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Research Article

Anti-Inflammatory Activities of Root Extracts of *Harungana madagascariensis* on Wistar Rat Model

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Abstract

The anti-inflammatory activities of *Harungana madagascariensis* was studied through the use of carrageenan-induced paw Edema. This has been a well-known model that has been used in accessing the anti-inflammatory action of lots of natural and synthetic compounds. This process induced an acute swelling that became maximal 3 - 5 hours after the injection and subsides by 24 hours. The ethanol extract of *H. madagascariensis* was given orally at doses of 200 mg/kg of H.M, 250 mg/kg of H.M. 100 mg of Aspirin was also used as a positive control. Extract was given 1hr before the induction of inflammation on the animal model (Wistar rat). The extract proved its anti-inflammatory potency by showing marked decrease in C-reactive protein in the ones treated when compared to the untreated. The differences between the treated and negative controls were statistically significant ($p < 0.05$) in all the parameters assessed (ESR, CRP and WBCC-total/differential) The result has given credence that *H. madagascariensis* extract has contents that blocked inflammatory mediators from interactions with its receptors thereby inhibiting/blocking inflammation. Hence, it could be said that H.M root extracts may possess anti-inflammatory properties.

Keywords: *Harungana madagascariensis; Anti-Inflammatory; Carrageenan; Erythrocyte Sedimentation Rate (ESR); C-Reactive Protein; White Blood Cell Count; Differential Count*

Introduction

In this study, we assessed the anti-inflammatory activities of root extracts of *H. madagascariensis* after establishing the toxicity levels. In the 19th century, inflammation was seen as an undesirable response that is considered harmful to its host. Metchnikoff postulated that it was an integral part of the healing process; however, inflammation has long been considered as a cornerstone of many pathological conditions [14]. In the world today, about 75% of useful bioactive plant that are produced pharmaceutically by scientific research is made possible via leads from traditional medicines [30]. Some scientific studies carried out in the years passed on plants have given leads that most of these plants are highly medicinal thereby giving credence to the folkloric claims that the plant H.M could be used in the management of inflammatory disorders. The possible mechanisms of most of the anti-inflammatory substances are a blockade of the pro-inflammatory

key enzymes (inhibiting further release of these key enzymes thereby blocking the interaction between mediators and their receptors. C-reactive protein is produced in the liver and adipose tissue, these in response to inflammatory stress and regulated by interleukin-6 [15].

Lots of steroids, especially glucocorticoids diminish inflammation or swelling by binding to glucocorticoid receptors. These drugs are often referred to as corticosteroids. Ibuprofen, aspirin and naproxen are the most popular examples of NSAIDs. Nonselective COX-inhibitors exact same action but maybe not by same mode of actions, hence not classified together with traditional NSAIDs. The counter-acting actions of cyclooxygenase (cox) enzyme inhibitors are to ameliorate pain. Prostaglandin synthesized by Cox enzymes creates inflammation. NSAIDs prevent the prostaglandin from being synthesized, thereby reducing or stopping the pain [12].

In Alzheimer's diseases, cardiovascular diseases, colonic adenomas and cancer, biosynthesis of prostaglandin has been proven in those pathologies [11,16] in all above, irritation will be both acute and chronic, the following obtain, an elevated vascular permeability, influx of leucocytes, and tissues repair [6].

Resolution of irritation is a well-controlled technique. The lack of ability to modify this system will result in out of control and continued irritation [13]. A cascade of events brings about counter-regulatory mechanisms that would transform macrophages from a greater classical cells to a greater activated opportunity cells thereby starting up healing [27].

