Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Analgesic effect of the methanol extract of *Garcinia kola* stem bark

[a]Kagbo, H. D.[*], [b]Nwafor, P.A.

a Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria
b Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria

ARTICLE HISTORY

Received: 17.11.2011

Accepted: 23.01.2012

Available online: 10.05.2012

Keywords:

Writhing test, Formalin test, Hot plate test, Tail Immersion test, Garcinia kola

***Corresponding author:**

Email: brighthope@rocketmail.com
Tel. : +2348032331949

ABSTRACT

This study sought to establish the analgesic properties of the methanol extract of the stem bark of *Garcinia kola* (Heckel) in mice using chemical and thermal test models of nociception. The hot plate, formalin, tail immersion and acetic acid-induced writhing tests were used to assess the analgesic effect of the extract in mice. Animals were pre-treated intraperitoneally with the extract in doses of 18, 36 and 72mg/kg. Acetylsalicylic acid was used as the reference drug; and one group of animal was given acetylsalicylic acid and 36mg/kg of the extract, in order to investigate the effect of the combination of the extract and the reference drug on nociception. The chemical models of analgesia showed significant inhibitions of acetic acid-induced writhing episodes, with percentage analgesic activity greater than acetylsalicylic acid; and significant decreases in formalin-induced sub-plantar licking time in the early and late phases. Furthermore, the extract produced significant increase in reaction time in the thermal models (tail immersion and hot plate tests). In all the models, the extent of analgesic activity in the group of animals where the extract was combined with acetylsalicylic acid was higher in magnitude than either acetylsalicylic acid or 36mg/kg of the extract given alone. The methanol extract of the stem bark of *Garcinia kola* (Heckel) markedly demonstrated analgesic action in mice. The result of this study scientifically justifies the rationale in the traditional use of this part of the plant to treat dysmenorrhoea.

INTRODUCTION

Garcinia kola Heckel (Guttiferae) commonly known as Bitter Kola is a medium-sized tree, which may sometimes, be up to 28 m in height. The bark is thick and brownish, and produces a yellow juice.

The leaves are broadly elliptic, acute or shortly acuminate at the apex. The fruits are reddish yellow and about 6 cm in diameter with 2 - 4 brown seeds embedded in an orange coloured pulp [1]

Garcinia kola is a highly valued plant in trado-medicine in Nigeria and most of West Africa. Different parts of the plant have been explored to treat various disease conditions. The seed of *G. kola* for instance, has been reported to have antitussive, purgative, anti-parasitic and anti-microbial effects.

It is also known to possess anti-hepatotoxic, antioxidant, hypoglycemic and aphrodisiac properties [2].

The bark of *G. kola* is taken orally for fever, cough, inflammation, respiratory tract disease and as an antihelmintic [3]. The dried root soaked in local beer is taken orally for the treatments of coughs, inflammation, liver cirrhosis, tooth decay and gonorrhoea [4]. Biological activity reported for the plant includes the use of the methanolic extract of the dried leaf as a molluscicide [5]. The tannin fraction of the dried stem bark of *G. kola* has been shown to have antibacterial activity against *Escherichia piracoli*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Staphylococcus aureus* [6].

The current study sought to subject the plant to antinociceptive screening, based on the fact that decoction and infusion of the stem bark is famous among traditional healers and birth attendants in the Ogoni area of Nigeria in treating gynaecological pains such as dysmenorrhoea.

MATERIALS AND METHOD

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 part (stem bark) was identified by Dr. Edwin-Wosu of Department of Plant Science and Biotechnology, University of Port Harcourt. Specimen vouchers (PSB 014) 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 45˚C.

The dried plant material was pulverised with a manual grinder. 100g of the pulverised plant material was soaked in 300ml of methanol 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 the Animals for assessment of Analgesic activity of the extract.

Adult male Swiss mice (2535 g) were obtained from the Faculty of Pharmacy Animal house, University of Uyo. They were housed in plastic cages, with food and tap water available *ad libitum* .

