

THE PHARMA INNOVATION - JOURNAL

Anti-inflammatory activity of methanol extract of *Hibiscus sabdariffa* in comparison with Aspirin

Agbai Emmanuel Onuka ^{1*}, MounmbegnaPouonkoussou Emmanuel ², Professor Egbe Philip Egbengu ³

1. Lecturer in Physiology, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Madonna University PMB 48 Elele, Rivers State, Nigeria.
[Email: emmanuelagbai207@yahoo.com]
2. Senior Lecturer in Biochemistry, Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, Madonna University Elele, Rivers State, Nigeria.
3. Professor in Physiology, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Madonna University, PMB 48 Elele, Rivers State, Nigeria.

Corresponding Author: Agbai Emmanuel Onuka, Email: emmanuelagbai207@yahoo.com

The anti-inflammatory activity of methanol extract of *hibiscus sabdariffa* in comparison with most widely used non-steroidal anti-inflammatory drug was studied in 25 adult albino rats. Acute inflammation was induced using 0.1 ml of egg albumin and was treated with 100, 200 and 400 mg/kg of the extract and aspirin (200 mg/kg). The sizes of the right hind paw of the twenty five rats were measured by threading a measuring tape around the foot for 2 hours. Results showed that 400 mg/kg of *hibiscus extract* significantly reduced ($P<0.05$) the paw size edema at 60 minutes (24.8 ± 0.17) compared to aspirin (27.4 ± 0.11). Aspirin showed late rapid onset of reduction of the paw size edema at 90 minutes (17.2 ± 0.16). There was percentage inhibition (48, 60, 95 and 96%) after treatment with 100, 200 and 400 mg/kg of *hibiscus sabdariffa* and aspirin (200 mg/kg) but percentage inhibition was more prominent only at high dose concentration of extract (400 mg/kg) and aspirin. Data suggest that the anti-inflammatory activity of extracts is concentration dependent and also as potent as aspirin.

Keyword: Hibiscus sabdariffa, aspirin, egg albumin, inflammation, anti-inflammation.

1. Introduction

From the ages past, edible plants had been therapeutic to different ailments. Roselle (*hibiscus sabdariffa*) or “Yakuwa/Karasu” in Northern Nigeria is an annual herb commonly cultivated in Borno State of Nigeria ^[1]. The aqueous calyx extract is consumed as a local drink “zobo” whereas the young shoots and leaves are eaten as vegetables. Studies have shown *hibiscus* flowers induced gastric acid secretion, ^[1] employed as an antihypertensive agent ^[2], possessed diuretic properties ^[3-4], increased antidiuretic hormone and reduced

plasma electrolytes after salt and water loading^[5], increased some hematological parameters in levodopa induced anemia ^[6], affected reproductive functions ^[7] and hepatoprotective ^[8] in alloxan induced diabetic rats.

Inflammation is a complex localized response to foreign substances such as bacteria or in some instances to internally produced substances ^[9] Inflammation can be acute or chronic; acute inflammation is rapid in onset and of short duration, lasting from a few minutes to as long as few days, and is characterized by fluid and

plasma protein exudation and a predominantly neutrophilic leukocyte accumulation whereas chronic inflammation is of longer duration, typified by influx of lymphocytes and macrophages with associated vascular proliferation and fibrosis ^[10].

Several non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, naproxen and indomethacin are been used as anti-inflammatory agents because of their ability to inhibit prostaglandin synthesis ^[11] inhibit the activity of cyclooxygenase and proinflammatory signaling pathways ^[12]. This remedy is without assault on some systems such as renal system ^[13] gastrointestinal tissues ^[14-15] and hematological parameters ^[16]. Several studies have reported anti-inflammatory activity of *hibiscus sabdariffa* ^[17-18], thus the present work was therefore designed to study the anti-inflammatory activity of methanol extract of roselle (*hibiscus sabdariffa*) in comparison with the most widelyused NSAID aspirin.