Nwobodo and Ezeigbo [21] had worked on the abortifacient effect of the leaves, stem and bark of H.M, the result attempted to prove that it could be used to induce abortion mainly in rats. The analgesic and anti-inflammatory properties of H.m was also researched by Nwodo [20] using the stem and bark of H.M. The result also attempted to prove that it could be used in cases of inflammation and pain. Moss [19] stated that safety should be considered before administration of herbal products, these necessitated the need to run an LD_{50} before commencement of this research. Most traditional medicine dealers use the plant *H. M.* in the treatment of most disease conditions such as anaemia, asthma, tuberculosis, fever, angina, diarrhoea, dysentery, syphilis, gonorrhoea, malaria, parasitic skin diseases, and in management of wounds [7,8,31].

Aim of the Study

The aim of this work was to ascertain the anti-inflammatory activities of root extract of *Harungana madagascariensis* on Wistar rat.

Materials and Methods

Plant materials/Prep/Extraction: The plant was sourced from Nenwe in Aninri local government area of Enugu state Nigeria, and brought for authenticated in Nnamdi Azikiwe University herbarium, voucher specimens No:NAUH 195A. The root of *H. madagascariensis* was chopped off and sun dried for 14 days and was coarsely powdered by the use of a pulverizer. The extraction was by means of maceration in 99% ethanol for forty-eight hours. The phytochemical analysis of the extract confirmed that it consists of alkaloids, tannins, saponins, flavonoids and glycosides [9].

Ethical approval

Ethical approval was obtained from the Faculty of Basic Medical Science, College of Health Science, Nnamdi Azikiwe University, Nnewi campus with ref: NAU/CHS/NC/FMBS/413. Rats handling and treatments conformed to guidelines of the Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC) for laboratory animal care and use.

Animals: 30 adult male Wistar rats weighing 170 ± 20g were used. The rats were housed in animal house of Nnamdi Azikiwe University, Nnewi campus, in plastic cages with stainless roofs in well ventilated room under standard condition (Room temperature, 12h light and

12h darkness cycles) with cleaning of the cages and changing of bedding done daily. The rats were fed, with free access to water and acclimatized for one week.

Acute toxicity test: The median lethal dose (LD_{50}) is the dose that killed 50% of the animals. The LD_{50} was calculated as the geometrical means of the maximum dose producing (a) 100% and minimum dose producing (b) 0% mortality = The animals were observed for possible manifestation of physical signs of toxicity such as decreased motor activity, decreased body/limb activity, decreased respiration and finally death. Record of a death was observed, once a death occurred, dosage was adjusted downwards. Testing was terminated when the upper limit (2000 - 5000 mg/kg) had been reached without mortality. The LD_{50} was calculated as below 2000 mg/kg [3].

The anti-inflammatory test

Carrageenan solution

500 ml of sterile 0.9% saline was poured into a 1-liter beaker and stirred with a bar. Slowly 2.5g (0.5%) - 10.0g (2.0%) carrageenan powder (Type IV Lambda, Sigma) was added to the beaker. The solution was heated to 90°C with stirring, but was not allowed to boil. The essence of the heating was to allow the carrageenan to dissolve; it took less than an hour to dissolve all the powder. The solution was poured into a clean 1-liter glass bottle equipped with a cap and sterilize by autoclaving. The solution was cooled at room temperature and aliquoted into sterile, 100-ml capped bottles. This solution was stable for at least a week because it was stored at 4°C after preparation.

