*Int.J.Adv.Microbiol.Health.Res.*2019; 3(1):21-28

International Journal of Advanced Microbiology and Health Research
ISSN: 2457-077X. Volume 3, Issue 1 (January-March 2019), PP. 21-28
Available online at www.ijamhr.com

Original Research Article

Antiinflammatory Activity of Stem Bark of *Rhamnus purpureus*

Charu Thakur[1*] and Bhairav Prasad[2]

[1&2]Vidya Jyoti Institution, Derabssi, SAS Nagar -140508, Punjab, India

Article Info

Received 1st March, 2019
Revised 6th March, 2019
Accepted 10th March, 2019
Published online 26th March, 2019

Keywords

- Rhamnus
- Antiinflammatory
- Carregenum

ABSTRACT

This study was facilitated to screen the anti-inflammatory effect of bark of the *Rhamnuspurpureus.Rhamnuspurpureus* frequently known as Gaunta . it is deciduous shrub originatedin the Himalayan range, Himachal Pradesh and Bhutan. In present study the stem bark of *Rhamnuspurpureus* was chopped and extracted in ethanol butenol, chloroform and evaluated for carregenum, cotton pallet induced granulloma in Wister Albino Rats. The results revealed that cruedethonolic extract showed maximum inhibition in caragenum induced rat paw edema followed by ethanol, butenol, and chloroform extract. The ethonolic extract of infective dose 200mg/kg body weight exhibit remarkable anti inflammatory activity with inhibition of 41.05%, 49.35%, 54.30%, and 54.77%, and butanolic extract showed significant results viz. 46.01%, 49.80%, 49.17%, 50.28%. and the standard drugs indomethacin also compared (10mg/ kg) showed inhibition of 50.69%, 51.63%, 50.46%, 51.78% after 1,2,3,and 4 hours of treatment respectively. The result indicates that the ethonolic extract as well as butonilic extract revealed strong inflammatory activity while chloroform showed less effective in comparision with indomethicin. The order of inhibition of carregenum induced rat paw edema was establish to be - indomethecine> ethanol >butonol>chloroform>. In conclusion to above study it has been seen that the *Rhamnuspurpureus* was found to have great anti-inflammatory response and can be substitute or alternative for synthetic or chemically derived drugs.

1. INTRODUCTION

The genus Rhamnus belongs to the family Rhamnaceae is a large genus of small trees and shrubs, commonly known as 'buckthorns', distributed chiefly in the temperate and warmer regions of the world. About ten species of Rhamnus occur in India [1].

RhamnuspurpueusEdgew locally known as 'Gaunta' is a deciduous shrub or small tree found in submontane to montane Himalaya, Himanchal Pradesh to Bhutan up to 1000-2400 m [2]. Its stem are dark purple-brown, 3-6m high; leaves aleternate, ovate-lanceolate, 7-14x2-2.4cm, serrulate, acuminate, membranous; flowers pale-white, ca 4mm across, 5-merous, in-axillary branches or simple racemes; peduncle 6-10cm long. Calyx cup-shaped; segement acute. Petals globose or obovate [2].

Rhamnus species have been reported to possess anti-inflammatory, anti-oxidant, anti-spamodic, cardio-stimulating, anti-ulcer and hypotensive activity [3-5].

Preliminary phytochemical screening of different parts (leaves, stem bark and roots) of R. purpureus showed that all these parts have similar chemical constituents but the higher concentration of the chemical constituents (monitored by TLC) was found in stem bark, therefore, pharmacological study of aqueous ethanol extract was carried out.

The aqueous ethanol extract showed significant anti-inflammatory and anti-oxidant activity, therefore, the ethanol extract was fractionated with CHCl3 and n-butanol and anti-inflammatory and anti-oxidant activity of aqueous ethanol, chloroform and butanol extract was carried out.

