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Pharmacology

Anti-Inflammatory Activity of Methanol Extract of *Garcinia kola* Stem Bark

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Abstract | **Original Research Article**

Background and Objective: *Garcinia kola* (Heckel) stem-bark is used by traditional medicine practitioners in Ogoni, Nigeria for treatment of dysmenorrhea and inflammatory disorders. The study was carried out to investigate the anti-inflammatory effects of the methanol extract of *G. kola* stem-bark. ***Materials and Methods:*** The extract was tested for anti-inflammatory effects against carrageenan and egg albumen-induced hind-paw oedema in rats and xylene-induced ear oedema in mice. ***Result****:* The extract showed significant ($p< 0.05 – 0.01$) and time dependent inhibitory effect against carrageenan and egg albumen-induced hind-paw acute inflammation. The highest dose of the extract (72mg/kg) showed a higher degree of inhibition of inflammation than acetylsalicylic acid (ASA). Combination of the extract with ASA did not produce an additive or synergistic effect. Furthermore, there was also a significant ($p<0.01$), dose-dependent suppressive action against acute ear inflammation induced by xylene. ***Conclusion:*** Several mechanisms, including inhibition of the action or release of pro-inflammatory mediators, appear to account for the anti-inflammatory effect of *G. kola* stem-bark. This study has provided a pharmacological confirmation that *G. kola* stem-bark has good anti-inflammatory properties, which therefore vindicate its ethnobotanical uses.

Keywords: Anti-inflammatory effect, Carrageenan, Paw edema, Xylene, Garcinia kola.

INTRODUCTION

Inflammation, which is functionally a protective response, can be considered as that complex series of events that develop when the body is injured either by mechanical or chemical agents or by self-destructive (autoimmune) process. The cardinal signs of inflammation include swelling, redness, heat, pain and altered function. During inflammation, leukocytes infiltrate in the site of injury and release mediators that help in decreasing the inflammatory response [1].

Garcinia kola (Heckel) is a medium-sized tree that grows in the subtropical and tropical lowland forests. It is popularly known as bitter kola or male kola [2].

Garcinia kola (Heckel) is an angiospermae belonging to the family Guttiferae, it is found in the West African sub region; from Sierra Leone to Southern Nigeria, mostly in moist conditions. It is a medium-sized tree mostly about 12m high and sometimes up to 28m in height. The bark is thick and brownish, yielding a yellow juice. The leaves are broadly elliptic, acute or shortly acuminate at the apex. The fruits are reddish yellow and about 6cm in diameter with 2 - 4 brown seeds embedded in an orange coloured pulp [3]. The fruit is a drupe of 5 - 10cm in diameter and weighs 30 - 50g. It is usually smooth and contains a yellow red pulp.

All parts of this "wonder kola" are useful in African ethnomedicine, for example; the dried fruit is used to treat arthritis [4]. The seeds are of value in the following conditions: diarrhoea, bronchitis and throat infections and liver diseases [5-7], other uses of the seed include treatment of cough and as anti-parasitic and anti-microbial agent [8, 9].

The bark of *G. kola* is taken orally for fever, cough, inflammation, respiratory tract disease and as an anthelmintic [10]. The antibacterial activity of the tannin fraction of the dried stem bark of *G. kola* against *Escherichia piracoli*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Staphylococcus* aureus has also been reported [11].

MATERIALS AND METHODS

Plant Material

The plant material used for this work was collected from a bitter cola (*G. kola*) TREE at Eliogbolo in the suburb of Port Harcourt city, Nigeria. The plant material was identified by Dr. Edwin-Wosu of Department of Plant Science and Biotechnology, University of Port Harcourt. Specimen vouchers were

made and deposited at the herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt.

Extract Preparation

The plant material was cut into smaller pieces and oven dried for 5 days at 43±2˚C. The dried plant material was pulverized with a manual grinder. The pulverized plant material was soaked in methanol in a 3:1 ratio and intermittently shaken. The mixture was kept for 72 hrs after which it was filtered through Whatman No. 1 filter paper and concentrated. The filtrate was stored in a refrigerator until required for use.

