12/10/01

Afr. J. Med. med. Sci. (2011) 40, 47-50

Analgesic activity of aqueous leaf extract of *Phyllanthus Amarus*

BO Iranloye[1], VB Owoyele[2], OR Kelani[2] and SB Olaleye[3]

Department of Physiology [1], Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Department of Physiology and Biochemistry[2], Faculty of Health Sciences, Universiy of Ilorin and Department of Physiology[3], Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria

Summary

Various doses of the aqueous extract of *Phyllanthus amarus* (AEPA) were investigated for analgesic and anti-inflammatory activities using both thermal and chemical models of pain assessment in rats. The extract caused a significant (P< 0.05) dose related increase inhibition of the carrageenan-induced paw oedema in the rats. The inhibition produced by 200mg/kg AEPA (70.20%) was significantly higher than that of the reference drug (Acetylsalicylic acid). The extract produced a marked analgesic activity by inhibiting both early and late phases of pain stimulus in Formalin-induced paw licking rats and also a significant and dose related increase in inhibiting the mean tail immersion duration (MITD) at varying water bath temperature (50°C, 55°C and 60°C). This study thus established the anti-inflammatory and analgesic activities of *Phyllanthus amarus.*

Keywords: *Phyllanthus amarus; analgesic; pain*

Résumé

Plusieurs doses des extraits aqueuses de *Phyllanthus amarus* (AEPA) étaient investigué pour des activités analgésique et anti-inflammatoire utilisant des modèles à la fois thermaux et chimiques de détermination de la douleur chez les rats. Les extraits causaient une dose importante (P< 0.05) lie a l'augmentation de l'edeme induit par la carrageenane chez les rats. L'inhibition produise par 200mg/kg AEPA (70.20%) était significativement plus élevé que ceux des médicaments de référence (L'acide Acétylsalicylique). Les extrait produisaient une activité analgésique L'extrait produisait une activité analgésique considérable en inhibant pendant la phase précoce et tardive la stimulation de la douleur induit par l'injection de la formaline au rats et augmentation croissante en inhibant la durée de la moyenne d'insertion de la queue (MITD) dans de l'eau a des températures variées (50°C, 55°C and 60°C). L'étude, ainsi a établit les activités anti-inflammation et analgésiques de *Phyllanthus amarus.*

Introduction

Phyllanthus amarus is an annual herb common in Sierra Leone, and the southern part Nigeria. It is often seen as weed on uncultivated land. It is called "*dobisowo*" or "*ehin olobe*" or "*eyin olubi n sowo*" in Yoruba part of Nigeria and "ngwu" by the Igbo tribe and "buchi oro" by the Asaba people, all in Nigeria [1]. Phyllanthus amarus has been used locally in the treatment of many diseases and in different countries all over the world. In Ivory Coast, it has a lot of magical uses; it is used to counter coastal pain and sore- throat. In Kenya, it is used to relieve stomach pain, in Angola; it is widely used against diarrhoea and amoebic dysentery. In Western part of Nigeria, it is used as an ingredient of "agbo" and infusion of its leaves is used for haemorrhoid. It has also been used traditionally in the treatment of jaundice, gonorrhea, and frequent menstruation, diabetes and also in the treatment of dysmenorrhea or menstrual pain [1]. Phyllanthus amarus.is tropically used as a poultice for skin ulcer, sores, swelling and itchiness [2, 3]. It has been shown that the methanoic extract of Phyllanthus amarus has a potential anti-oxidant activity and so inhibit lipid peroxidation as well as reduce blood glucose in alloxan-diabetic rats [4]. It is also used in the treatment of viral hepatitis [5] and it inhibits HIV-1 replication [6]. Anti-mutagenicity of methanoic extract of Phyllanthus amarus was reported in Salmonella typhimuriumo [7].

No report is available in literature to establish the analgesic use of this plant. Based on the traditional use of this plant in relieving pain especially among the Yoruba tribe of Nigeria (who believe that consumption of this leaf induces labour and reduce dysmenorrhea) this study therefore investigated the effect of various concentrations of the aqueous extract of the leaves of

Correspondence: Dr. Bolanle O. Iranloye, P.O.Box 21, Marina, Lagos. Nigeria. E-mail: bolasunkanmi45@yahoo.com

Phyllanthus amarus on pain (thermally and chemically induced) as well as its anti-inflammatory activities.