The anti-inflammatory activities of *Harungana madagascariensis* was studied through the use of carrageenan-induced paw Edema. This was a widely used model that accesses the anti-inflammatory action of lots of natural and synthetic compounds [2,23]. This model was highly distinct with respect to acute inflammation and showed a high reproducibility than the use of Agar powder [17,28]. In the second phase, carrageenan induced inflammation around 3hrs after carrageenan injection, Prostaglandins were the key players that made the second phase to occur [24]). Paw edema was induced by administering 0.1 ml of 1% carrageenan's in 0.9% Nacl (saline) into the plantar surface of the right hind paw of the animal [12]. This induced an acute swelling that became maximal 3 - 5 hours after the injection and subsides by 24 hours [22,33]. The subcutaneous injection of carrageenan produced local inflammation, designated by the 5 cardinal signs: hypersensitivity, redness, swelling, heat, and loss of function [29,33]. The animals were kept in five groups of 6 animals per group 1. (Negative control (Carrageenan induced paw edema without treatment), group 2. Carrageenan induced paw edema treated with 200 mg/kg of H.M, group 3; Carrageenan induced paw edema treated with 250 mg/kg of H.M, group 4, positive control (Carrageenan induced paw edema treated with 100 mg/kg of Aspirin) and group 5; water/feed only) The animals were induced with inflammation after 1hr of administration of H.M extract and 100 mg/kg of Aspirin and using a digital Vernier Caliper the paw edema was calculated at 0.5h, 1hr, 2hrs, 3hrs, 4hrs, 5hrs and 6 hrs. After the 6^{th} hour the blood samples of animal models were collected to analyse for C-reactive protein, Erythrocyte sedimentation rate and white blood cell count, total and differential.

Differential WBC counts

Whole blood specimens were received in EDTA vacutainer tubes. The differential WBC counts were assessed via the Technicon H-1 automated haematology analyzer (Technicon Instruments Corp, Terrytown, NY, USA) [31,32].

Histological assessment

The liver samples were excised and washed with normal saline. Then, the livers were fixed immediately in 10% formalin solution. The tissue biopsies were processed with automated tissue processor and embedded in paraffin. Sections 5 µm thickness were cut with rotary microtome (Leica RM 212 RT). Cut sections were floated on a water bath of 45°C and were placed on a hot plate of 65°C to allow the sections stick firmly on the slide. The tissue sections were then tainted using haematoxylin and eosin (HE) for morphology assessment [26]. Histological sections were examined using Leica Light microscope (Leica DM 750) and were photographed.

Analysis of data

Data were expressed as mean ± S.E.M. A One-way ANOVA was used for the comparison between the test groups and the control. Analysed data was presented using tables. A P scale less than 0.05 was considered statistically significant.

Results

Group	Paw Edema Mean ± SEM	P-Value	Body Weight Mean ± SEM	P-Value	ESR Mean ± SEM	P-Value	CRP Mean ± SEM	P-Value
N C	5.43 ± 0.02		174 ± 4.0		5.0 ± 0.32		5.46 ± 0.47	
200 mg/kg of H.M	3.02 ± 0.09	0.00	164 ± 4.0	0.49	3.2 ± 0.20	0.002	3.34 ± 0.18	0.00
250 mg/kg of H.M	3.09 ± 0.04	0.00	172 ± 4.9	0.10	3.4 ± 0.24	0.006	3.34 ± 0.20	0.00
PC	3.02 ± 0.08	0.00	174 ± 4.0	1.0	3.2 ± 0.37	0.002	2.5 ± 0.08	0.00

Table 1: *Study on the effect of H. madagascariensis extract on paw edema, body weight, c-reactive and erythrocyte sedimentation rate when compared with negative control.*

Result showed that Paw edema, ESR and CRP in the group that received 200 mg/kg, 250 mg/kg of H.M and Positive control when compared to the Negative control were statistically significant (p < 0.05). NC: Negative control; PC: Positive control; n: 6.

Group	Paw Edema Mean ± SEM	P-Value	Body Weight Mean ± SEM	P-Value	ESR Mean ± SEM	P-Value	CRP Mean ± SEM	P-Value
PC	3.02 ± 0.08		174 ± 4.0		3.2 ± 0.37		2.5 ± 0.08	
200 mg/kg of H.M	3.02 ± 0.09	1.00	164 ± 4.0	1.00	3.2 ± 0.20	0.4	3.34 ± 0.18	0.174
250 mg/kg of H.M	3.09 ± 0.04	0.934	172 ± 4.9	0.486	3.4 ± 0.24	0.4	3.34 ± 0.20	0.174
NC	5.43 ± 0.02	0.00	174 ± 4.0	0.997	5.0 ± 0.32	0.4	5.46 ± 0.47	0.00

Table 2: *Study on the effect of H. madagascariensis extract on Paw edema, body weight, C-reactive protein and Erythrocyte sedimentation rate when compared with positive control.*

Result showed that Paw edema, ESR and CRP in the group that received 200 mg/kg, 250 mg/kg of H.M when compared to the Positive control were not statistically significant (p < 0.05). NC: Negative Control; PC: Positive control; n: 6.

