www.ajbrui.net

Afr. J. Biomed. Res. Vol.18 (September, 2015); 217- 223

Full Length Research Paper

Analgesic and Central Nervous System Depressant Activities of Kolaviron (a *Garcinia kola* biflavonoid complex)

Ibironke G.F and Fasanmade A.A

Department of Physiology College of Medicine University of Ibadan Nigeria

ABSTRACT

This study evaluated the analgesic and CNS depressant activities of kolaviron (KV) .The analgesic potential was measured using thermal (hot plate and tail withdrawal) and chemical (acetic acid-induced writhing) algesiometric tests, while the CNS depressant activity was evaluated by observing the reduction of locomotor and exploratory activities in the open- field the and whole board tests respectively at a doses of 50, 100 and 200mg/kg. The animals were also pre-treated with the ATP-K+ sensitive receptor antagonist, glibenclamide and L-arginine for the evaluation of the mechanism of its analgesic activity. The results showed that KV extract significantly ($p<0.05$) exhibited a dose dependent antinociceptive activity as shown by the prolongation of both the tail withdrawal and hot plate latencies in the thermal tests and a reduction in the number of writhes in the acetic acid-induced writhing test . Pre- treatment with glibenclamide and L- arginine was found to potentiate the previously observed analgesia as shown by greater reductions in the number of writhes in the acetic acid –induced writhing test. The extract at the same doses demonstrated a CNS depressant activity as indicated by a reduction in the number of head dips in the hole board test and a fall in the rearing, total locomotion and grooming frequencies in the open-field test. These results suggest the involvement of the nitrergic and ATP-K+ sensitive pathways in kolaviron analgesia and the possession of central nervous depressant activities.

Key words: analgesia, neurobehavior, kolaviron, algesiometric tests

INTRODUCTION

Kolaviron is a biflavonoid complex from *Garcinia kola* seeds belonging to the family Guttiferae.The tree is widespread in green forests and is found along the Atlantic coast from the democratic republic of Congo to Ghana .

Kolaviron is a mixture of three compounds , Garcinia biflavonoid GB1, GB2 and Kola flavanone in a ratio of 2:2;1 (Iwu and Igboko ,1982) . Phytochemical analysis revealed the presence of flavonoids , xanthones, benzophenones, saponins and tocopherols (Okereke *et al*,2014) The health effects of flavonoids have been attributed to their actions as anti-oxidants, free radical scavengers and inhibition of peroxidase reactions (Lichem *et al*,2006) .The extract has been credited with many therapeutically proven values , for example, it has been used in West Africa as part of several preparations for treating diseases such as edema, bronchitis, abcesses and cough (Ayensu,1993). Several

*Corresponding author: (*E-mail):*
gibironk@yahoo.com
Tel: +234-8058826713

Date Received: March, 2015
Date Accepted:, June, 2015

Abstracted by:
Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

other reports on its therapeutic efficacy have also been published , for example, the analgesic and anti-inflammatory activities (Olaleye *et al* ,2000), protection against testicular damage (Olayinka and Ore, 2014) ,ameliorative effects against reproductive toxicity in rats (Adaramoye *et al*, 2012), neuroprotective effect (Ijomone *et al*, 2013), protection against dextran sulphate induced colitis (Farombi *et al*, 2013) , scavenging potentials (Olatunde *et al*, 2012) and cardioprotective activities (Nwane-*et al* al, 2014).

However the underlying mechanisms accounting for its analgesic actions and its pshychoactive activities are yet to be fully characterized, this study therefore looked into the effect of the extract on pain perception and neurobehavior in mice

MATERIALS AND METHODS

Plant material: Seeds of *Garcinia kola* were bought from Bodija market in Ibadan, Oyo state, Nigeria. They were identified by the taxonomist at the Forestry Research Institute at Jericho in Ibadan where a voucher specimen was deposited.

Preparation of extract: The kolaviron extract was prepared according to Iwu (1990).The full details of KV extraction from Garcinia kola seeds have been documented (Olaleye 2005, Olaleye and Farombi, 2006). Briefly, powdered seeds were extracted with light petroleum ether in a soxhlet extractor for 24 hours. The deffated dried marc was repacked , concentrated and diluted to twice its volume with ethyl acetate to give a yellow solid known as kolaviron , a mixture of Garcinia biflavanones GB1,GB2 and kolaflavanonone (KF) .