2. Materials and Methods

A total of twenty five adult albino rats (200-220 g) was purchased from the Animal house of the Department of Physiology, University of Nigeria. The animals were randomly selected and grouped into five groups of five members each. They were housed individually in a wire mesh cage (under temperature of 27-30 °C, 12 hours light and 12 hours dark cycle) in the Animal House of Department of Human Physiology, Madonna University Elele. They were acclimatized for two weeks, fed with normal rat chow (Guinea Feeds, Nigeria) and had access to tap water *ad libitum*. The Animal Ethical Committee of Department of Human Physiology, Madonna University approved all the protocols of the study.

2.1 Extract Preparation

The calices of *hibiscus sabdariffa* were obtained from a local market in Enugu State. They were taxonomically identified in the herbarium of the Faculty of Pharmaceutical Sciences of Madonna University Elele. The calices were sun dried, grounded into a fine powder using a manual

blender (corona) and stored in air tight container. 200 mg/kg of the powdered form was macerated in 600 ml of methanol and placed in a mechanical shaker for 48 hours, after which the extract was filtered with a clean white handkerchief. The filtrate was then concentrated using a rotator evaporator (Buchi), and was further concentrated to dryness at 40 °C in an electric oven (Gallenkamp). After drying, it was stored in the refrigerator at 4 °C until it was needed for use.

2.2 Drug Preparation

3000 mg of aspirin tablet was purchased from the Pharmacy store of the institution. Four tablets (1200 mg) were dissolved in 12 ml of distilled water to make a concentration of 100 mg/kg according to the manufacturer's description.

2.3 Induction of Paw Edema

Egg white induced paw edema was employed in the study. Before induction of inflammation, the size of the right hind paw of the twenty five rats was measured by threading a measuring tape around the foot. Acute inflammation was produced by injecting the fresh egg albumin (0.1 ml) into the plantar surface of all the rats right hind paw according to a modified method ^[19-20].

The test extract was administered at different doses (100, 200, 300 mg/kg) with or without the standard drug aspirin (200 mg/kg). The paw size of the rats right hand paw was measured at different intervals (30, 60, 90, and 120 minutes) considering the half-life of aspirin. The inhibitory activity was calculated according to formula by Perez ^[21]:

$$\text{Percentage inhibition} = 100(1 - (a - x/b - y))$$

Where

a= the mean paw volume of treated animals after egg albumin injection

x= the mean paw volume of treated animals before egg albumin injection

b=the mean paw volume of control animals after egg albumin injection

y= the mean paw volume of control animals after egg albumin injection

Table 1: Experimental Grouping and Treatment Protocols for Experimental and Control Cases

Groups (n = 5)	Egg albumin (ml)	HSE (mg/kg)	Standard drug Aspirin (mg/kg)
Group I (Control)	0.1 ml	-	-
Group II	0.1 ml	100 mg/kg	-
Group III	0.1 ml	200 mg/kg	-
Group IV	0.1 ml	400 mg/kg	-
Group V	0.1 ml	-	200 mg/kg

2.4 Statistical Analysis

All statistical analysis was performed using SPSS (version 15.0). Data was analyzed using one way analysis of variance (ANOVA). The mean values of each of the parameters measured in all the groups were compared with those of Group I (Control) for any significant difference using Turkey's multiple comparison. P values of 0.05 or less were taken as statistically significant.

3. Results and Discussion

Table 2 showed the percentage inhibition of paw size) at 30 minutes, 60 minutes, 90 minutes and 120 minutes in adult mice after treatment with HSE (100, 200, 400 mg/kg) in comparison with aspirin (200 mg/kg). Figure 1 showed that paw size was significantly reduced at 60 minutes in Group IV ($P < 0.05$) compared to other groups. There was also a statistically significant difference ($P < 0.05$) at 90 minutes in Group IV and V compared to Group I, II, and III. There was statistically significant difference ($P < 0.05$) at 120 minutes in all the groups (II, III, IV, and V) compared to Group I with percentage inhibition of 48%, 60%, 95% and 96% in groups II, III, IV and V.