1.1. Anti-inflammatory Activity of R. purpureus

1.1.1. Inflammation

The inflammation is the body's response to tissue injury [8]. Inflammation is resistance of the organism and its tissue to injurious stimuli that lead to the local accumulation of plasmatic fluid and blood cells. Though it is a protectionsystem the complex events and mediators drawn in in the inflammatory reaction can be incorporated, maintained or motivated by many diseases such as vasomotor rhinnorrhoea, rheumatoid arthritis, and atherosclerosis. [9-11].

Inflammation is caused due direct damage (cuts, sprains), chemical such as acid, ischemia and cell necrosis, allergic reaction, physical agents such as injuries or burns. Inflammation usually involves a series of events which can be categorized under three phases viz. acute transient phase, delayed sub acute phase and chronic proliferate phase. In the first phase, inflammatory exudates develop due to improved vascular permeability and cause local edema. Afterward the migration of leukocytes and phagocytes from blood to vascular tissue which is the second phase, in the third phase; tissue degradation is followed by fibrosis. As a result endogenous mediators librates like histamine, serotonin, bradykinin, prostaglandins etc.is called inflammation . Prostaglandins are omnipresent substances that designate and transform cell and tissue responses involved in inflammation. These mediators even in small quantities can elicit pain response [12].

Inflammation can be classified based on duration of inflammation as acute and chronic inflammation. Acute inflammation begins within seconds to minutes following the injury of tissues. The damage may be purely physical, or it may involve the activation of an immune response [13].

The cardinal signs of acute inflammation are heat, redness, swelling, pain and loss of function. Acute inflammation is initiated by the cells. At the onset of an infection, burn or injuries, the cells undergo activation and release inflammatory mediators responsible for the clinical signs of inflammation. Vasodilation and its resulting increased blood flow cause the redness and increased heat. Increased permeability of the blood vessels results in an exudation of plasma proteins and fluids into the tissue (edema), which apparent itself as swelling. Some other released mediators for instance bradykinin enhance the feeling of pain. This mediator moleculesbradykinin also change the blood vessels to allow the immigration of leukocytes, chiefly neutrophils, exterior of the blood vessels into the tissue.

The acute inflammation reaction requires steady stimulation to be persistent Inflammatory mediators are quickly degraded in the tissue due to having short half lives. Chemical mediators of inflammation include histamine, prostaglandins, leukotriene B4, and nitric oxide. Histamine, the main mediator of inflammation, when tissue is injured released from mast cells, basophils, and platelets, causes transient dilation of arteriole and the smooth muscles contraction, increases permeability in venules and is the primary cause of increased vascular permeability in the first hour of injury. Prostaglandins released from mast cells are responsible for vasodilation, fever and pain. The leukocyte adhesion and activation mediated by Leukotriene B4, allowing them to bind to the endothelium and migrate across it. Nitric oxide which is nearby synthesized by endothelium and macrophages during the activity of the enzyme Iwhich is responsible for the skin inflammation [14].

Chronic inflammation has slow inception and keep on for weeks or more. The symptoms of the chronic inflammation are not as severe as acute inflammation, but the condition is dangerous and constant. An acute inflammationdevelop into chronic if the immune system is incapable to get rid the foreign agents from the body. In some cases of constant infections, such as tuberculosis, and autoimmune diseases, chronic fatigue will occur directly. The chief cells occupied in chronic infection are macrophages and lymphocytes [14].

In order to figure out the inflammatory development, antagonists of mediators are usually in use in both Ayurveda and Allopathy treatment. The majority of the anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperanalgesic, effective vasodilators and also contribute to swelling and pain. For the treatment of inflammatory diseases analgesic and anti-inflammatory agents are required. For curing diseases rather than chemically derived drugs herbal drugs are routinely usedhaving no or less

side effects. Now a daysInflammation has turn into the spotlight of global scientific research because of its implication in practically all human and animal diseases. Inflammatory diseases include different types of rheumatic disorders such as rheumatic fever, rheumatoid arthritis, ankylosing spondylitis, polyarthritis nodosa, systemic lupus erythematosus and osteoarthritis. Therefore, new anti-inflammatory and analgesic drugs lacking these side effects are being researched as alternatives to NSAID and opiates [15, 17].