Materials and methods

Leaves of Phyllanthus amarus were obtained locally in Ilorin, Nigeria and certified/authenticated by F.A Oladele Professor of Botany at the Dept of Botany. University of Ilorin.

Plant materials

The leaves were dried and grinded to fine powder. 50g of the powdered leaves were stirred into 2L of the distilled water and left for 24hours. This was then filtered and concentrated (1ml of the extract being equivalent to 20mg of the starting material) and the extract regenerated at 4°c

Animal

75 adult male albino rats weighing between 150g and 170g were obtained from the animal house, Department of Veterinary Medicine, University of Ibadan, Nigeria. They were then housed in cages at room temp at the Department of Physiology, University of Ilorin and allowed free access to rat cubes and water.

Analgesic activity

The aqueous extract of Phyllanthus amarus was evaluated for analgesic activity in rat using Formalin [8] and the tail immersion test [9].

Fomalin-induced paw licking in rat

Rats were treated orally with 50,100 and 200mg/kg body weight of aqueous extract of Phyllanthus amarus (AEPA), reference drug (5mg/kg indomethacin) and vehicle (10ml/kg of distilled water). 60mins after treatment in each group, 20ìl of 3% Formalin was then injected into the dorsal surface of the left hind paws as described by Hunskaar and Hole [8].

The rats were observed for 30minutes. The time spent by the rat in licking the injected paw was recorded as the licking time. Observations were made for the first 5mins post-formalin, i.e. 0-5mins (early phase) and for the last 10min starting at the 20th min post Formalin injection (late phase).

Tail Immersion Test

Rats were treated orally with 50,100,200mg/kg body weight of the aqueous leaf extracts of Phyllanthus amarus (AEPA), reference drug (5mg/kg body weight indomethacin) and vehicle (10ml/kg body weight distilled water) 60mins before the measurement of extract effect. Water was heated to different temperature 50 ± 1.0°C, 55 ± 1.0°C and 60 ± 1.0°C in 3 different water baths. Rats were wrapped carefully with towel leaving only the head and tail free. The tail was then immersed gently in the hot water bath. The time taken for the animal to flick his tail out of each of the water baths was obtained and the average, expressed as the mean tail immersion duration (MTID) was recorded before and after treatment. The percentage protection was then calculated using the ratio:

$$\frac{\text{(Mean duration) Control} - \text{(Mean duration) treated}}{\text{(Mean duration) Control}} \times 100$$

Anti-inflammatory activity

The effect of oral administration of 50,100 and 200mg/kg body weight of aqueous extract of Phyllanthus amarus, 150mg/kg body weight acetylsalicylic acid (Dysprin - Reckitt and Coleman) and vehicle (distiled water 10ml/kg) on the hind-paw oedema induced by subplantar injection of 0.1ml of 1% carrageenan.was evaluated as described by Winter *et al* [10]. Paw-oedema was measured using cotton thread wrapped round the paw and measuring the circumference with a metre rule [11]. Measurements were done immediately before and 3 hours following carrageenan injection.

The inhibitory activity was calculated using the formula below:

$$\text{Percentage Inhibition} = \frac{(Ct - Co)\text{Control} - (Ct - Co)\text{treated}}{(Ct - Co)\text{ Control}} \times 100$$

Inhibitory activity at 3 hours was taken as a measure of oedema.

Statistical analysis

All values were expressed as Mean ± S.E.M. for the experiment on formalin. Statistical comparison was done using the student's-test. Pair t-test was used to compare pain responses before and after treatment with extract, reference drug or vehicle in the tail flick test.

Result

Analgesic activity

Formalin-induced paw licking in rats

The aqueous extract of Phyllanthus amarus (50,100 and 200mg/kg) caused significant ($p < 0.05$) inhibition of both the early and late phases of Formalin-induced pain response. The mean pain scores (licking response) in

both the early and late phases for rats treated with 50mg/kg AEPA were not significantly different ($p > 0.05$) from that of the vehicle treated (control) rats. (Table1)

Table 1: Effect of AEPA on formalin-induced paw licking in rats.