In the present study, 400 mg/kg of HSE exerted a maximum percentage inhibition (95%) effect on egg albumin induced paw edema at 120 minutes

compared to aspirin (96%). At 60 minutes, HSE exerted a significant reduction (24.8 ± 0.17) on egg albumin induced paw edema compared Group V (27.4 ± 0.11) treated with aspirin. From the result (table 2), HSE has an early rapid onset of inhibition compared to aspirin. At 90 minutes, aspirin (200 mg/kg) significantly reduced the paw size, suggesting that aspirin has a late rapid inhibitory effect. Animal model used in acute inflammation is biphasic; first phase (about 1 – 2 h) which involved inflammation mediated by the release of serotonin and histamine and increased prostaglandins in the surroundings of the damaged tissues, and the second phase (3–5 h) involving the release of kinins mainly prostaglandins [21]. The results (figure 1) indicated that the inflammation occurred in the first phase (1–2 h) because the effect of egg albumin induced paw edema showed a decrease at 120 minutes corroborating with report by Crunkhorn [21].

In the present study, 400 mg/kg of HSE exerted a maximum percentage inhibition (95%) on egg albumin induced paw edema at 120 minutes similar to aspirin (96%). At 60 minutes, only 400 mg/kg of HSE exerted a significant reduction (24.8 ± 0.17) on egg albumin induced paw edema.

Table 2: Percentage inhibition of paw size (mm) in response to HSE (100, 200, 400 mg/kg) and aspirin (200 mg/kg) in adult mice

Groups	Initial Size	Mean Change in Paw Size (mm)				Inhibition (%)
		30 mins	60 mins	90 mins	120 mins	
Group I	15.6±0.11	28.6 ± 0.11	30.2 ± 0.13	32.4 ± 0.13	31.6 ± 0.18	-
Group II	15.6 ± 0.18	28.8 ± 0.17	26.6 ± 0.11	24.2 ± 0.17	24.0 ± 0.09	*48 %
Group III	15.6 ± 0.11	28.8 ± 0.09	25.6 ± 0.18	23.0 ± 0.20	22.0 ± 0.14	*60 %
Group IV	14.2 ± 0.17	28.0 ± 0.14	24.8 ± 0.17*	20.4 ± 0.17*	15.0 ± 0.11	*95 %
Group V	14.8 ± 0.09	28.0 ± 0.14	27.4 ± 0.11	17.2 ± 0.16*	15.5 ± 0.11	*96 %

Values are means ± Standard Error. *P <0.05 compared with value for Group I (Control).

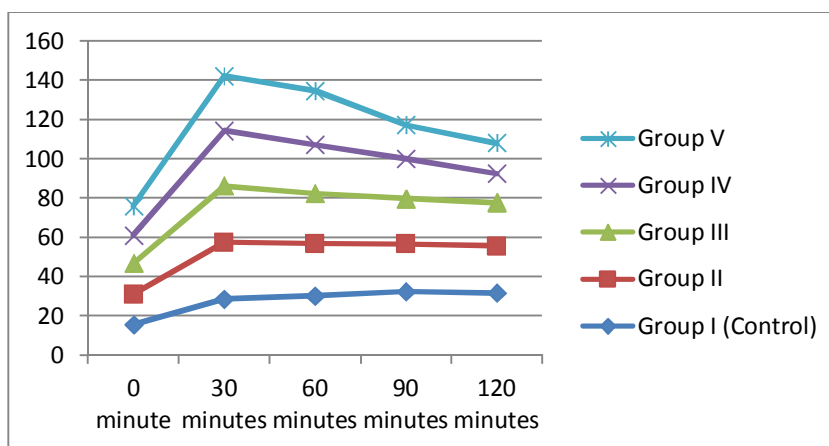


Fig 1: Paw size in response to HSE (100, 200, and 400 mg/kg) and aspirin (200 mg/kg) at 30, 60, 90 and 120 minutes

From the result (Table 2), it seemed that the increased dose concentration of HSE has an early rapid onset of inhibition compared to aspirin. At 90 minutes, the action of aspirin (200 mg/kg) was observed, which significantly reduced the paw size, suggesting that aspirin may have a late rapid inhibitory effect. Animal model used in acute inflammation is reported to be biphasic; first phase (about 1–2 h) which involved inflammation mediated by the release of serotonin and histamine and increased prostaglandins in the surroundings of the damaged tissues, and the second phase (3–5 h) involving the release of kinins mainly prostaglandins [22]. The results indicated that the inflammation (acute

inflammation) occurred in the first phase (1 – 2 h) because the maximum percentage inhibition was observed at 120 minutes even at different dose concentration of HSE.