Chemicals: The following drugs and chemicals were used: Acetic acid (BDH, UK), glibenclamide (Sarnoti-Aventis,Nigeria) . All other drugs were of analytical grade obtained from a local pharmaceutical outfit in the city of Ibadan, Nigeria .

Animals: Adult male Swiss mice (20-25g) obtained from the animal house , College of Medicine , University of Ibadan ; Nigeria were used for the study .They were housed in cages under standard laboratory conditions (room temperature and 12h light/dark cycle) and fed with mouse cubes (Ladokun feeds ,Nig . Ltd, Ibadan) and water ad libitum .

Antinociceptive assay

The animals were grouped 1-V and administered 10ml/kg normal saline, 50,100 and 200mg/kg extract per oral (p.o) and 10mg/kg indomethcin intraperitoneally (i.p) to groups 1-V respectively for the antinociceptive assay.

Tail withdrawal test: The technique of D´Armour and Smith (1941) was used .Briefly , the animal was gently held with a hand towel and the terminal 3cm of the tail was gently lowered into a water bath maintained at a temperature of 50±0.2^{o}C . The time taken for the animals to flick its tail out of hot water was taken as the tail flick latency .

Hot plate test: The technique proposed by Eddy and Leimbach (1953) as modified by Ibironke *et al* (2004) was employed .The animals were divided into five main groups (1-V) and treated as follows : Groups 1-V were given normal saline (10ml/kg), 50, 100 and 200mg/kg KV extract per oral and 10mg/kg indomethacin intraperitonally (i.p) respectively .One hour later, the animals were placed individually on a hot plate maintained at temperature of 52±0.2^{o}C and the time taken for the animals to start licking the paws or jump off the hot plate was taken as the hot plate latency .At no time was any animal allowed to stay on the hot plate for more than 60s to avoid excessive tissue damage .

Acetic acid writhing test: The method of Siegmund *et al* (1957) as modified by Konster *et al* (1959) was employed .The animals were grouped and treated as described above .Thirty minutes after the various treatments the animals were given 0.2ml of 3% acetic acid solution i.p. to induce the characteristic writhing . The number of writhes occurring within a 10-minute period after injection was calculated for each group.

Behavioral evaluation

A similar grouping to the one used in the nociceptive essay was used except that the reference group (V) was given 1mg/kg diazepam as the reference drug.

The hole- board test: This test was carried out using a wooden board measuring 40×40cm with 16 evenly spaced holes (File and Pellow,1985)) The mice were grouped 1-V and then given 10ml/kg normal saline,50,100 , 200mg/g KV extract all p.o and 1mg/kg diazepam (i.p) respectively .One hour later the mice were placed on the hole- board apparatus and the number of head dips into the holes in a 5min trial was counted . At the end of each test, the board was cleaned was cleaned with 70% alchohol to eliminate olfactory bias.

Open –field test: The open-field box is a rectangular arena composed of a hard floor measuring

36cm×36cm×26cm and made up of a white painted wood .The floor was divided by permanent red markings into 16 equal squares at the bottom .Generally spontaneous motor activity was monitored for 30min in the open- field as described by Ajayi and Ukponman (1994) .The animals were similarly grouped 1-V and treated with 10ml/kg normal saline,50,100,200mg/kg KV extract (p.o.) and 1mg/kg diazepam (i.p) respectively .One hour later the animals were each placed in one corner of the box and the total locomotion (number of floor units entered with all four paws), rearing frequency (number of times the animal stood up on its hind limb or with the fore limbs against the wall of the observation box or free in the air) and grooming frequency (number of body cleaning with paws or picking of the body and pubis with mouth and face washing actions) within each 10 min interval were recorded .The arena was cleaned with 70% ethanol to eliminate olfactory bias and allowed to dry before introducing a fresh animal .

Statistical analysis: Values were expressed as mean ± SEM. The data were analyzed using student´s t –test .A value of $p<0.05$ was considered significant.

RESULTS

Tail withdrawal test: Administration of kolaviron resulted in a prolongation of the tail withdrawal latency which was significant ($p<0.05$) at 100 and 200mg/kg dose levels compared with control (fig 1) The response of the reference drug , indomethacin was greater than that of the 200mg/kg KV ($p<0.05$ Vs $p<0.01$) compared with control.