Group	Neutrophils Mean ± SEM	P-Value	Lymphocyte Mean ± SEM	P-Value	Monocyte Mean ± SEM	P-Value
NC	23.4 ± 0.82		75.8 ± 1.50		0.2 ± 0.2	
PC	37.8 ± 0.58	0.00	64.0 ± 0.45	0.59	2.2 ± 0.58	1.12
200 mg/kg of H.M	32.2 ± 1.02	0.03	72.2 ± 1.62	0.99	2.6 ± 0.81	0.04
250 mg/kg of H.M	50.8 ± 2.89	0.00	53.0 ± 4.09	0.07	3.0 ± 0.71	0.02

Table 3: *Study on the effect of H. madagascariensis extract on neutrophils, lymphocytes and monocytes when compared with negative control.*

Result showed that Neutrophils and Monocytes in the group that received 200 mg/kg, 250 mg/kg of H.M and Positive control when compared to the Negative control were statistically significant (p < 0.05). Nb. The Lymphocytes was not statistically significant. NC: Negative control; PC: Positive control; n: 6.

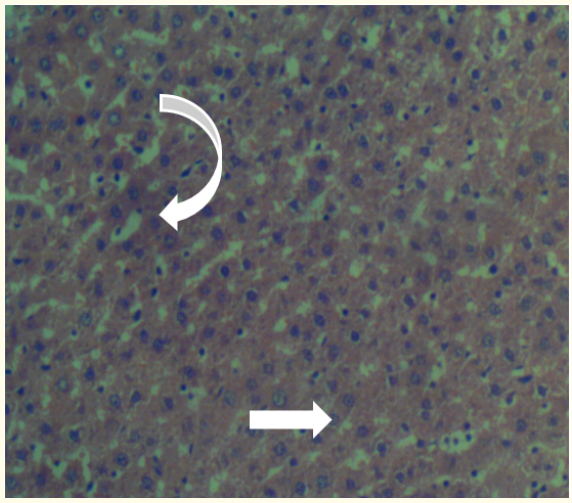

Figure 1: *(Control): Photomicrograph of liver tissue shows morphology consistent with liver histology. The sinusoids (curved arrow) and hepatocytes (arrow) are normal with no obvious sign of injury (H&Ex400).*

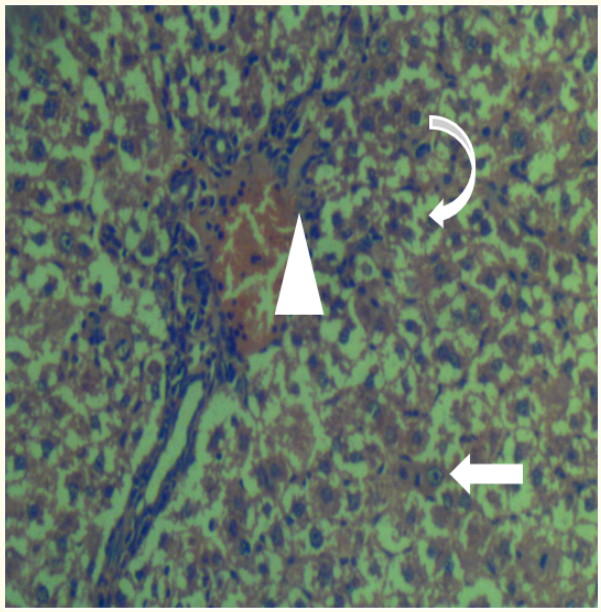

Figure 2: *Received 250 mg/kg of H.M: Photomicrograph of liver tissue shows moderately distorted morphology not consistent with liver histology. The portal triad shows moderate congestion with moderate infiltration of inflammatory cells (arrowhead), hepatocytes show moderate degeneration with mild clumping of chromatin (arrow), while the interstitial tissue shows remarkable degeneration with mild vacuolation (curved arrow) (H&E x400).*