Inflammatory mediators, serotonin, histamine and prostaglandins could be responsible for the increase in the paw size, which was significantly reduced at 120 minutes after treatment with varying doses of HSE. The mechanism of action could possibly involve inhibition of serotonin, histamine and prostaglandins by HSE because study has showed that aqueous extract of *hibiscus sabdariffa* directly inhibited inflammatory and/or metabolic pathways responsible for monocyte chemoattractant protein (MCP-1) production [17].

Regulation of leukocyte migration and activation by chemokines are recognized as potentially important functions in the induction of acute and chronic inflammatory reactions. Experimental studies reported that RANTES and MCP-1 injected in the rat provoked mast cells activation, caused eosinophil and macrophage recruitment, stimulated prostaglandin E2 generation and increased histidine decarboxylase mRNA expression in a dose dependent manner [23]. *Hibiscus sabdariffa* extracts have also been reported to cause attenuation of expression of pro-inflammatory mediators at the gene, protein and cytokine level in a concentration-dependent manner [24]. This agreed with our observation that percentage inhibition in egg albumin induced paw edema observed (Table 2) by the HSE was concentration dependent and HSE mechanistically inhibited the inflammatory mediators responsible for the first phase of inflammation.

Furthermore, cyclooxygenase is reported to catalyze the conversion of arachidonic acid to the prostaglandins [25], and these prostaglandins are also implicated in acute inflammation [22]. Polyphenols from *hibiscus sabdariffa* is reported to cause down-regulation of cyclooxygenase 2 both in vitro and in vivo [26]. Therefore, HSE anti-inflammatory activities are not only mediated via inhibition of serotonin, histamine and prostaglandins but also via attenuation of cyclooxygenase 2 activity thereby causing a significant percentage inhibition of egg albumin induced paw edema more potently at increased dose concentration of HSE.

4. Conclusion

We have proven the safety of HSE in experimental animals at different dose concentrations in experimental animals, [5-8] therefore, it is wise to conclude that HSE is therapeutically potent and safe in the treatment of acute inflammation compared to aspirin.

5. Acknowledgment

The authors are particularly grateful to Mr. Raymond Okonkwo for his laboratory assistance

6. References

1. Enyikwola O, Otukonyong EE, Hussainy AY, Akinniyi JA. Effect of saline extracts of roselle (*Hibiscus sabdariffa*) on gastric acid secretion in albino rats. Niger J Physiol Sci 1993; 9(1-2):45-50
2. Mojiminiyi FBO, Dikko M, Muhammad BY, Ojobor PD, Ajagbonna OP, Okolo RU *et al.* Antihypertensive effect of aqueous extract of the calyx of *hibiscus sabdariffa*. Fitoterapia 2007; 78:292-297
3. Alarcon-Alonso J, Zamilpa A, Aguilar FA, Herrera-Ruiz M, Tortoriello J, Jimenez-Ferrer E. Pharmacological characterization of the diuretic effect of *hibiscus sabdariffa* Linn (Malvaceae) extract. J Ethnopharmacol 2012; 139:751-756
4. Jimenez-Ferrer E, Alarcon-Alonso J, Aguilar-Rojas A, Zamilpa A, Jimenez-Ferrer CI, Tortoriello J *et al.* Diuretic effect of compounds from *hibiscus sabdariffa* by modulation of aldosterone activity. Planta Med 2012; 78(18):1893-1898.
5. Agbai EO, Nwanegwo CO. Effect of methanolic extract of *hibiscus sabdariffa* on antidiuretic hormone and some electrolytes after salt and water loading in albino wistar rats. J Agric Vet Sci 2013; 5(2):123-132
6. Agbai EO, Nwanegwo CO. Effect of methanolic extract of *hibiscus sabdariffa* on some hematological parameters in levodopa induced anemia. J Biological Science and Bio conservation 2013; 5(2):44-53
7. Agbai EO, Nwanegwo CO. Effect of methanolic extract of *hibiscus sabdariffa* on male reproductive functions in alloxan diabetic albino rats. J Agricultural Science and Policy Research 2013; 3(2):9-23
8. Agbai EO, Nwanegwo CO. Hepatoprotective effect of *hibiscus sabdariffa* in alloxanized mice. J Science and Multidisciplinary Research 2013; 5(2):169-175
9. Barrett KE, Barman SM, Boitano S, Brooks HL. In Ganong WF. Review of Medical Physiology. Ed 23, Lange Medical Publication, 2010; 75.
10. Kumar V, Abbas AK, Fausto N, Mitchell RN. In: Robins Basic pathology, Ed 8, Saunders Elsevier, 2007, 32.
11. Klassen CD. Cassarett and Doull's Toxicology: the basic science of poison. Ed 6, The McCraw Hill Companies Inc. New York, 2001.
12. Morris T, Stables M, Hobbs A, de-Souza P, Colville-Nash P, Wamer T *et al.* Effects of low-dose aspirin on acute inflammatory responses in humans. J Immunol 2009; 183:2089-2096
13. Calder IC, Funder CC, Green CR, Ham KN, Tange JD. Comparative nephrotoxicity of aspirin and phenacetin derivatives. Br Med J 1971;4:518

