

Anti-inflammatory Activity of Methanolic Extract of *Hibiscus sabdariffa* on Carrageenan Induced Inflammation in Wistar Rat

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ABSTRACT:

Background: *Hibiscus sabdariffa* is used for medicinal purposes, especially in alternative medicine. It is a folk remedy for abscesses, cancer, cough, debility, dyspepsia, dysuria, fever, hangover, heart ailments, neurosis, scurvy and strangury. This present study was carried out to investigate the anti-inflammatory activity of methanolic extract of *hibiscus sabdariffa* in adult wistar rat.

Methods: A total of Twenty-five (25) of both sexes rats weighing between 180 - 200 g were randomly divided into five (5) groups of five (5) animals each. Group 1 received only 1 ml distilled water which served as control. Group 2 were induced with inflammation using (0.1ml/kg b.w) carrageenan without treatment while Group 3 and 4 were induce with inflammation and treated with low dose (250 mg/kg b.w) and high dose (500 mg/kg b.w) of extract of *hibiscus sabdariffa* respectively. Group 5 served as the positive control group, they were treated with (20mg/kg b.w) diclofenac after carrageenan induced inflammation. The paw diameter of each rat was measured before and after the inflammation then after treatment.

Results: There was significant reduction ($p < 0.05$) in paw diameter in the group that received high dose (500 mg/kg b.w) of methanolic extract of *hibiscus sabdariffa* from Mean \pm SEM of 0.566 \pm 0.023 to 0.414 \pm 0.009 as compared with the untreated group.

Conclusions: The decrease in paw diameter in the high dose group showed that methanolic extract of *hibiscus sabdariffa* possess anti-inflammatory properties against carrageenan induced inflammation similar to the action of diclofenac. The therapeutic effect of this extract will encourage its use in the treatment of inflammation.

Keywords: *Hibiscus sabdariffa*, anti-inflammatory, carrageenan, diclofenac, paw diameter.

I. INTRODUCTION

The use of plants in medicine goes far back as thousands of years and still continues today (Arora and Kaur, 1999). Many plants are used for the treatment of different diseases and many possess antimicrobial activities against *E. coli* (Arora and Kaur, 1999). *Hibiscus sabdariffa* (common name is Roselle) is a medicinal plant in the Malvaceae family which originated from Egypt (Ali *et al*, 2005). Its original Arabic name is Karkade. In Hausa, the plant is locally called “yakuwa”, the seed, “isontea” and the fresh calyx, “soboroto”. The yorubas call the leaaves “amukan” and the flowers “ishapa”. Other common names are; Sorrel, Jamaica Sorrel, Lozey, Cabitutu, Vinuela, oseille de Guinee, pink lemonade flower, Vinagrillo and Afrika Bamyasi. *Hibiscus* is considered to be a tropical plant that grows annually or perennially with woody-based subshrub, growing to 2–2.5 m (7–8 ft) tall. The leaves are deeply three- to five-lobed, 8–15 cm (3–6 in) long, arranged alternately on the stems. The flowers are 8–10 cm (3–4 in) in diameter, white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base, 1–2 cm (0.39–0.79 in) wide, enlarging to 3–3.5 cm (1.2–1.4 in), fleshy and bright red as the fruit matures which it takes about six months to mature. All parts of *Hibiscus sabdariffa* are used for medicinal purposes, especially in alternative medicine (Ali *et al*, 2005). It is a folk remedy for abscesses, cancer, cough, debility, dyspepsia, dysuria, fever, hangover, diuretic, mild laxative, heart ailments, neurosis, scurvy and strangury (Lin *et al*, 2011). Recent scientific work has established the protective effect of the dried flower extract of *Hibiscus sabdariffa* (Tseng *et al*, 1996), anti-inflammatory activity (Dafallah and Mustapha, 1996), anti-hypertensive effect of the dried calyx extract (Adegunloye *et al*, 1996) and anti-mutagenic effect (Chewonarin *et al*, 1999). *Hibiscus sabdariffa* has been reported to have antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, sedative,