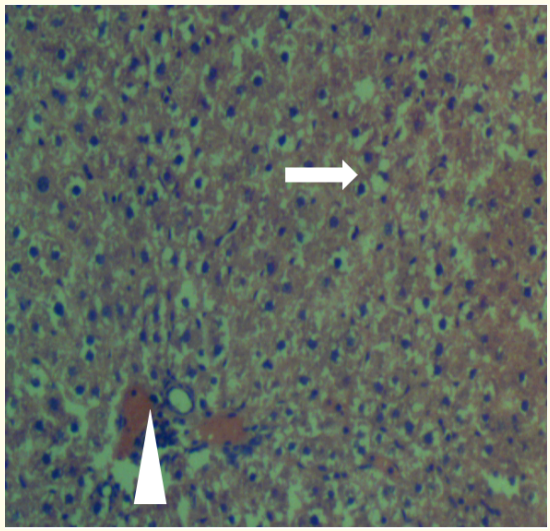

Figure 3: *Received 200 mg/kg of H.M: Photomicrograph of liver tissue shows moderately distorted morphology not consistent with liver histology. There is unremarkable moderate congestion with mild infiltration of inflammatory cells (arrowhead) while hepatocytes show moderate degeneration with mild clumping of chromatin (arrow (H&E x400).*

Discussion

Carrageenan-induced paw edema was a biphasic response. The first phase was mediated by the release of histamine, serotonin and kinins and the second phase was due to the release of prostaglandin-like substances within 2 - 3 hours [32]. Drugs that inhibited carrageenan-induced paw edema could work by inhibiting leukocyte migration and prostaglandin synthesis [5]. C-reactive protein (CRP) was one of the common test parameters used in clinical practice to assess, diagnose, and predict inflammation. However, the role played by CRP in physiological processes has not clearly been understood apart from being a great marker of inflammation [25]. CRP has a half-life of about 19hours which are maintained constant at all health and disease conditions [14].

Oral administration of ethanol extracts of roots of *H. madagascariensis* did show that there was a significant difference in Paw Edema, Erythrocyte sedimentation rate and C-reactive protein in the animal models that received 200 mg/kg of H.M, 250 mg/kg of H.M and 100 mg/kg of Aspirin when compared to Negative control (inflammation without treatment). Where p-value < 0.05 was considered to be statistically significant. These showed that the extract does possess products or anti-inflammatory properties. The mechanism possibly was an increase in monocytes and neutrophils which brought about a cascade of events that led to the release of C-reactive protein from the liver and adipose tissue. The extract possibly acted by stopping the interaction of this inflammatory mediator with its receptor thereby reducing the inflammation proper. The result showed that in all the animal models treated with the extract, the paw edema was drastically decreased when compared to the ones not treated, an indication that the extract could have acted by blocking the release of proinflammatory mediators thereby stopping its interaction with its receptor. Moreover, when comparing the positive control to the extract, it was not statistically significant. One could say that the extract was as potent as positive control (100 mg/kg of Aspirin).

Inflammation did bring about a cascade of events that led to mobilisation of white blood cells to the wound site (Neutrophils and Monocytes) to respond to the damaged tissue in order to help fight off any unwanted pathogens thereby initiating healing process. Look-

ing at table 3 which was the effect of *H. madagascariensis* on Neutrophils, Lymphocytes and monocytes when compared with the negative (group that was induced inflammation without treatment), the result showed that all the other groups were statistically significant when compared to the negative. During inflammation, neutrophils and monocytes are mobilized to the inflamed site leading to cascade of events that brings about the release of C-reactive proteins from the liver and adipose tissue. C-reactive protein increase was in response to inflammatory stress and this is regulated by interleukin-6. This study showed that there was a decrease in the number of C-reactive protein in the groups treated with the extract when compared to the untreated further collaborating [35] which said that when the inflammation or tissue destruction is resolved, CRP levels fall, making it a useful marker for monitoring disease activity.

