test	Negative
9.	Amino acids	Ninhydrin Test	Negative
10.	Diterpenes	Copper Acetate Test	Negative

HRBC membrane stabilizing activity

The hypotonic solution induced Human RBC membrane stabilizing activity was carried out using different concentrations of extract and standard dexamethasone. In this spectrophotometric study, the results obtained are presented in Table 2. The enzymes released by lysosomal membranes into cytosol in inflammation causes damage to surrounding tissues and produce various disorders. Human red blood cells (HRBC) resembles with lysosomal membranes and NSAIDs stabilizes the lysosomal membrane and or inactivates the lysosomal enzymes. Similarly these drugs also stabilize RBC and prevents release of haemoglobin when subjected to hypotonic stress. Therefore, HRBC membrane stabilization may prove a useful assay to assess anti-inflammatory activity *in vitro* (Bag *et al.*, 2013) [2]. The hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components (Hossain *et al.*, 2015) [7].

In present study the extract of *C. infortunatum* showed dose dependant HRBC membrane stabilization activity at various concentrations when compared with standard drug dexamethasone. The inhibition was concentration dependant and at 100 µg/ml concentration the inhibition of lysis was highest

Table 2: The results obtained are presented

Concentration (µg/ml)	Membrane stabilization (%)	
	Dexamethasone	Extract
10	56.33	46.67
20	54.67	49.67
40	67.00	48.33
80	69.67	56.00
100	75.33	62.67

Inhibition of protein denaturation (Anti-arthritic activity)

The hydroethanolic extract of leaves of *C. infortunatum* has shown 25.71, 32.5, 46.07, 57.5 and 65.36 percent inhibition of protein denaturation at 10, 20, 40, 80 and 100 µg/ml concentrations, respectively (Table 3).

Inflammation is a complex process involving pain along with increased vascular permeability, increased protein denaturation and membrane alteration. Denaturation of protein is caused by inflammatory conditions like arthritis (Umapathy *et al.*, 2010) [19]. In present study, the hydroethanolic extract of leaves of *C. infortunatum* has appreciably inhibited the protein denaturation at various concentrations. In earlier studies it was observed that the anti-arthritic activity of *C. infortunatum* was comparable with standard NSAID acetyl salicylic acid at 500 µg/ml concentration (Ripon *et al.*, 2016) [14].

The presence of biologically active phytochemicals in plants has been documented to be responsible for the pharmacological activities of medicinal plants (Henneh *et al.*, 2008) [6]. In a series of experiments, the phenyl propanoids: isoeugenol and eugenol present in the leaf oil of *Pimenta dioica* (L.) Merr. (Myrtaceae) demonstrated considerable anti-oxidant and is used for treating inflammation in Jamaica (Williams *et al.*, 2008) [21]. Flavonoids such as quercetin are known to be effective in reducing acute inflammation. Certain flavonoids possess potent inhibitory activity against a variety of enzymes such as proteinkinase C, protein tyrosine kinases, phospholipase A2, phosphodiesterases (Parvin et al., 2015) [13].

In our previous work, the leaves of the plant *C. infortunatum* demonstrated presence of flavonoids in preliminary pytochemical analysis and Thin Layer Chromatography. The extract was found to have 74.50 µg/ml of quercetin equivalent and Total Flavonoid Content (TFC) was estimated as 6.07% when determined by Aluminium chloride colorimetric method. It also showed antioxidant potential with DPPH scavenging action having IC_{50} value of 0.047±0.003 mg/ml (Limsay et al., 2018) [11].

Table 3: Show the Inhibition of protein denaturation

Concentration (µg/ml)	Inhibition of protein denaturation (%)	
	Dexamethasone	*C. infortunatum*
10	42.86	25.71
20	45.36	32.5
40	61.07	46.07
80	80.36	57.5
100	94.29	65.36

5. Conclusion

The results obtained in present *in vitro* study reveal that the leaves of the plant *Clerodendrum infortunatum* possess anti-inflammatory activity. In present and previous studies at department, the plant *C. infortunatum* was found to have many useful active phytoconstituents including flavonoids, which plays a major role in neutralizing the oxidative damage. The findings in the study support the use of this plant in traditional system of medicine in inflammatory conditions and therefore requires further systematic efforts for *In vivo* studies.

Acknowledgements

The authors express their sincere gratitude towards Associate Dean of Nagpur Veterinary College and Dr. S. P. Chaudhari, Professor and Head, Dept. of Veterinary Public Health, Nagpur Veterinary College, Nagpur for encouragement and necessary support for the work.