Treatment with doses	Licking time (seconds) Early phase	Late phase
Vehicle (Distilled water 10ml/kg)	50.8 ± 2.6	16.4 ± 2.3
50mg/kg AEPA	48.4± 2.77	15.6 ± 2.1
100mg/kg AEPA	41.4 ± 2.6	9.2 ± 1.5
200mg/kg AEPA	34 ± 2.77	7.6 ± 2.3
Reference(5mg/kg/ Indomethacin)	23.2 ± 1.56	7.2 ± 1.9

Latency of tail immersion in rats

In Table 2 it is shown that treatment with the vehicle did not have any significant ($p > 0.05$) effect on the latency of the mean tail immersion duration (MITD) before and 60min after treatment. AEPA at all doses tested showed significant ($p < 0.05$) and dose related increase in MITD.

Table 2: Effect of various doses of aqueous extract of Phyllanthus amarus (AEPA) on the mean tail immersion duration.

Treatment with doses	Mean Reaction Time (Seconds) ± S.E.M. Pre-treatment	Post-treatment
Vehicle (Distilled water)	6.2 ± 1.13	6.8 ± 1.04
50mg/kg AEPA	6.3 ± 0.16	8.07 ± 0.5*1 (18.68%)
100mg/kg AEPA	6.75 ± 0.11	9.5 ± 0.58** (41.18%)
200mg/kg AEPA	6.4 ± 0.16	12.6 ± 0.66** (85.29%)
Reference (Indomethacin 5mg/kg)	6.85 ± 0.2	16.74 ± 0.2** (146 18%)

**p<0.05, **p< 0.001 (c.f. vehicle, paired t-test, n=10 Values in parenthesis represent percentage protection.*

Anti-inflammatory activity.

The anti-inflammatory potencies of aqueous extract of Phyllanthus amarus are shown in Table 3. The extract significantly ($p < 005$) and progressively inhibited the inflammatory oedema. The maximum inhibition of oedema attained in rats pre-treated at 100mg/kg AEPA (59.97%) is not significantly ($p > 0.05$) different from that given by 150mg/kg Acetylsalicylic acid (60.02%). The inhibition produced by 200mg/kg AEPA (70.20%) was significantly higher than that of the reference drug (Acetylsalicylic acid).

Table 3: Effect of various doses of aqueous extract of Phyllanthus amarus (AEPA) on carrageenan-induced paw oedema in rats.

Treatment with doses	Paw size in mm (Mean ± S.E.M.)	Inhibition (%)
Control (Distilled water 10ml/kg)	3.23 ± 0.07	-
AEPA 50mg/kg	2.84 ± 0.04	39.42%
AEPA 100mg/kg	2.61 ± 0.07	59.97%
AEPA 200mg/kg	2.49 ± 0.05	70.20%
Acetylsalicylic Acid (150mg/kg)	2.72 ± 0.04	60.02%

Discussion

In this present study the analgesic and anti-inflammatory properties of aqueous leave extract of Phyllanthus amarus has been established.

Aqueous leave extract of Phyllanthus amarus has been found to contain some bioactive constituents such as saponins, flavonoids, tamins, alkaloids, sugar and vitamins (E & A) [12]. Flavonoids have been shown to have a dichotic, laxative, anti-spasmodic, anti-hypertensive and anti- inflammatory actions [13]. The analgesic and anti-inflammatory activities of this extract may then be linked with the presence of an anti-oxidative constituent – flavonoids. Flavonoids have been reported to poses anti-oxidant and antiradical properties [14, 15]. The carrageenan rat paw edema is a suitable method used in evaluating anti-inflammatory drugs. The mechanism involved in the genesis of the carrageenan - induced edema can cause the release of prostaglandins and kinnins [16]. Inflammatory pain results from the release of hyperanalgesic mediators like prostaglandin and catecholamines which are believed to act by regulating the pain receptor sensitivity [17]. Thus the anti-inflammatory mechanism of action of Phyllanthus amarus may also be due to prostaglandin synthesis inhibition as described for anti-inflammatory mechanism of aspirin-like drugs [18].