The Neutrophils and Monocytes however increased in response to inflammation. This increase in neutrophils and monocytes however did not increase the CRP in the group treated; this could better be explained as the role played by the extract in inhibiting the interaction of inflammatory mediators with its receptors. A nonspecific response to inflammation, Erythrocyte sedimentation rate, was also considered in this research to further give credence to this property or better still anti-inflammatory activities of H.M. The result as explained in table 1 showed that when the extract was compared to a negative control it was statistically significant. Table 2 which is the comparison of the extract to a positive control showed that it was not statistically significant when a nonspecific parameter for inflammation (ESR) was checked. The liver histology of the untreated and the treated did show that whereas the control had a liver whose morphology was not distorted, the treated showed a mild infiltration of inflammatory cells. The inflammatory cells released from the liver as shown by the liver histology was in response to agar powder which was used to induce inflammation. However, the inflammatory cells release was mild.

Conclusion

The result of root extract of *H. madagascariensis* has shown to be effective in management of inflammation thereby making it a potential anti-inflammatory drug yet to be synthesise. Hence the interest to extract the active ingredients for better pharmacological uses in the future.

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Bibliography

1. Black S., *et al*. "C-reactive Protein". *Journal of Biological Chemistry* 279.47 (2004): 48487-48490.
2. Boominathan R., *et al*. "Anti-inflammatory evaluation of Ionidium suruticosam Ging in rats". *Journal of Ethnopharmacology* 91 (2004): 367-370.
3. Bruce RD. "An up-and down procedure for Acute Toxicity testing". *Toxicological Science* 5 (1985): 151-157.
4. Caner Gunaydin and S-Sirri Bilge. "Effects of Nonsteroidal Anti-Inflammatory Drugs at the molecular level". *The Eurasian Journal of Medicine* 50.2 (2018): 116-121.
5. Di Rosa M and Willoughby DA. "Screens for anti-inflammatory drugs". *Journal of Pharmacy and Pharmacology* 23 (1971): 297-298.
6. Eddouks M., *et al*. "Animal Models as Tools to Investigate Antidiabetisc and Anti-Inflammatory Plants". *Evidence-Based Complementary and Alternative Medicine* (2012): 142087.

7. Emea Pubic statement on thiomersal containing medicinal products. The European Agency for the Evaluation of medicinal products". *Human Medicine Evaluation Unit* (1999).

8. Erah PO., *et al*. "Prescribing practise in two health care facilities in Warri, Southern Nigeria. A comparative study". *Tropical Journal of Pharmaceutical Research* 2.1 (2003): 175-182.

9. Fabrice Kengni., *et al*. "*In vitro* antimicrobial activity of *Harungana madagascariensis* and Euphorbia prostrata extracts against some pathogenic salmonella sp". *International Journal of Biological and Chemical Sciences* 7.3 (2013): 1106-1118.

10. Gewurz H., *et al*. "Structure and functions of the penetraxins". *Current Opinion in Immunology* 7 (1995): 54-64.

11. Gupta M., *et al*. "Antiinflammatory evaluation of leaves of Plumeria acuminate". *BMC Complementary Medicine and Therapies* 6 (2006): 36.

12. Gyani Devi Gupta and Ram S Gaud. "Anti-inflammatory activity of Tenoxican gel on carrageenan induced paw oedema in rats". *Indian Journal of Pharmaceutical Sciences* 68.3 (2004).

13. Headland SE and Norling LV. "The resolution of inflammation: Principles and challenges". *Seminars in Immunology* 27 (2015): 149-160.