References

1. Ahmed F, Shahid IZ, Biswas UK, Roy BA, Das AK, Chaudhuri MSK. Anti-inflammatory, antinociceptive, and neuropharmacological activities of *Clerodendron viscosum*. Pharmaceutical Biology 2007;45(7):587-593.
2. Bag A, Bhattacharya SK, Pal NK, Chattopadhyay RR. Anti-inflammatory, anti-lipid peroxidative, antioxidant and membrane stabilizing activities of hydroalcoholic extract of *Terminalia chebula* fruits. Pharmaceutical Biology 2013;51(12):1515-1520.
3. Fransworth N. Biological and phytochemical screening of plants. Journal of Pharmaceutical Sciences 1996;55(3):225-273.
4. Geetha DH, Indhiramuthu Jayashree, Rajeswari M. *In vitro* anti-arthritic activity of *Elaeocarpus serratus* Linn. (Elaeocarpaceae). International Journal of Pharmaceutical Sciences and Research 2014;6(6):2649-51.
5. Ghosh D. Bhant- A weed having multifarious medicinal properties. Science and Culture 2012;78(3, 4):174-176.
6. Henneh Isaac Tabiri, Robertson Akrofi, Elvis Ofori Ameyaw, Daniels Konja, George Owusu, Bashiru Abane, et al. Stem bark extract of *Sterculia setigera* Delile exhibits anti-inflammatory properties through membrane stabilization, inhibition of protein denaturation and prostaglandin E_2 activity. Journal of Pharmaceutical Research International 2018;22(5):1-11.
7. Hossain Mohammed Munawar, Mohammad Shah Hafez Kabir, Abul Hasanat, Md. Imtiazul Kabir, Tanvir Ahmad Chowdhury, Abu Sayeed Md et al. The Pharma Innovation Journal 2015;4(1):76-80.
8. Keskes Henda, Sahla Belhadj, Lobna Jlail, Abdelfattah El Feki, Mohamed Damak, Sami Sayadi et al. LC-MS-MS and GC-MS analyses of biologically active extracts and fractions from Tunisian Juniperus phoenice leaves. Pharmaceutical Biology 2017;55(1):88-95.
9. Kumar V, Abbas AK, Aster JC. Robbins Basic Pathology 9th Edition, Elsevier India Pvt. Ltd., New Delhi 2013.
10. Lacopino AM. What is the role of inflammation in the relationship between periodontal disease and general health? Journal of California Dental Association 2008;74(8):695.
11. Limsay RP, Somkuwar AP, Kakde VK, Shirankar SS, Jagdale RA. The phytochemical study and antioxidant potential estimation of flavonoid rich fraction of *Clerodendrum infortunatum* leaves. Multilogic in science 2018;8(XXVI):268-271.
12. Mizushima Y, Kobayashi M. Interaction of anti – inflammatory drugs with serum proteins, especially with some biologically active proteins. Journal of Pharmacology 1968;20:169-173.
13. Parvin, Shahnaj, Nandita Das, Nusrat Jahan, Most. Afia Akhter et al. Evaluation of *in vitro* anti-inflammatory

and antibacterial potential of *Crescentia cujete* leaves and stem bark. BMC Research Notes 2015;8:412.
14. Ripon SS, Mahmood A, Chaudhaury MM, Islam MT. A possible anti-atherothrombosis activity via cytoprotective trait of the *Clerodenrum viscosum* leaf methanol extract. Insight in Biomedicine 2016;1(2):1-6.
15. Rosenthaler L. The chemical investigation of plants 1st Edition, Bell and Son's, London, 1930, 36.
16. Sadique J, Al-Rqobab NA, Bughaith MF, Et-Gindy AR. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterapia 1989;60:525-532.
17. Sarveswaran R, Jayasuriya WJABN, Suresh TS. *In vitro* assays to investigate the anti-inflammatory activity of herbal extracts: a review. World Journal of Pharmaceutical Research 2017;6(17):131-141.
18. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. International Pharamceutica Sciencia 2011;1(1):98-106.
19. Umapathy E, Ndebia EJ, Meeme A, Menziwa P, Nkeh-Chungag BN, Iputo JE. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. Journal of Medicinal Plants Research 2010;4(9):789-795.
20. Vegad JL. Textbook of Veterinary General Pathology 2nd Edn. International Book Distributing Co., New Delhi 2007.
21. Williams LAD, Connar AO', 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 Medical Journal 2008;57(4):327-331.