Attention is being focused on the investigation of the worth of plant-based drugs used in the traditionalherbel medicine because they are cheap, have little side effects and as said by WHO, about 80% of the world population still rely mainly on herbal remedies [15, 17].

2. MATERIALS AND METHODS

2.1 Preparation of Extract

The stem bark of R. purpureusEdgew locally known as 'Gaunta' was collected from Kamad region district Uttarkashi, Uttrakhand, in the month April. The plant was identified by Dr. Sumer Chand, Systematic Botany Divison, FRI, Dehradun, Uttrakhand, India. A receipt specimen (No. 70) was deposited in the herbarium of Department of Botany, Govt P.G. College Uttarkashi, Uttrakhand. The stem bark are chopped in to small pieces and dried under shade, and powdered in a grinder. The powdered plant material (3.0 kg) was defatted by petroleum ether (60-80ºC) for 72 h. The defatted plant material was dried in room temperatures and extracted with Ethanol (95%) for 72 h. The Ethanol extract was resolute under reduced pressure at a low temperature (40-50°C) yields crude ethanol extract (71.3g). A suspension of ethanol extract (60.0g) was made with water, which was successively partitioned with chloroform and n-butanol. The chloroform and butanol layers were separated out and concentrated under reduced pressure afforded chloroform (21.5 g) and n-butanol extract (27.3g), respectively.

2.2Animals Used

The either sex ofAlbino Wistar rat weighing between 150-200 g were, purchased from CCS Haryana Agriculture University, Hisar, Haryana, India, used in current study and were The animals had free admittance to food and water and were maintained under restricted temperature (27±20C) and 12 h: 12 h light and dark cycle. Primary body weight of each animal was recorded. Institutional Animal Ethics Committee (IAEC), Department of Pharmacy, GGD University, Bilaspur, had permitted the experimental protocol and care of animals which has taken as per the guiding principle of CPCSEA, Department of Animal Welfare, Government of India.

For determination of acute-inflammation the most commonly used method is Carrageenan-induced paw edema in rats. This model is based upon the principle of liberation of various inflammatory intermediaries by carrageenan [20].

Carrageenan produce the edema in the rat paw is biphasic event. The primary phase is recognized to the release of histamine and serotonin. The release of prostaglandins, protease and lysosomes occurs in the secondary phase and carrageenan induced granuloma pouch model (Sub acute model) and carrageenan induced air pouch model (chronic model).

2.3 Acute toxicity studies

Acute toxicity study was performed as per the guidelines of OECD-423 .The experiment substance was injected in a single dose by an intubation canula. In each step three animals were used. The experiment substance was administered orally at a doses ranging from 5, 50, 300 and 2000 mg/kg body weight. Animals were observed individually after dosing once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, mortality were observed. All test animals were subjected to gross necropsy. No signs of toxicity were recorded. The doses selected were 50, 100 and 200 mg/kg [35].

2.3.1Carrageenan–Induced Rat Paw Edema Method

The animals were divided into eight groups of six animals each. The first group (control) was treated with 5% acacia i.e. vehicle only 10ml/kg and second group (standard) was treated with indomethacin (10mg/kg). The third, sixth and ninth group received ethanol, chloroform and n-butanol extract at 100mg/kg, body weight; fourth. Seventh

and tenth group received ethanol, chloroform and n-butanol extract 200mg/kg, respectively and fifth, eighth and eleventh group received ethanol, chloroform and n-butanol 400mg/kg, body weight respectively.

All the drugs were given orally half an hour before the administration of carrageenan suspension with the help of an oral catheter. One percent suspension of carrageenan in 5% acacia was prepared and 0.1 ml of it was injected in sub plantar region of right hind paw of each rat. The paw volume was measured immediately after injection (i.e. 0 hrs) and after 1, 2, 3 and 4 hrs with the help of Plethysmometer. The average paw swelling in rats treated with plant extracts was compared with control and standard group [20].