The mice were fasted for 1218 h before the tests. They were taken out of the animal house and acclimatized to the laboratory environment for about 2 hours 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 current guidelines of the International Association for the Study of Pain, for the use of animals in pain research [7].

Effect of extract on thermally-induced pain (Hot plate) in mice.

Thermally-induced pain using the hot plate[8, 9] was adopted in this study. The hot plate was maintained at 55±0.5°C. Animals were placed in a glass beaker of 50cm in diameter on the heated surface, and the time(s), in seconds, between placement and shaking, licking of the paws or jumping was recorded as the index of response latency. A latency cut-off time of 30s was used to prevent tissue damage. Thirty six mice were divided into six groups of six mice per group. Groups 2 - 4 were administered with 18, 36 and 72 mg/kg; (i.p.) of the extract respectively. Group 5 was given 100 mg/kg of acetylsalicylic acid (ASA) (i.p.). Group 6 was pre-treated with ASA followed by 36mg/kg of extract 10min later. Group 1 served as control. All pre-treatments were given 30min before placing the animal on the hot plate.

Effect of extract on formalin-induced hind paw licking in mice

In the formalin-induced hind-paw licking test [10], twenty microlitre (20µl) of 2.5% formalin solution (0.9% formaldehyde) was made up in phosphate buffer solution (PBS concentration: NaCl, 137mM, KCl, 2.7mM and phosphate buffer, 10mM) and injected subcutaneously under the surface of the right hand paw and the animals were individually placed in transparent observation chambers. The animals in groups 2-4 were pre-treated with the extract (18, 36 and 72mg/kg; i. p. respectively). Group 5 received acetylsalicylic acid (100mg/kg; i. p.) alone while Group 6 received acetylsalicylic acid (100mg/kg; i. p.) and 36mg/kg of extract, 10mins later. After 30mins, all the animals were challenged with buffered formalin. Group 1 served as control and was given saline (10ml/kg; i. p.). The mice were observed for 30 min after the injection of formalin, and the amount of time spent licking the injected paw was recorded and considered as indicative of pain.

Effect of extract on tail immersion test in mice.

The tail immersion method [11; 12] was adopted for this test. Thirty-six mice were randomized into six groups, with each group containing six mice. The groups were pre-treated as follows: Group 1 served as control and was injected with normal saline (10ml/kg; i.p.). Groups 2 4 were administered with 18, 36 and 72mg/kg; (i.p.) of the extract respectively, Group 5 was treated with acetylsalicylic acid (100mg/kg; i. p.) and Group 6 was given acetylsalicylic acid (100mg/kg; i. p.) followed by 36mg/kg; (i. p.) of the extract 10min later. The lower two-thirds of the tail of the mice were immersed in a beaker containing water maintained at 50 ± 0.5 °C. The reaction time was taken to be the time (in seconds) that the mice withdraw their tail from the water due to the pain sensation. The mice were exposed to hot water for no longer than 30 s to avoid tissue injury.

Effect of extract on acetic acid-induced writhing in mice

In this test [10] 3% acetic acid was injected into the peritoneal cavities of the mice, which were placed in a large glass cylinder for observation. The animals were divided into six groups of six mice per group. Group 1 served as control and was injected with saline (10ml/kg), while groups 2 - 4 were pre-treated with 18, 36 and 72 mg/kg of extract intraperitoneally (i. p.) respectively. Group 5 was administered with acetylsalicylic acid (100mg/kg; i. p.) only, while group 6 was given acetylsalicylic acid (100mg/kg, i. p.) and 10min later was treated with the extract (36mg/kg; i. p.). After 30min, acetic acid was administered to all groups. The number of writhing movements (characterised by constriction of abdominal muscle and/or with the stretching of hind limbs was counted for 30mins as an indication of nociceptive behaviour; whereas antinociception (analgesia) was expressed as the reduction in the number of abdominal constrictions observed between control animals and mice pre-treated with the extract [13]. The percentage analgesic activity was calculated using the method below [9].

$$\text{i.e. Percentage analgesic activity} = \frac{Nc - Nt}{Nc} \times 100\%$$

Where :

Nc is the average number of stretches of the control group

Nt is the average number of stretches of the test drug group.