Tail flick test : The effects of kolaviron on the tail flick latency are as shown in Fig 2 . The results showed that kolaviron (50-200mg/kg) exhibited a significant ($p<0.05$) inhibition of pain perception as shown by a prolongation of the hot plate latency .The inhibition produced by the 200mg/kg dose of the extract was comparable with that of the reference drug , indomethacin .(10mg/kg) .

Acetic acid writhing test: The results showed that the extract (100 and 200mg/kg) and the reference drug (10mg/kg) significantly ($p<0.05$) reduced the number of writhes produced by acetic acid compared with the control (Fig. 3). Inhibition at the 50mg/kg dose level was not significant.

Effect of ATP-K+ sensitive receptor antagonist and L-arginine pre-treatment on kolaviron antinociception: The results are as shown in Figs 4 and 5. Pre –treatment with glibenclamide (Fig. 4) and L-arginine (Fig. 5) resulted in greater reductions in the number of writhes produced by kolaviron alone.

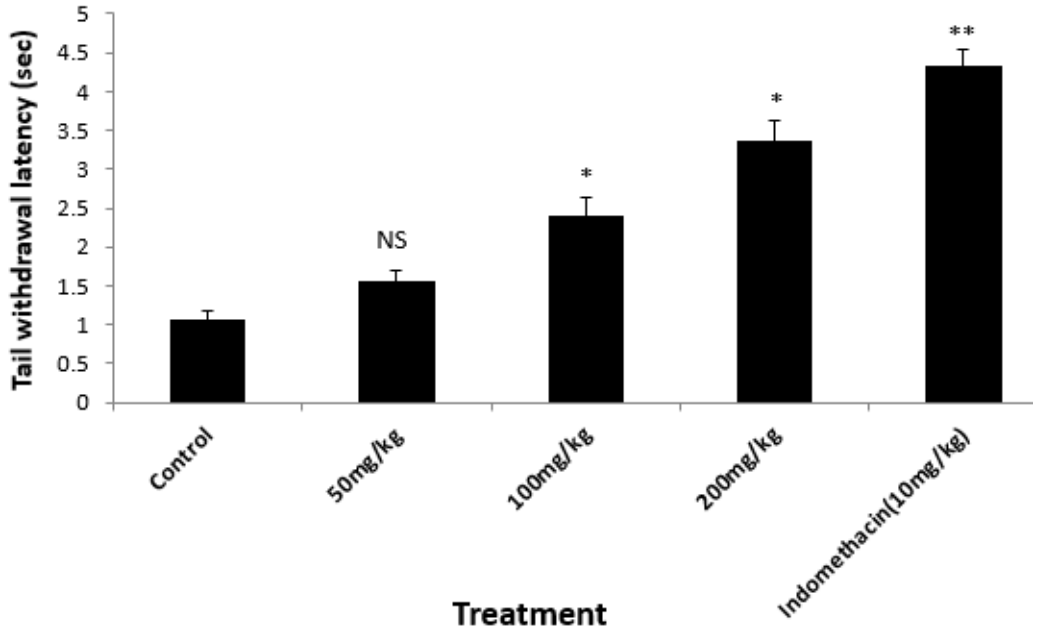

Figure 1:
Effect of kolaviron on tail withdrawal latencies. Values are means ± SEM, n =6. *P< 0.05, **P< 0.01, NS= Not significant compared with control