The total percent change in edema was considered by the formula:

% edema inhibition = [(Vc – Vt) / Vc] X 100
Where, Vt = Volume of treated group;
Vc = Volume of control group

2.3.2 Cotton Pellet-Induced Granuloma

The rats were divided into eight groups (n = 6) as discussed under acute model. The rats were anesthetized and an incision was made on the lumbar region by blunted forceps, a subcutaneous tunnel was made and a sterilized cotton pellet (100 ± 1 mg) was inserted in the groin area. From the day of insertion of cotton pellet all the animals received either vehicle (1% CMC) or indomethacin or plant extracts vocally depending upon their respective grouping for seven successive days [20]. On 8th day,in anesthesized rats the cotton pellets were removed and dried to consant mass.

2.4Chronic toxicity studies:

2.4.1Carrageenan induced air-pouch model

The rats were divided into different groups (n = 6) as discussed in acute model. Air-pouch was produced in accordance with the manner described by Salvemini et al. 1996 [36]. In brief rats were anesthetized and air cavities were formed by subcutaneous insertion of 20 ml of sterile air into the intrascapular area of the back (that is, 0 day). Ansupplementary 10 ml of air was inserted into the cavity of every 3rd day (3rd and 6th day) to maintain the space open. On the 7th day, 2 ml of 1% solution of salinedissolved incarrageenan was injected into the pouch to stimulate an inflammatory response. The rats were orally pre-treated with either vehicle or CEE/CFM/BFM or indomethacin 2 h earlier to the injection of carrageenan. The second dose of treatment was continual after 24 h of the first treatment. 48 h after carrageenan injection, the rats were anesthetized with ether and the pouch was carefully opened by a small slit. The volume of exudates has collected and measured. An aliquot of the exudate was used for differential cell count (neutrophils and monocytes) using a manual cell counter after staining with Wright's stain.

Statistical analysis

The results were articulated as Mean ± SEM. Statistical analysis was done by using one way ANOVA followed by Dunnett,s test.

3. RESULT

3.1 Effect on Carrageenan Induced Rat Paw Edema

The effect results crude ethanol, chloroform extract and btanol extract are shown in table 1. The results showed that the crude ethanolic extract showed maximum inhibition in carrageenan induced rat paw edema followed by butanol and chloroform extracts.

The crude ethanolic extract showed considerable ($P < 0.05$) anti-inflammatory activity with inhibition of 41.05%, 49.39%, 54.50% and 54.77% after 1hr, 2hr, 3hr and 4hr of treatment, respectively at the dose of 200mg/kg body weight whereas the n-butanol extract showed 46.01%, 49.80%, 49.17 and 50.28% inhibition after 1hr, 2hr, 3hr and 4hr of treatment at 200mg/kg dose, respectively. The standard drug indomethacin (10mg/kg) showed the inhibition of 50.69, 51.63%, 50.46%, and 51.78% after 1hr, 2hr, 3hr and 4hr of treatment, respectively.

The effects of crude ethanol extract at the dose of 100 and n-butanol extract at the dose of 200 mg/kg body weight at 4 hr after treatment were found comparable to that of indomethacin whereas the ethanol extract at the dose 200 mg/kg body weight at 3hr, and 4hr were found greater than that of standard drug indomethacin. Furthermore, it is evident from the table 3.1 that the anti-inflammatory activity of these extracts was found

dose and time dependent. These results indicate that the ethanol extract as well butanol extract showed strong anti-inflammatory activity where as chloroform extract showed weak activity. The order of inhibition of carrageenan induced rat paw edema has found to be indomethacin>ethanol>butanol>chloroform.