Statistical analysis

The data obtained were analyzed using the GraphPad Prism Software program Version 5.0 and expressed as a mean ± S.E.M. Statistically significant differences between groups were calculated by the application of an analysis of variance (ANOVA) followed by the Dunnett's Multiple Comparison Test. *p*-values less than 0.05 ($p < 0.05$) were used as the significance level.

RESULT & DISCUSSION

Effect of extract on thermally-induced pain (Hot plate) in mice

The result of thermally-induced pain in mice is as shown in Fig. 1. The extract exhibited a dose-dependent protection (increase in reaction time) against thermally induced pain in mice. This effect was statistically significant ($p<0.001$)

Effect of extract on formalin-induced hind paw licking in mice

The result is as shown in Table 1. The extract showed an inhibitory effect on formalin-induced hind paw licking in mice.

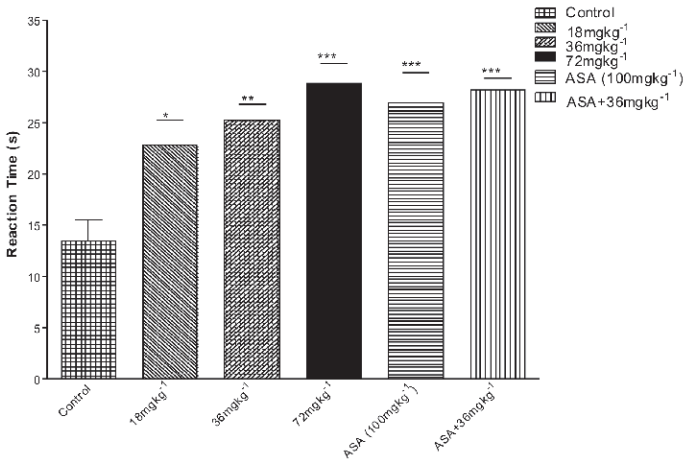

Fig. 1: *Effect of extract on thermally-induced pain (Hot plate) in mice*

Values represent Mean ± SEM; (n=6)
Significance relative to control: * p<0.05, *** p<0.001
ASA = Acetylsalicylic acid

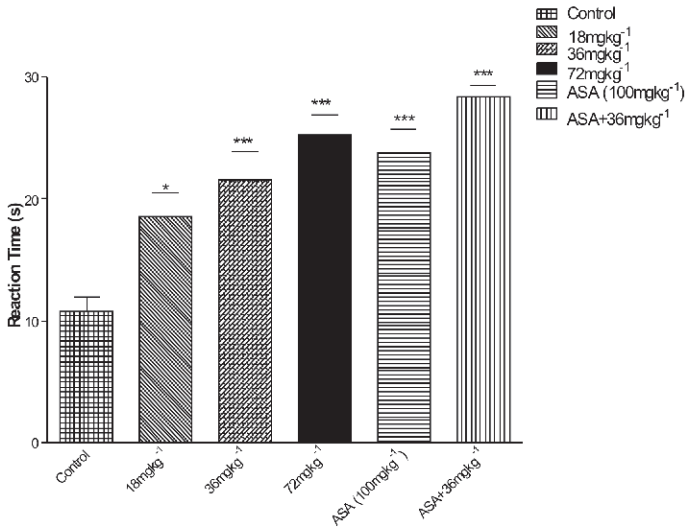

Fig 2 *Effect of extract on tail-flick response latency time in mice*

Values represent Mean ± SEM, (n=6)
Significance relative to control: * p<0.05, *** p<0.001
ASA = Acetylsalicylic acid

Table No.1 : Effect of extract on formalin-induced hind paw licking in mice.