stomachic and tonic activities (Olaleye, 2007). It is used in the treatment of hypertension (Wang *et al*, 2000), hypocholesterolemic (Olaleye, 2007), anti-oxidative and hepatoprotective effects in animals (Wang *et al*, 2000). Delphinidin 3-sambubioside, a Hibiscus anthocyanin, induces apoptosis in human leukaemia cells through oxygen reactive species-mediated mitochondrial pathway. The flowers of *Hibiscus sabdariffa* contain gossypetine, hibiscetine and sabdaretine, anthocyanins such as delphinidin-3-sambubioside, cyaniding-3-sambubioside and delphinidin -3- glucose, flavonoids such as hibiscitrin and hibiscetin and polyphenols (Lin *et al.*, 2007), Studies have shown the role of polyphenolic acid, flavonoids and anthocyanins that may act as antioxidants or have other mechanisms contributing to the cardio protective actions (Rimm and Stamfer, 2000). The chemistry of the calyx revealed that per 100 g, it contains 49 calories, 84.5% water, 1.9 g protein, 0.1 g fat, 12.3 g total carbohydrate, 2.3g fibre, 1.2 g ash, 1.72 mg Calcium, 57 mg Potassium, 2.9 mg Iron, and 14 mg Ascorbic acid. Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Ferraro *et al*, 2007). It is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Anti-inflammatory response refers to the property of a substance or treatment that reduces inflammation. The calyx of *Hibiscus sabdariffa* contains ascorbic acid which is a very good anti-inflammatory agent (Mahadevan *et al*, 2008). In Nigeria and some parts of the West Africa were this plant *Hibiscus Sabdariffa* are grown, there has been reports from herbal medicinal partners indicating that *Hibiscus Sabdariffa* is used in the treatment of pain and inflammation by boiling leaves and drinking orally, hence this present study was aimed at investigating the anti-inflammatory activity of methanolic extracts of the calyx of *Hibiscus sabdariffa* using the laboratory model: Carrageenan induced inflammation in wistar rat.

II. MATERIALS AND METHODS

Experiment animals

Twenty-five wistar rats of both sexes weighing between 180 - 200 g were obtained from the animal house of the Department of Physiology, University of Jos. They were kept in the Animal House of Bingham University for two weeks of acclimatization and maintained under standard conditions, temperature, food and clean water was given *ad libitum*.

Extract preparation

The fresh *Hibiscus sabdariffa* leaves were obtained in Wuse market, Abuja metropolis, Federal Capital Territory. It was washed thoroughly and dried under room temperature. The extraction of *Hibiscus sabdariffa* leaves was done using three hundred and sixty grams (360 g) of the ground leaf sample in soxhlet extractor with methanol in Department of Chemistry, Bingham University, Kodape, Karu, Nasarawa State, Nigeria. The sample was packed in the column, mounted on a retort stand to balance the apparatus. The collection point which constitutes a conical flask 2/3 filled with methanol and placed on a heating mantle at a temperature of hundred degrees celsius. The methanolic extracts were then transferred to a rotatory evaporator at 3000 rev/min at 95 degrees celsius for one hour then stored in a sample bottle.

Extract administration

The extract was given orogastrically using oropharyngeal cannula, this was given according to the average body weight of animal in each group. Group I received only 1 ml distilled water which served as control, Group II were induced with inflammation using (0.1ml/kg b.w) carrageenan without treatment, Group III and IV received with low dose (250 mg/kg b.w) and high dose (500 mg/kg b.w) of extract of *hibiscus sabdariffa* respectively after they were induce with inflammation while Group V served as the positive control group, they were treated with (20 mg/kg b.w) diclofenac after carrageenan induced inflammation. The paw diameter of each rat was measured before and after the inflammation then after treatment using an analogue vernier calliper.

Statistical analysis

The values are recorded as mean \pm SEM at $p < 0.05$ significant difference using student t-test.

III. RESULT AND DISCUSSION

There was significant reduction ($p < 0.05$) in the paw diameter in the high dose (500 mg/kg) of methanolic extract of *hibiscus sabdariffa* treated group from Mean \pm SEM of 0.566 \pm 0.023 to 0.414 \pm 0.009 when compared with the untreated group as shown in the table I. The Positive control group; treated with (20 mg/kg) diclofenac also shows a significant reduction ($p < 0.05$) in the paw diameter of treated group from Mean \pm SEM of 0.580 \pm 0.026 to 0.346 \pm 0.004 as compared with the untreated group as shown in the table I. The low dose (250

mg/kg) of methanolic extract of *hibiscus sabdariffa* treated group shows no reduction in paw diameter when compared with the untreated group as shown in the table I.

Table I: shows the effect of methanolic Extract of *hibiscus sabdariffa* and Diclofenac on carrageenan induced inflammation.