3.2 Effect on Cotton Pellet Granuloma in Rats

The effect of ethanol, chloroform and butanol extract on granuloma formation in cotton pellet method was presented in table 2. The ethanol extract showed maximum inhibition of 51.06 (at 200 mg/kg) and (54.62% (at 200 mg/kg) in granuloma formation followed by butanol extract with inhibition of 52.74% and 54.55% at 100 mg/kg and 200 mg/kg dose, whereas the chloroform extract showed inhibition of 54.08%. The standard indomethacin showed 54.55% inhibition in granuloma formation . The order of inhibition was found to be ethanol>indomethacin=n-butanol>chloroform extract.

3.3 Effect on exudate volume, neutrophil and monocyte count in carrageenan induced air pouch inflammation.

The effect of crude ethanol, chloroform and butanol extracts on exudates volume, neutrophil, and monocyte count in carrageenan induced air pouch in showed that these extracts elicited significant ($P<0.05$) dose dependent reduction in exudates volume and infiltration of netrophil and monocytes into the air pouch compared to control group (table 3). Indomethacin at a dose of 10 mg/kg body weight also showed significant ($P < 0.05$) result. The butanol extract showed significant effect on observed parameters than ethanol and chloroform extracts when compared with standard group. The activity was found to be in following order: Indomethacin>butanol>ethanol>chloroform extracts.

4. DISCUSSION

As we discussed Inflammation is component of the complex biological reaction of vascular tissues to harmful stimuli such as pathogens, foreign particle, damaged cells, or irritants. Even though it is bodies defense mechanism, the complex procedures and mediators implicated in the inflammatory reaction can be incorporated, maintained or aggravated by many diseases [9].

The inflammation is the process to repair the damage or at least to perimeter it and also to eliminate the cause. Causes of inflammation includes direct damage, chemical such as acids, ischemia and cell necrosis of infarction and infections [8].

An acute inflammatory modelCarragenan-induced rat paw edema is involved several mediators released in series [37, 38].

In primary phase during the first 1.5 hr is attributable to the liberation of inflammatory mediators histamine and serotonin. The second phase mediated by bradykinin from 1.5 to 2.5 hr and third phase, in which the mediator is likely to be prostaglandin, occurs from 2.5 to 5 hrs after carragenan injection [39].

The carragenan-induced paw edema in rats is known to be sensitive not to lipoxygenase inhibitors but sensitive to cyclooxygenase inhibitors . This experiment has been used to assess the effect of NSAIDs which principally reduce the cylclo oxygen as implicated in prostaglandins synthesis [39].

In the current study, the crude ethanolic and butanolic extracts at the doses of 100 and 200 mg/kg extensively decreased the rat paw edema induced by carragenan in all phases, suggesting that the probablemethod of action of these test extracts may involve in reduction of these inflammatory mediators liberate in all phases.

The cotton pellet-induced granuloma formation in rats is a model involved with chronic inflammation. The inflammatory responses had been divided into transudative, exudative and proliferative phases, these three phases are represents chronic inflammation. The fluid engrossed by the pellet greatly inclined the wet weight of the granuloma, and the dry weight associated well with quantity of granulomatous tissue formed [40-41].

In Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non steroidal anti-inflammatory drugs reduce the size of granuloma which results from cellular reaction by

inhibiting granulocyte infiltration, preventing production of collagen fibers and suppressing mucopolysaccharides [42-43].

In the currentlearning, the crude ethanolic, butanolic and chloroform extract at all experienced doses extensively reduced cotton pellet-induced granuloma development in rats, representing that the transudative, exudative and proliferative phases of inflammation were moderately inhibited.

To facilitate the efficacy of the plantextract against proliferative phase of inflammation, we preferred carrageenan induced air pouch model of rat in which tissue deprivation and fibrosis occurs. In the repair system of inflammation, there is production of macrophages, neutrophils, fibroblasts and growth of small blood vessels, which are the vital sources of forming aextremelyvascularised reddish mass, termed granulation of tissue [44-45].