Dose (mg/kg)	5mins	10mins	15mins	20mins	25mins	30mins
Normal saline + Formalin	24.50±1.93	5.17±1.28	6.00±1.16	3.17±1.08	3.67±0.72	5.00±1.39
8 mgkg^{-1} Extr + Formalin	9.00±0.73***	1.05±0.10***	1.33±0.21***	0.67±0.33*	0.67±0.31*	0.50±0.22***
36 mgkg^{-1} Extr + Formalin	7.00±0.37***	0.80±1.16***	1.00±0.37***	0.50±0.22*	0.17±0.17**	0.33±0.21***
72 mgkg^{-1} Extr + Formalin	7.17±1.25***	0.50±0.25***	0.50±0.34***	0.33±0.21*	0.50±0.34**	1.67±1.67***
ASA (100 mgkg^{-1})	8.67±0.99***	0.67±0.33***	0.50±0.34***	0.50±0.22*	1.67±1.67**	1.67±1.67***
ASA + 36 mgkg^{-1} Extr + Formalin	4.17±0.91***	0.45±0.42***	1.67±1.67***	0.33±0.21*	1.67±1.67**	0.00±0.00***

Values represent Mean ± SEM, (n=6)
Significance relative to control: * p<0.05, *** p<0.001
ASA = Acetylsalicylic acid

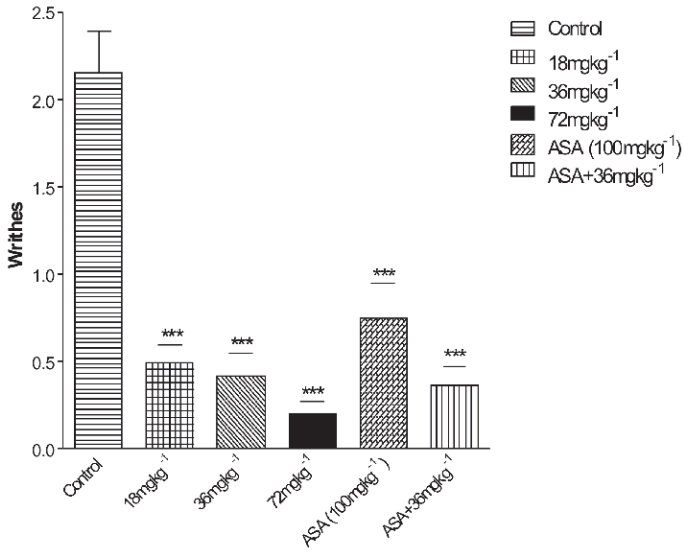

Fig. 3 *Effect of extract on acetic acid-induced writhing in mice*

Values represent Mean ± SEM; (n=6)
Significance relative to control: *** p<0.001
ASA = Acetylsalicylic acid

This inhibition which was statistically significant is dose dependent, with maximal effects observed in the 10th and 20th min respectively.

Effect of extract on tail-immersion test in mice

The result of tail-immersion test in mice is as shown Figs. 2. The extract showed inhibitory effect on tail-flick response. The latency time was prolonged. This effect was dose-dependent and statistically significant (p<0.05 0.001)

Effect of extract on acetic acid-induced writhing in mice

The result is as shown in Fig. 3. The extract inhibited the writhing reflex induced by acetic acid. The maximal reduction was in the 10th min of the test. The overall percentage analgesia and mean writhes were dose dependent and statistically significant, relative to control (p<0.001)

Garcinia kola stem bark extracts showed distinct analgesic effects.

In the writhing test, the acute peritonitis caused by the intraperitoneal administration of acetic acid produced a response characterised by contraction of the abdominal muscles followed by elongation of the body and extension of the forelimbs. This has been associated with increased levels of prostaglandins in the peritoneal fluids [12] and it is a sensitive procedure to establish peripherally acting analgesics [14]. From the above results, *Garcinia kola stem bark* extracts has a stronger peripherally acting analgesic action at the lower doses (18 and 36mg/kg) that significantly reduced acetic acid-induced writhing responses, with a higher percentage of writhing inhibition (analgesia) than acetylsalicylic acid (aspirin).