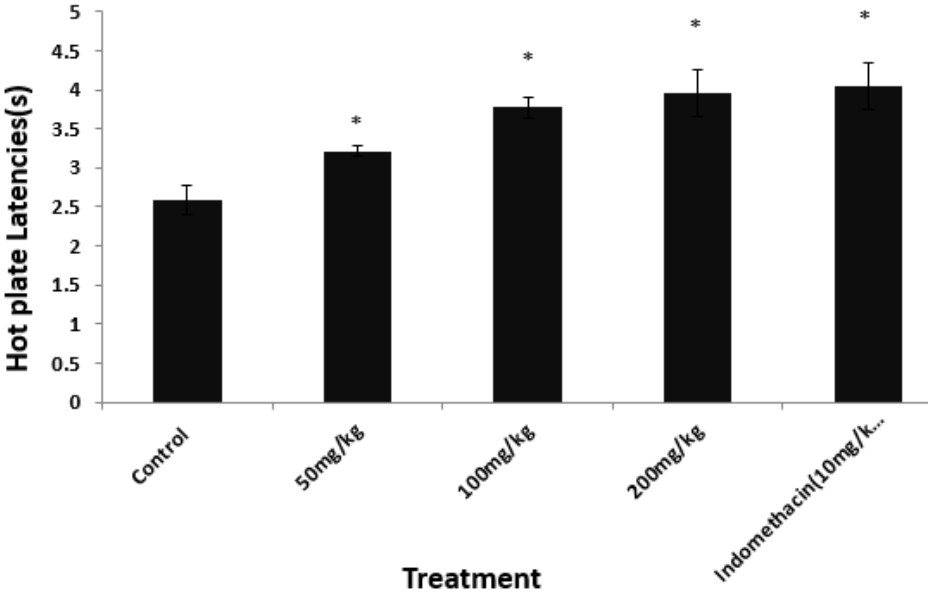

Figure 2
Effect of kolaviron on hot plate latencies in rats. *Values are means± SEM, n =6. *P<0.05, NS= Not significant compared with control

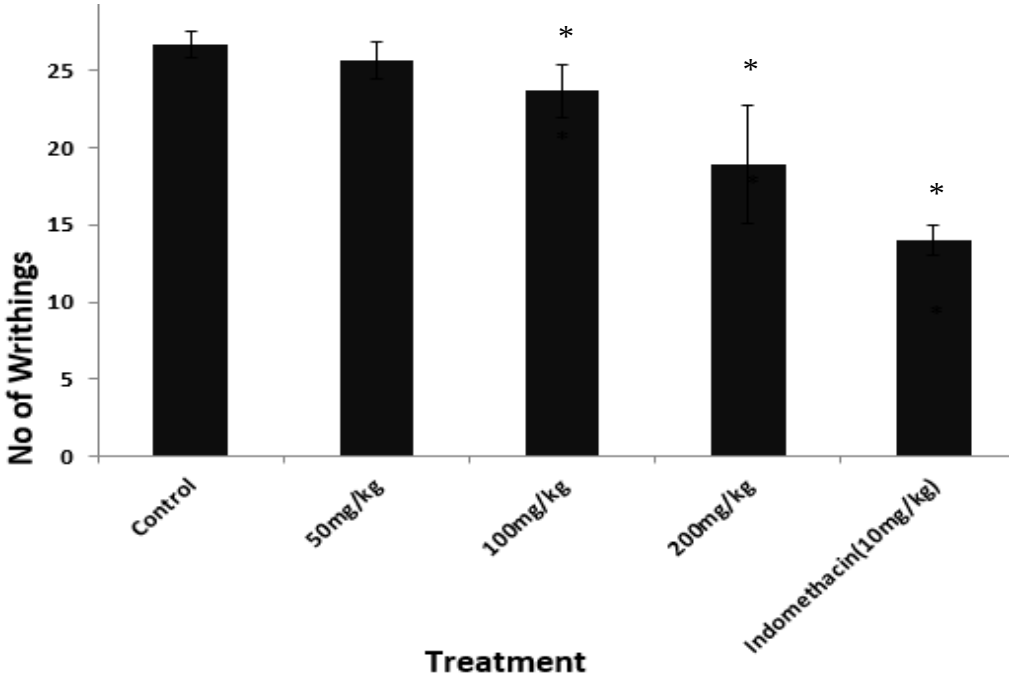

Figure 3
Effect of kolaviron on the number of writhings in the acetic acid induced writhing test. Values are mean ± SEM , n =6. * P<0.05 compared with control, NS= Not significant compared with control.

Hole-board test

The results showed that the extract (50-200mg/kg) significantly (p<0.05) caused a reduction in the number of head dips compared with the control (Fig. 6). The increase in the number of head dips by the reference drug was not significant.

Open –field test

Locomotion frequency : Administration of Kolaviron resulted in a reduction of the locomotion frequency (fig 7) which was only significant at the 200mgdose level compared with the control.