In chronic model the crude ethanol, chloroform and n-butanol extracts extensively reduced infiltration of neutrophils and monocytes. These results designate that the experiment extracts may modify the action of endogenous factors that are involved in the relocation of these substances ar the location of inflammation.

There is risingfacts that lysosomal enzymes participate an important function in the progress of acute and chronic inflammation [46-49] process. It is thus noticeable that the ant-inflammatory effect produced by these extracts due to inhibition of the lysosomal enzymes, sattlethe membrane or by changing the action of endogenous factors that are implicated in the movement of these substances at the location of inflammation.

5. CONCLUSION

In the present study the crude ethanol and its n-butanol fraction showed strong anti-inflammatory activity than that of chloroform extract, which indicate that the active constituents that produced anti-inflammatory effect are present in higher concentration in the ethanol and n-butanol, extracts than that of chloroform extracts. The anti-inflammatory effect produced by ethanol, chloroform and n-butanol extracts of R. purpureus is noticeable that the ant-inflammatory effect produced by these extracts due to inhibition of the lysosomal enzymes, sattlethe membrane or by changing the action of endogenous factors that are implicated in the movement of these substances at the location of inflammation.

REFERENCES

1. Annons, ‘The Wealth of India’ (Raw materials), (1984),Vol-IX (Rh-So), pp 1, PID, CSIR, New Delhi.
2. Gaur R. D, Flora of District Garhwal, Trans Media, Srinagar Garhwal (1999).
3. Ram S.N, Dwived S.P.D, Pande V.B, and RaoY.V, Fitoterapia, 68:3, 273 (1989).
4. Goel R.K, Dwived S.P.D, Pandey V.B, and Rao Y.V, Indian Journal of Experimental Biology,26:2, 121 (1988).
5. Adzet T, Iglesios J, Rossel G, and Torrent M.T, Plant. Med. Phytother, 14:3, 164 (1980).
6. Chopra R. N, Nayar S. L, and Chopra I. C, (1986) ,Glossary of Indian Medicinal Plants (Including the Supplement) CSIR, New Delhi.
7. Tiwari J. K, Gairola A, Tiwari P, and Ballabha R, Asian J. Exp. Biol, 3:4, 778-784 (2012).
8. Gould B. E (2002), Inflammation and Healing In “Pathophysiology and Health Professions’ (2nd ed, Gould B E eds), Philadelphia, USA, W.B. Saunder, pp (192-199).
9. Gupta M, Mazumdar U.K, Kumar R.S, and Kumar T.S, Iran. J. Pharm. Therap, 2: 30-34 (2003).
10. Henson P.M, Murphy R.C. (1989), Mediators of the inflammatory process.(6th ed). Amsterdam, Elsevier.
11. Sosa S, Balicet M.J, Arvigo R, Esposito R.G, Pizza C, and Altinier G.A, J. Ethanopharmaco., 8, 211-215 (2002).
12. Anilkumar M, Ethnomedicine, A Source of Complementary Therapeutics, (2010) (Ed- D. P. Chattopadhyay), Research Signpost, Trivandrum, Kerala, India pp.(267-293).
13. Steven S.A, and Lowe J, (2000), Tissue responses to damage, In ‘Pathology’ (2nd ed, Steven S.A, and Lowe J eds), Edinburgt, Scotland, Butterworth Heine mann. Pp. 30-38.
14. Wu C.C, Curr. Med. Chem., 3, 217-222 (2004).
15. Dharmasiri J. R, Jayakody A. C, Galhena G, Liyanage S. S. P, and Ratnasooriya W D, J Ethnopharmacol ,87, 199-206 (2003).