The Formalin test has two distinctive phases: the early and the late phases that can possibly indicate different types of pain [*10*].

It is a useful method for elucidating the mechanism of analgesia [15],[16].

The early phase, often called neurogenic or non-inflammatory pain, is a result of direct stimulation of nociceptors and reflects centrally mediated pain; the late phase, termed inflammatory pain, is due to local inflammation caused by a release of inflammatory and hyperalgesic mediators [10] [17]. The late phase responses are known to be attenuated by cyclooxygenase inhibitors, such as acetylsalicylic acid, because of their actions that ultimately diminish the mediators of inflammation and pain, such as prostaglandins [16] [14].

In the current study, the extract produced significant antinociceptive activity in the early and late phases of the formalin test. The inhibition of nociception in the late phase of the test suggests that an antiinflammatory action is contributory to the antinociceptive activity of *G. kola* stem bark.

Furthermore, the initial phytochemical screening of the extract in another study (unpublished) showed that it has moderate flavonoid content. Flavonoids are known to inhibit the cyclooxygenase and/or the lipoxygenase pathways of arachidonate metabolism [18]. Hence, the presence of flavonoids may be contributory to the analgesic activities of the extract.

The thermal model of the tail flick test is a spinal reflex; it also identifies central analgesics, whose actions involve the higher centers [16] .

The test differentiates between central and peripheral analgesics [19].

In this test, an increase in the reaction time is generally considered to be an important parameter for evaluating central antinociceptive activity [12].

CONCLUSION

G. kola stem bark extract showed a dose dependent increase in tail-flick response latency time, indicating increasing access to the higher centres with increase in dose.This result was corroborated by the hot plate test, which is a specific, supraspinal central antinociceptive test that also utilise the method of thermal induction of pain.These results therefore suggest that *G. kola* stem bark extract has central antinociceptive effect with two components: spinal, as shown from the tail immersion test [20], and supraspinal, as demonstrated by the hot plate method [21] [22]. Further studies may reveal the exact mechanism(s) of action responsible for the analgesic of the extract, but from the foregoing, the antinociceptive action of the stem bark of *G. kola* is due to both central and peripheral mechanisms.

ACKNOWLEGEMENT

The authors gratefully acknowledge the assistance of Ms. Sifonobong John Akpan and Mr. Nsikan Malachy Udo of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo.

REFERENCES

1. Keay R. W. J., Onochie C. F. A., Stanfield, D. P. Trees of Nigeria. Clarendon press Oxford, 1989 p 108-10.

2. Akpantah, A. O., Oremosu, A. A., Noronha. C. C., Ekanem, T. B. & Okanlawon, A. O. Effects of Garcinia kola seed extract on ovulation, oestrous cycle and foetal development in cyclic female Sprague-Dawley rats. Nig. J. Physiol. Sci. 2005, 20 (1-2): 58-62

3. Gill, L. S. and Akinwumi C. Nigerian Folk Medicine: Practises and Beliefs of Ondo people. J. Ethnopharmacol. 1986 18(3): 259-66.

4. Iwu, M. M., Igboko, O. A., Elekwa, D. K., Tempesta, M. S. Prevention of thioacetamide-induced hepatotoxcity of biflavanones of Garcinia kola. Phytother. Res. 1990, 4(4): 157-9.

5. Okonji, C. O. and Iwu, M. M. Control of Schistosomiasis using Nigerian medicinal plants as molluscicides. Int. J. Crude Drug Res. 1988, 26(4): 246- 52.

6. Lutete, T., Kambu, K., Ntondele, D., Cimanga, K., Luki, N. Antimicrobial activity of Tannins. Fitoterapia 1994, 65 (3): 267-78.

7. Zimmermann, M. Ethical considerations in relation to pain in animal experimentation. Acta Physiol. Scand. Suppl. 1986, 554: 22123.