14. Derry S, Loke YK. Risk of gastrointestinal haemorrhage with long term use of aspirin: meta-analysis. *BMJ* 2000; 321:1183
15. Weil J, Collins-Jones D, Langman M, Lawson D, Logan R, Vessey M, Wainwright P. Prophylactic aspirin and risk of peptic ulcer bleeding. *BMJ* 1995; 310:827
16. Merchant MA, Modi DN. Acute and chronic effects of aspirin on hematological parameters and hepatic ferritin expression in mice. *Indian J Pharmacol* 2004; 36(4):226-230.
17. Beltran-Debon R, Alonso-Villaverde C, Aragonés J, Rodríguez-Medina I, Rull A, Micol V *et al.* The aqueous extract of *hibiscus sabdariffa* calices modulates the production of monocyte chemoattractant protein-1 in humans. *J Phytomedicine* 2010; 17(3-4):186-191
18. Ali MK, Ashraf A, Biswas NN, Karmakar UK, Afroz S. *Journal Zhong Xi Yi Jie He Xue Bao.* 2011; 9(6):626-631
19. Okoli CO, Akah PA. A pilot evaluation of the anti-inflammatory activity of *Culcasia scandens*, a traditional antirheumatic agent. *J Altern Compl Med* 2000; 6(5):423-427.
20. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in the hind paw of rat as an assay for anti-inflammatory drugs. *Proc Soc Biol Med* 1962; 11:544-547.
21. Perez GRM. Anti-inflammatory activity of *Ambrosia artemisiaefolia* and *Rhoeo spathacea*. *Phytomed* 1996; 3(2):163-167.
22. Crunkhorn P, Meacock SCR. Mediators of inflammation induced in the rat paw by carrageenan. *Br J Pharmacol* 1971; 42:392-402.
23. Conti P, DiGioacchino M. MCP-1 and RANTES are mediators of acute and chronic inflammation. *Allergy Asthma Proc* 2001; 22(3):133-137.
24. Watson CY, Williams LL, Waterman JT. Modulation of pro-inflammatory genes by swine confinement facility dust exposure and phytochemical treatments in human airway cells. Paper presented at American Thoracic Society 2011 International Conference May 13-18, 2011 Denver Colorado. DOI:10.1164/ajrcm-conference
25. Seibert K, Masferrer J, Zhang Y, Gregory S, Olson G, Hauser S *et al.* Mediation of inflammation by cyclooxygenase-2. *Agents Actions Suppl.* 1995; 46:41-50.
26. Kao ES, Hs JD, Wang CJ, Yang SH, Cheng SY, Lee HJ. *J Biosci Biotechnol Biochem* 2009; 73(2):385-390.