Preparation of Animals for the Study

Adult albino mice and rats (weighing 20-30g and 165-190g respectively) were used in this study. The animals were obtained from the Faculty of Pharmacy Animal house, University of Uyo, Nigeria. They were housed in plastic cages and maintained under standard laboratory conditions (12h light and dark cycles) with food and water *ad libitum*. They were taken out of the animal house and acclimatized to the laboratory environment for about 2h prior to commencement of the tests. All efforts were made to minimize discomfort. The care and handling of these animals were carried out in strict compliance with the guidelines of the International Association for the Study of Pain, for the use of animals in pain and pain-related research [12].

Effect of extract on carrageenan-induced hind-paw oedema in rats

Six groups of rats (each group with six animals) were fasted for 18 hours with water given *ad libitum*, except during experimentation. Group 1 served as control group and was administered with distilled water, 10ml/kg (i.p.). Groups 2- 4 were pre-treated with 18, 36 and 72mg/kg; (i.p.) of the extract respectively. Group 5 was treated with acetylsalicylic acid (ASA), the reference drug. Group 6 was pretreated with ASA and 10 min later 36mg/kg; (i.p.) of the extract was given.

All pre-treatments were done 1hr before the injection of the phlogistic agent (carrageenan suspension). Oedema was induced by injecting 0.1 ml of 10% (w/v) carrageenan suspension subcutaneously (s. c.) into the sub plantar region of the right hind paw of the rats according to the method described by Winter *et al.* [13]. The paw circumference was measured before administering carrageenan (C_0) and every 30min thereafter for 5h (C_t). The paw size was measured with digital vernier callipers. The difference between C_t and C_0 within a given time represents the degree of inflammation. The percentage of inhibition of oedema was calculated using the formula below [14]:

$$\text{Percentage inhibition} = \frac{(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}}{(C_t - C_0)_{\text{control}}} \times 100$$

Where:

$\mathbf{C_t}$ = paw circumference at time $_t$; $\mathbf{C_0}$ = paw circumference before carrageenan injection; $\mathbf{C_t - C_0}$ = oedema

Effect of extract on fresh egg albumin-induced hind-paw oedema in rats

Adult albino rats were randomized into six different groups (with six animals per group). The rats were of either sex; and they were used for the experiment after 18h fast and were only deprived of water during experiment. Inflammation of the hind paw was induced by injecting 0.1 ml of fresh egg albumin (s.c.) into the sub-plantar surface of the hind paw [15, 16]. The control group was pre-treated with distilled water (10ml/kg,i.p.) and the reference group received 100mg/kg ASA (i.p.). The test groups of rats were pre-treated with 18, 36, 72 mg/kg(i.p.) of the extract respectively. A sixth group was pre-treated with 100mg/kg; (i.p.) ASA followed by 36mg/kg; (i.p.) extract after 10min. The basal paw circumference was measured with digital vernier callipers before administering the fresh egg albumin (C_0) and every 30min thereafter for 5h (C_t). The difference between C_t and C_0 within a given time represented the degree of inflammation [17]. Results were expressed as percentage of inhibition of oedema and calculated according to the standard formula [14].

$$\text{Percentage inhibition} = \frac{(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}}{(C_t - C_0)_{\text{control}}} \times 100$$

Where:

C_t = paw circumference at time $_t$; C_0 = paw circumference before fresh egg albumin injection; $C_t - C_0$ = oedema

Effect of extract on xylene-induced ear oedema in mice

Thirty-six (36) mice of both sexes were randomized into six (6) groups of six animals each, fasted for 18h with water (only deprived during experimentation). The test groups of the mice were pre-treated with 18, 36 and 72mg/kg; (i.p.) of the extract respectively. The reference group was pre-treated with 100mg/kg; (i.p.) ASA while the control group was treated with distilled water (10ml/kg,i.p). A sixth group was pre-treated with 100mg/kg; (i.p) acetylsalicylic acid followed by 36mg/kg; (i.p) extract after 10min. All pre-treatments were done 30 minutes before the induction of inflammation. Inflammation was induced in mice by topical administration of 2 drops of xylene at the inner surface of the right ear. The xylene was left to act for 15 min. The animals were then sacrificed under light ether anaesthesia and both ears cut off and weighed. The difference between the ear weights was taken as oedema induced by the xylene [18, 19].

Statistical analysis

Results were expressed as mean ± SEM. Statistical significance was determined by one way ANOVA followed by Dunnett's multiple comparison post-test using Graphpad Prism 3.0.