	Control	Carrageenan (0.1ml/kg b.w)	Low dose (250 mg/kg)	High dose (500 mg/kg b.w)	Diclofenac (20 mg/kg b.w)
Paw Diameter					
Before Treatment	0.346	0.502	0.504	0.566	0.580
	± 0.004	± 0.009	± 0.029	± 0.023	± 0.026
After Treatment	–	–	0.522 ± 0.042	0.414* ± 0.009	0.346* ± 0.004

Values are mean ± S.E.M (n=5): * indicate significant (p<0.05) different when compared with the untreated groups

The study showed that low dose (250 mg/kg b.w) treatment with the methanolic extract of *hibiscus sabdariffa* had no effect on the carrageenan induced inflammation after two hours of treatment. There was a decrease in paw diameter in high dose (500 mg/kg b.w) of methanolic extract of *hibiscus sabdariffa* treated group which is also in agreement with the findings of (Dafallah and Mustapha, 1996). *Hibiscus sabdariffa* is known to have ascorbic acid as one of its phytochemicals which has been proposed to have an anti-inflammatory activity (Mahadevan *et al*, 2008). Apart from this phytochemical, it also consists of flavonoids such as hibiscitrin and hibiscetin and polyphenols (Lin *et al.*, 2007) and other minerals that have been shown to used in the treatment of hypertension (Wang *et al*, 2000; Rimm and Stamfer, 2000), also having hypocholesterolemic effect (Olaleye, 2007), anti-oxidative and hepatoprotective effect (Wang *et al*, 2000).

IV. CONCLUSIONS

The result of this study therefore suggests that the methanolic extract of *Hibiscus sabdariffa* exhibited therapeutic properties that were only significant at high dose of treatment. Therefore the therapeutic effect of this extract will encourage its use in the treatment of inflammation.

REFERENCES

- [1]. Adegunloye, B.J., Omoniyi, J.O., Ajabonna, O.P. Mechanisms of the blood pressure lowering effects of the calyx extract of *Hibiscus sabdariffa* in rats. African Journal of Medical Science (1996). 25: pg 235–238.
- [2]. Ali BH, Al Wabel N, & Blunden G. "Phytochemical, Pharmacological and toxicological aspects of *Hibiscus sabdariffa* Linn: a review". Phytotherapy Research (2005). 19 (5): 369-75.
- [3]. Arora D and J Kaur: Antimicrobial activity of spices, International Journal of Antimicrobial Agents (1999). 12: pg 257-262.
- [4]. Chewonarin, T., Kinouchi, T., Kataoka, K., Arimochi, H., Kuwahara, T., Vinitketkumnuen, U., Ohnishi, Y: Effects of roselle (*Hibiscus sabdariffa* Linn.), a Thai medicinal plant, on the mutagenicity of various known mutagens in *Salmonella typhimurium* and on formation of aberrant crypt foci induced by the colon carcinogens azoxymethane and 2-amino-1-methyl-6-phenylimidazol [4,5-b] pyridine in F344 rats. Food Chemical Toxicology (1999). 37 (6): pg 591-601.
- [5]. Dafallah, AA. and Al-Mustafa, Z.: Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. American Journal Chin Medicine (1996) 24 (3-4): 263-269.
- [6]. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. "Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation". Clinical Expert Immunology. (2007). Pg 147.
- [7]. Lin, HH; Chen, JH; Wang, CJ. "Chemopreventive properties and molecular mechanisms of the bioactive compounds in *Hibiscus sabdariffa* Linne". Current medicinal chemistry (2011) 18 (8): 1245–54.
- [8]. Lin, T., Lin, H., Chen, C., Lin, M., Chou, M., Wang, C. Hibiscus sabdariffa extract reduces serum cholesterol in men and women. Nutrition Research. (2007). 27:140-145.
- [9]. Mahadevan N, Shivali and Pradeep Kamboj. Hibiscus Sabdariffa Linn.-An overview (2008).
- [10]. Olaleye, MT. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. Journal of Medicinal Plants Research. (2007). 1(1): 009-013.
- [11]. Rimm, EB. and Stamfer, MJ. Antioxidants for vascular disease. Medicine Clinical North America. (2000). 84: pg 239-249.
- [12]. Tseng, TH., Wang, CJ., Kao, ES., Chu, CY. Hibiscus protocatechuic acid protects against oxidative damage induced by tert-butylhydroperoxide in rat primary hepatocytes. Chemical Biology Interaction (1996). 101: 137–148.
- [13]. Wang, CJ., Wang, JM., Lin, WL., Chu, CY., Chou, FP. and Tseng, TH. Protective effect of *Hibiscus* anthocyanins against tert butyl hydroperoxide- induced hepatic toxicity in rats. Food Chemical Toxicology. (2000). 38 (5): pg 411-416.