16. Park J. H, Son K. H, Kim S. W, Chang H. W, Bae K, Kang S. S, and Kim H. P, Phytother Res,18, 930-933 (2004).
17. Kumara N. K. V. M. R, (2001), Identification of strategies to improve research on medicinal plants used in Sri Lanka. In: WHO Symposium. University of Ruhuna, Galle, Sri Lanka. pp(12-14).
18. Ogunyemi A.O, (1979), The origin of herbal cure and its spread; proceedings of a Conference on African Medicinal Plants. Sofowora A. (Ed.) University Press, Ile-Ife, 20-22.
19. Okunrobo L, Usifoh C, Ching P,andBariweni M, Journal of Pharmacology, 7:1, 27 (2009).
20. Winter C. A, RisleyE,andNuss G, Proceedings of the Society for Experimental Biology and Medicine, 111,544-547 (1962).
21. Vinegar R, Schreiber W, and Hugo R, Journal of Pharmacological Experimental Therapeutics, 66, 96-103 (1969).
22. Crunkhon P, and Meacock S, British Journal of Pharmacology, 42, 392-402 (1971).
23. Chatpaliwar V. A, Johrapurkar A. A, Wanjari M. M, Chakraborty R. R,andKharkar V. T, Indian Drugs, 39, 543- 545 (2002).
24. Whittle B. A, British Journal of Pharmacology and chemotherapy, 22, 246-253 (1964).
25. Miles A. A, and Miles E,Journal of Physiology, 118, 228-257 (1992).
26. Junping K, Yun N, Wang N, Liang L, and Zhi-Hong H, Biological and Pharmaceutical Bulletin, 28, 176-180 (2005).
27. Amann R, Schuligoi R, Lanz I, and DonnererJ, Europian Journal of Pharmacology, 279, 227-231 (1995).
28. Romay C, Ledon N, and Gonzalez R., Inflammation Research, 47, 334-338 (1998).
29. Selye H, The Journal of the American Medical Association, 152,1207-1213 (1953).
30. Fukuhara , and Tsurufuji S, Biochemical Pharmacology, 18,475-484 (1969).
31. Turner R., Screening Method in Pharmacology: Anti-Inflammatory agent, 158 (Academic Press New York, 1965).
32. Crunkhon P, and Meacock S,British Journal of Pharmacology, 42, 392-402 (1971).
33. Goldstein S. A, Shemano L, Daweo R, and Betler J,Archives Internationales De DeThérapie, 165, 294-301 (1976).
34. Vogel H, in Drug Discovery and Evaluation, 725-771 (Springer, 1996).
35. OECD: OECD guideline for testing of chemicals: Acute Oral Toxicity, Acute Toxic Class Method, Environmental Health and Safety Monograph Series on Testing and Assessment No 423 (2001).
36. Salvemini D, Wang Z. Q, Bourdon D. M, Stern M. K, and Currie M. G, Journal of Pharmacology,303, 217-224 (1996).
37. DiRosa M, Giroud J.P, and Willoughby D.A, J. Pathol., 104, 15+29 (1971).
38. Olajide O.A, Makinde J.M, and Awe S.O, J. Ethnopharm., 66, 113-117 (1999).-
39. DiRosa M, J. Pharm and Pharmacol, 24:2, 89-102 (1972).
40. Lowry O. H, Rosebrough N. J, Far A. L, and Randall R. J, The Journal of Biological Chemistry ,193, 265- 275 (1951).
41. Castro J, Saseme H, Sussman H, and Bullette P,Life Sciences ,7, 129-136 (1968).
42. DellaLoggiaA, Tubaro A, Dri P, Zilli C, and DelNegro P, Progress in clinical and biological research ,213, 481-486 (1968).
43. Alcaraz, M. J, and Jimenez M. J,Flavonoide, an anti-inflammatory agents, 59, 25-38 (1988).
44. Bhattacharya S, Pal S, and Nag Chaudhauri A. K, Phytotherapy Research,6, 255-301 (1992).
45. Swingle K. F, Anti-inflammatory agents, Vol. 2,33-47 (Academic Press, 1974).
46. Anderson AJ, Bocklehurst WE, and Wills A. L, Pharmacological Research Communications,3, 13-17 (1971).
47. Shen TY, Topics in medicinal chemistry. Vol. 1 29-38 (Wiley Interscience, 1967).
48. Weissmann G, Annual Review of Medicine18, 97-101 (1967).
49. Jannoff A, and Zweifach B. W,Journal of Experimental Medicine,120,747-752 (1964).
50. Nair R. B, Ravishankar B, Vijayan N. P, Sasikala C. K, and Saraswathy, V. N, Journal of Research in Ayurveda and Siddha9, 46-50 (1988).
51. Mantena R.K.R, Wijburg O.L.C, Vindurampulle C, Bennett-Wood V.R, Walduck, A, Drummond G.R, Davies J.K, Robins-Browne R.M, and Strugnell R.A, Cell. Microbiol. 10 :5, 1058–1073 (2008).