8. Lanhers, M. C., Fleurentin, J., Dorfman, P., Motrier, F., Pelt, J. M. Analgesic, antipyretic and anti-inflammatory properties of Euphorbia hirta. Planta Medica 1991, (57) 225231.

9. Zhang Li, Juan-Juan Hu, Jian-Wei Lin, Wei-Shuo Fang, Guan-Hua Du. Anti-inflammatory and analgesic effects of ethanol and aqueous extracts of Pterocephalus hookeri (C.B. Clarke) Höeck J. Ethnopharmacol. 2009, 123: 510 - 14

10. Nwafor, P. A; Jacks, T. W. and Ekanem, A. U. Analgesic and Anti-inflammatory Effects of Methanolic Extract of Pausinystalia macroceras Stem Bark in Rodents. Int. J. Pharmacol. 2007 3(1): 86 - 90.

11. Wang, Y. X., Gao, D., Pettus, M., Phillips, C., Bowersox, S. S. Interactions of intrathecally administered ziconotide, a selective blocker of neuronal N-type voltage-sensitive calcium channels, with morphine on nociception in rats. Pain 2000 ,(84), 271281.

12. Vilela , F. C , Marina de Mesquita Padilha, Lucas dos Santos-e-Silva, Geraldo Alves-da-Silva, Alexandre Giusti-Paiva. Evaluation of the antinociceptive activity of extracts of Sonchus oleraceus L. in mice. J. Ethnopharmacol. 2009, 124: 30610

13. Collier, H. O., Dinneen, L. C., Johnson, C. A., Schneider, C., The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br. J. Pharmacol. Chemother 196832: 295310.

14. Chakraborty, A., Devi, R. K., Rita, S., Sharatchandra, K., Singh, T. I. Preliminary studies on anti-inflammatory and analgesic activities of Spilanthes acmella in experimental animal models. Ind. J. Pharmacol. 2004; 36: 148-50

15. Shibata, M., Ohkubo, T., Takahaski, H., Inoki, R. Modified formalin test: characteristic biphasic pain response. Pain 1989, 38: 34752.

16. Nonato F. R, Barros ,T. A. A , Lucchese ,A. M, Oliveira C. E. C, dos Santos R. R , Soaresa M. B. P , Villarreal ,C. F. Antiinflammatory and antinociceptive activities of Blechnum occidentale L. extract. J.Ethnopharmacol. 2009, 125: 1027

17. Yaksh, T. L., Ozaki, G., McCumber, D., Rathbun, M., Svensson, C., Malkmus, S., Yaksh, M. C. An automated flinch detecting system for use in the formalin nociceptive bioassay. J. Appl. Physiol. 2001, 90: 2386402.

18. Kwak, W. J., Moon, T. C., Lin, C. X., Rhyn, H. G., Jung, H. J., Lee, E., Papyriflavonol A from Broussonetia papyrifera inhibits the passive cutaneous anaphylaxis reaction and has a secretory phospholipase A(2)-inhibitory activity. Biol. Pharm. Bull. 2003 26, 299302.

19. Asongalem, E. A., Foyet, H. S., Ekobo, S., Dimo, T., Kamtchouing, P. Anti-inflammatory, lack of central analgesia and antipyretic properties of Acanthus montanus (Ness) T. Anderson. J. Ethnopharmacol. 2004, 95: 6368.

20. Luttinger, D. Determination of antinociceptive efficacy of drugs in mice using different water temperatures in a tail-immersion test. J. Pharmacol. Methods 1985, 13: 351357.

21. Yaksh, T. L., Rudy, T. A. Studies on the direct spinal action of narcotics in the production of analgesia in the rat. J. Pharmacol. Exp. Ther. 1977, 202: 41128.

22. Yeung, J. C., Yaksh, T. L., Rudy, T. A. Concurrent mapping of brain sites for sensitivity to the direct application of morphine and focal electrical stimulation in the production of antinociception in the rat. Pain 1977: 2340.