Recently, the potential anti-oxidant activity of this extract was shown [5]. The analgesic evaluation methods in this study were chosen to test the different nociceptive stimuli (thermal and chemical). The result

showed that progressive increase concentration of the aqueous leave extract of Phyllanthus amarus protects the animals from pain produced by both chemical (formalin-induced paw licking) and thermal (tail immersion) stimuli. This confirms in part the antecdotel use of Phyllanthus amarus in pain relief like dysmenorrhea (belief to be caused by prostaglandin) and stomach pain. Further study is needed to give the analgesic activity profile of the extract.

References

1. Adjahloun and Ake Assi: Useful plants of West Africa. In: H.M. Biokill. (Eds.), 1972 vol 2. 119.
2. Bharatiya V.B. Selected medicinal plants of India. Bombay. Tata press, 1992; 235-237.
3. Nadkami, K. M.. India material medica, vol. 1. Bombay popular prakashan private Ltd. 947-948.
4. Raphael, K.R., Sabu, M.C. and Kuttan, R., Hypoglycaemic effects of methanol extract of Phyllanthus amarus Schum and Thonn on alloxan-induced diabetesmellitus in rats and its relation with antioxidant potential. Indian J Exp Biol. 2002 Aug; 40(8), 905-909.
5. Kiemer A. K,.Hartung T., Huber C., Vollmar A.M. Phyllanthus amarus has anti-inflammatory potential by inhibition of iNOS, COX-2, and cytokines via the NF-kappaB pathway. J Hepatol. 2003; 38(3): 289-297.
6. Notka, F., Meier, G.R. and Wagner, R. Inhibition of wild-type human immunodeficiency virus and reverse transcriptase inhibitor-resistant variants by Phyllanthus amarus., Antiviral Res.; 2003; 58(2):175-186.
7. Raphael, K.R., Ajith, T.A., Joseph, S and Kuttan, R., Anti-mutagenic activity of Phyllanthus amarus Schum & Thonn in vitro as well as in vivo Teratog Carcinog Mutagen. 2002; 22(4):285-291.
8. Hunskar S. and Hole, K.,. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1997; 30: 103 –114.
9. Owoyele B.V., Olaleye S.B., Oke J.M. and Elegbe R.A:, Anti-inflammatory and analgesic activities of leaf extracts of Landolphia owariensis Afr. J.Biomed. Res. 2001; Vol.4: 131-133.
10. Winter, C.A., Risley, E.A. and Nuss C.W., Carrageeenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biol. and Med. 1962; 111: 544-547.
11. Bamgbose S.O.A and Noamesi B.K.,: Studies on cryptolepine inhibition of carrageenan-induced oedema. Planta medica 1981; 2: 392 –396.
12. Harbone. J.B. Bioactive Constituents of plants. A guide to modern tech. of plant analysis, 1973; 46-201
13. Okuda T. Flavonoids. In chemistry of organic natural products. Ed. By H. Mitsuhashi,O.Tanaka. S. Nazoe and M. Nagai. Nankodo, Tokyo. 1961; 219-228.
14. Robaki, Glyglewski R.J. Flavonoids are scavengers of superoxide anim. Biochem. Pharmalcol. 1988; 37: 837.841.
15. Birs. W., Heller W., Michael C. and Satan M. Flavonoids as antioxidant determination of antiradical efficiencies. Methods litcymal, 1991; 186: 343 –353.
16. Garcia Leme J., Nakamura L., Leite M. P. Rocha E and Silva, M. Pharmacological analysis of the acute inflammatory process induced in rat's paw by local injection of carrageenan and by heat-ing. Br. 1. Pharmacol.,1973; 48: 88-96.
17. Ferreria, S. H. Prostaglandins, aspirin-like drugs and analgesia. Nat. New Biol.,1972; 240: 200-203.
18. Ferreria, S. H., Moncada, S. and Vane, 1. R. Further experimentation to establish that the anal-gesic action of aspirin-like drugs depends on the in-hibition of prostaglandin biosynthesis. Br. 1. Pharmacol., 1973; 47: 48-58.

Received: 27/05/10
Accepted: 15/12/10

12/10/11