14. Hurley JV. "Acute Inflammation". *British Journal of Surgery* 60 (1972): 588588.

15. Hurlimann J., *et al*. "The liver as the cite of C-reactive protein formation". *Journal of Experimental Medicine* 123.2 (1966): 365-378.

16. Lipsky PE. "The clinical potential of cyclooxygenase-2–specific inhibitors". *The American Journal of Medicine* 106 (1999): 51S-57S.

17. Mahajan A., *et al*. "Effect of concurrent use of cimetidine and anti-inflammatory agents in experimental models of peptic ulcer and inflammation". *Indian Journal of Pharmacology* 16 (1984): 132.

18. Molgaard P., *et al*. "Perceptions about Malaria transmission and control using anti-malaria plants in Mola, Kariba ,Zimbabwe". *Nigerian Journal of Natural Products and Medicine* 5.1 (2001).

19. Moss. "Recent studies of Mycotoxins". *Journal of Applied Microbiology* 84.1 (1998): 62s76s.

20. Nwobodo Ed and Ezeigbo JC. "Abortifacient effects of aqueous extracts of roots of *Harungana madagascariensis* Orient". *Journal of Medicine* 22.4 (1992): 261-265.

21. Nwodo OFC. "Antibiotic and anti-inflammatory analgesic activities of *Harungana madagascariensis* stem bark". *International Journal of Crude Drug Research* 27 (1989): 137-140.

22. Otterness IG and Moore PF. "Carrageenan foot edema test". *Methods in Enzymology* 162 (1988): 320-327.

23. Panthong A., *et al*. "Anti-inflammatory, analgesic and antipyretic activities of the extract of gamboge from Garcinia hanburyi Hook f". *Journal of Ethnopharmacology* 111 (2007): 335-340.

24. Patil KR and Patil CR. "Anti-inflammatory activity of bartogenic acid containing fraction of fruits of Barringtoniaracemosa Roxb. in acute and chronic animal models of inflammation". *Journal of Traditional and Complementary Medicine* 7 (2017): 86-93.

25. Pepys MG and Hirschfield GM. "C-reactive protein: a critical update". *Journal of Clinical Investigation* 111.12 (2003).

26. Preece Ann. A manual for Histologic technicians (1972).

27. Reville K., *et al*. "Lipoxin A4 redistributes myosin IIA, Cdc42 in macrophages: implications for phagocytosis of apoptotic leukocytes". *Journal of Immunology* 176.3 (2006): 1878-1888.

28. Sarkhel S. "Evaluation of the anti-inflammatory activities of Quillaja saponaria.Mol. saponin extract in mice". *Toxicology Reports* 3 (2016): 1-3.

29. Stein C., *et al*. "Unilateral inflammation of the hindpaw in rats as a model of prolonged noxious stimulation :alterations in behaviour and nociceptive thresholds". *Pharmacology Biochemistry and Behavior* 31 (1988): 445-451.

30. Tomoko N., *et al*. "Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant staphylococcus aureus". *Journal of Health Science* 48 (2002): 273-276.

31. Tona L., *et al*. "Antiamoebic and Phytochemical screening of some Congolelese medicinal plants". *Journal of Ethnopharmacology* 61 (1998): 57-65.

32. Vinegar R., *et al*. "Biphasic development of carrageenin oedema in rats". *Journal of Pharmacology and Experimental Therapeutics* 166 (1969): 96-103.

33. Winter CA., *et al*. "Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Antiinflammatory Drugs". *Experimental Biology and Medicine* 111 (1962): 544-547.

34. Ying SC., *et al*. "Reactivity of anti-human C-reactive protein (CRP)and serum amyloid P component (SAP) monoclonal antibodies with limulin and pentraxins of other species". *Immunology* 76.2 (1992): 324-330.

35. Young B., *et al*. "C-reactive protein: a critical review". *Pathology* 23 (1991): 118-124.

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