P values of <0.05 were considered significant.

RESULTS

Effect of extract on carrageenan-induced hind-paw oedema in rats

The extract showed inhibitory effect against carrageenan-induced acute inflammation. This suppression of carrageenan-induced rat hind-paw oedema significantly time dependent (p< 0.05 – 0.01), (Table 1).

Table-1: Effect of extract on carrageenan-induced hind paw oedema in rats

	Initial	30	1h	1.5h	2h	2.5h	3h	3.5h	4h	4.5h	5h
Control.	3.50 ±0.21	12.0 3±0.32	11.88 ±0.50	11.50 ±0.06	11.30 ±0.51	10.90 ±0.86	10.70 ±0.50	10.35 ±0.21	9.95 ±0.11	9.70 ±0.35	9.80 ±0.33
18mgkg^{-1}	3.55 ±0.11	11.50 ±0.05^{a}	9.75 ±0.06^{b}	9.50 ±0.04^{b}	9.10 ±0.08^{c}	8.70 ±0.03^{c}	7.90 ±0.13^{c}	6.80 ±0.22^{c}	5.95 ±0.54^{c}	4.20 ±0.45^{c}	3.90 ±0.40^{c}
36 mgkg^{-1}	3.40 ±0.33	10.58 ±0.20^{a}	9.50 ±0.90^{b}	9.20 ±0.66^{c}	8.80 ±0.11^{c}	7.90 ±0.30^{c}	6.80 ±0.55^{c}	5.70 ±0.20^{c}	4.90 ±0.60^{c}	3.95 ±0.80^{c}	3.65 ±0.14^{c}
72mgkg^{-1}	3.85 ±0.25	10.15 ±0.33^{c}	8.20 ±0.23^{c}	8.00 ±0.52^{c}	7.50 ±0.30^{c}	6.70 ±0.05^{c}	6.40 ±0.02^{c}	5.50 ±0.06^{c}	4.85 ±0.64^{c}	4.10 ±0.10^{c}	3.90 ±0.09^{c}
ASA(100 mgkg^{-1})	3.62 ±0.40	10.25 ±0.61^{b}	9.30 ±0.35^{c}	9.10 ±0.21^{c}	8.60 ±0.30^{c}	7.80 ±0.45^{c}	6.90 ±0.52^{c}	5.70 ±0.65^{c}	4.80 ±0.22^{c}	4.20 ±0.71^{c}	3.92 ±0.20^{c}
ASA+ 36mg/g	3.20 ±0.07	10.23 ±0.55^{b}	9.10 ±0.55^{c}	8.70 ±0.66^{c}	7.70 ±0.45^{c}	6.50 ±0.31^{c}	5.40 ±0.25^{c}	4.85 ±0.54^{c}	3.95 ±0.09^{c}	3.85 ±0.05^{c}	3.45 ±0.31^{c}

Values represent Mean ± SEM; (n= 6)

Significance relative to control: a = p >0.05, b = p <0.05, c = p <0.01

ASA = Acetylsalicylic acid

Effect of extract on fresh egg albumin-induced hind paw oedema in rats

The extract had a suppressive effect on fresh egg-induced rat hind paw oedema (Table 2). The inhibitory effect was significantly time dependent (p< 0.05 – 0.01).