Table 1: Effect of Ethanol, butanol, and chloroform extracts of stem bark of *R. purpureus*on cotton pellet granuloma in rats.

Treatment	Dose	Weight of dry cotton pellet (mg)	% Inhibition
Control	Vehicle	85.95	--
Indomethacin	10mg/kg	39.47[a]	54.08
Crude Ethanolic Extract(CEE)	100mg/kg	65.27[ab]	24.06
	200mg/kg	54.77[ab]	36.28
	400mg/kg	39.00[a]	54.62
Chloroform Fraction(CFM)	100mg/kg	57.92[ab]	32.62
	200mg/kg	42.07[a]	51.06
	400mg/kg	39.07[a]	54.55
Butanol Fraction (BFM)	100mg/kg	47.38[a]	44.88
	200mg/kg	40.62[a]	52.74
	400mg/kg	39.07[a]	54.55

Values are given as mean ±SEM (n=6)[a]P<0.05 as compared to control; [b]P<0.05 as compared to Indomethacin treated group.

Table 2: Effect of Ethanol, butanol, and chloroform extracts extracts of stem bark of *R. purpureus*on excudate volume, neutrophil and monocyte count in carrageenan induced air-pouch inflammation.

Treatment	Dose	Exudate volume	Neutrophils (X 10cells)	Monocytes (X 10cells)
Control	Vehicle	3.17±0.021	213.33±6.67	88.67±4.25
Indomethacin	10mg/kg	0.65±0.034[a]	68.00±2.38[a]	38.50±2.81[a]
Crude Ethanolic Extract(CEE)	50mg/kg	2.95±0.148[b]	185.83±3.75[a]	70.33±3.86[ab]
	100mg/kg	2.62±0.040[a]	166.17±6.26[ab]	61.00±3.82[ab]
	200mg/kg	1.17±0.056[ab]	113.17±4.30[ab]	56.33±2.46[ab]
Chloroform Fraction(CFM)	50mg/kg	2.57±0.033[ab]	137.67±2.51[ab]	59.17±2.48[ab]
	100mg/kg	1.58±0.048[ab]	130.17±1.28[ab]	55.67±2.06[ab]
	200mg/kg	1.48±0.060[ab]	122.83±1.17[ab]	51.00±1.59[ab]
Butanol Fraction (BFM)	50mg/kg	1.5±0.045[ab]	114.17±1.40[ab]	55.00±2.49[ab]
	100mg/kg	1.1±0.037[ab]	98.33±1.82[ab]	48.17±2.34[a]
	200mg/kg	0.95±0.022[ab]	95.50±1.38[ab]	45.00±3.51[a]

Values are given as mean ±SEM (n=6) [a]P<0.05 as compared to control; [b]P<0.05 as compared to Indomethacin treated group.

Corresponding Author: Dr. Charu Thakur
Assistant Professor, Vidya Jyoti Institution, Derabassi, SAS Nagar -140508, Punjab, India.
E-mail: mbdcharuthakur@gmail.com

How to cite this article:
Thakur C and Prasad B. Antiinflammatory Activity of Stem Bark of *Rhamnus purpureus*. Int. J. Adv. Microbiol.Health.Res., 2019; 3(1):21-28.

Source of Financial Support: Nil, **Conflict of interest:** Nil.