Table-2: Effect of extract on fresh egg albumin-induced hind paw oedema in rats

Initial		30	1h	1.5h	2h	2.5h	3h	3.5h	4h	4.5h	5h
Control	4.46± 0.61	10.36± 0.24	10.16± 0.45	9.85± 0.63	9.75± 0.17	9.64± 0.24	9.24± 0.17	8.84± 0.15	8.34± 0.06	7.54± 0.13	7.33± 0.15
18 mg/kg	4.25± 0.01	9.04± 0.53^{b}	8.96± 0.24^{b}	8.43± 0.11^{c}	7.63± 0..71^{b}	7.33± 0.26^{b}	6.72± 0.50^{c}	6.06± 0.23^{c}	5.86± 0.32^{c}	5.27± 0.16^{c}	4.75± 0.23^{c}
36 mg/kg	3.25± 0.40	8.34± 0.36^{c}	8.04± 0.25^{c}	7.70± 0.14^{c}	7.30± 0.64^{c}	6.60± 0.42^{c}	6.29± 0.23^{c}	5.67± 0.34^{c}	5.07± 0.55^{c}	4.66± 0.14^{c}	3.85± 0.05^{c}
72 mg/kg	4.17± 0.11	8.32± 0.16^{c}	7.82± 0.12^{c}	7.32± 0.08^{c}	6.71± 0.08^{c}	6.30± 0.08^{c}	5.90± 0.06^{c}	5.29± 0.06^{c}	5.08± 0.06^{c}	4.40± 0.14^{c}	4.35± 0.05^{c}
ASA 100mg/kg	3.35± 0.52	8.95± 0.12^{b}	8.32± 0.06^{c}	7.91± 0.15^{c}	7.30± 0.65^{c}	6.84± 0.76^{c}	6.29± 0.14^{c}	5.68± 0.03^{c}	4.78± 0.15^{c}	4.17± 0.05^{c}	3.86± 0.16^{c}
ASA+ 36mg/kg	3.16± 0.13	8.63± 0.22^{c}	8.22± 0.27^{c}	7.82± 0.13^{c}	7.10± 0.44^{c}	6.69± 0.77^{c}	5.88± 0.08^{c}	5.28± 0.04^{c}	4.73± 0.14^{c}	4.37± 0.11^{c}	3.56± 0.65^{c}

Values represent Mean ± SEM; (n= 6)

Significance relative to control: a = p >0.05, b = p <0.05, c = p <0.01

ASA = Acetylsalicylic acid

Effect of extract on xylene-induced ear oedema in mice

The effect of extract on xylene-induced ear oedema in mice is as shown in Table 3. The extract exhibited inhibitory effect on acute ear inflammation induced by xylene. The percentage suppressive effect was statistically significant (p<0.01) in a dose related manner.

Table-3: Effect of extract on xylene-induced ear oedema in mice

Dose (mg/kg)	Mean weight of right ear (g)	Mean weight of left ear (g)	Increase in ear weight (g)	% Inhibition
Control (normal saline) 0.2ml	0.082 ± 0.003	0.045 ± 0.002	0.037±0.001 (45.1%)	-
18	0.068 ± 0.002	0.043 ± 0.002	0.025±0.002 (36.8%)[c]	32.43
36	0.064 ± 0.002	0.040 ± 0.004	0.024±0.002 (37.5%)[c]	35.14
72	0.056 ± 0.022	0.039 ± 0.000	0.017±0.001 (30.4%)[c]	54.10
ASA(100)	0.058 ± 0.002	0.042 ± 0.000	0.016±0.001 (27.6%)[c]	56.76
ASA + 36	0.050 ± 0.002	0.040 ± 0.002	0.010±0.001 (20%)[c]	72.97

Values represent Mean ± SEM; (n=6)
Significance relative to control: [C] $p<0.01$
ASA = Acetylsalicylic acid

DISCUSSION

The anti-inflammatory activity of *Garcinia kola* was evaluated in this study using the carrageenan, egg albumin and xylene tests. Carrageenan-induced inflammation is triphasic; the first phase consists of an initial release of histamine and serotonin; the second phase is mediated by kinins; and the third phase involves prostaglandins [20, 16, 21]. Egg albumin-induced oedema results primarily from the release of histamine and serotonin [22, 23]. The xylene ear oedema test is useful for the evaluation of anti-inflammatory steroids and is said to be less sensitive to non-steroidal anti-inflammatory agents [24, 23]. The model is thus linked to the activity of phospholipase A_2 (PLA_2) [25].

In this study, *G. kola* showed significant inhibitory effect on rat paw oedema development in all the phases of carrageenan and egg albumin-induced inflammation. This suggests that the extract possibly acts by inhibiting the release and/or actions of vasoactive substances (histamine, serotonin and kinins) and prostaglandins. The effectiveness of *G. kola* in the xylene-induced oedema test is therefore suggestive of the possible involvement of PLA_2 inhibition in its mechanism of anti-inflammatory action. Flavonoids which are some of the constituents of the extract have anti-inflammatory property [26, 27].

CONCLUSION

The results obtained from this investigation showed that that the stem bark of *G. kola*, has good anti-inflammatory properties and could be of value in the management of inflammations. This study therefore vindicates its ethnobotanical use. This effect may be due to its phytochemical constituents.

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