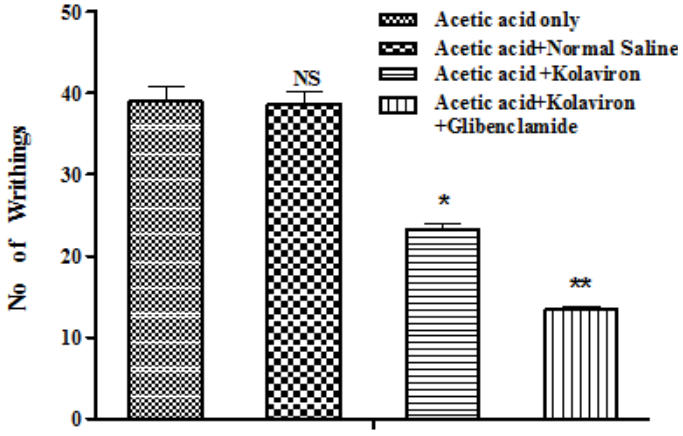

Figure 4
Effect of glibenclamide (3mg/kg) pre-treatment on kolaviron antinociception in the acetic acid- induced abdominal writhing test in mice. (NS=Not Significant, *p<0.05, **p<0.01).Values are expressed in means ± SEM (n=6)

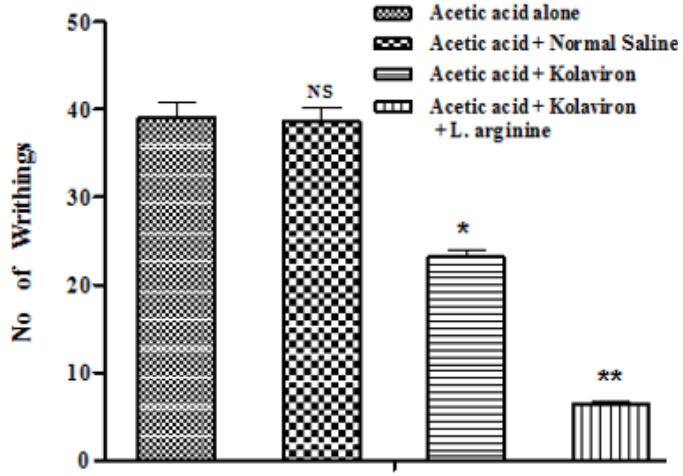

Figure 5
Effect of L- arginine (600mg/kg) pre-treatment on kolaviron antinociception in the acetic acid- induced abdominal writhing test in mice. (NS=Not Significant, *p<0.05, **p<0.01).Values are expressed in means ± SEM (n=6)

Grooming frequency: Graded doses of KV extract resulted in reductions in the grooming frequency which were significant at different dose levels (50 and100mg/kg, $p<0.05$ and 200 mg/kg, $p<0.01$) compared with the control (fig 8) .The grooming frequency was insignificantly increased by the reference drug, diazepam

Rearing frequency: The rearing frequency was significantly (50 and 100mg/kg, $p<0.05$ and 200mg/kg, $p<0.01$) reduced by the administration of the KV extract compared with the control. (Fig. 9). However, the rearing frequency was insignificantly increased above the control value by the reference drug, diazepam compared with the control

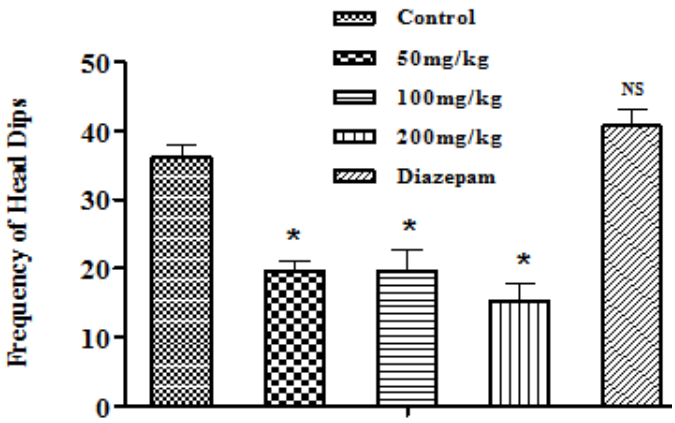

Figure 6
Effect of graded doses of kolaviron on the frequency of head dips in the hole board test. Values are expressed in means ± SEM (n=6). *p<0.05, NS=not significant, compared with the control.

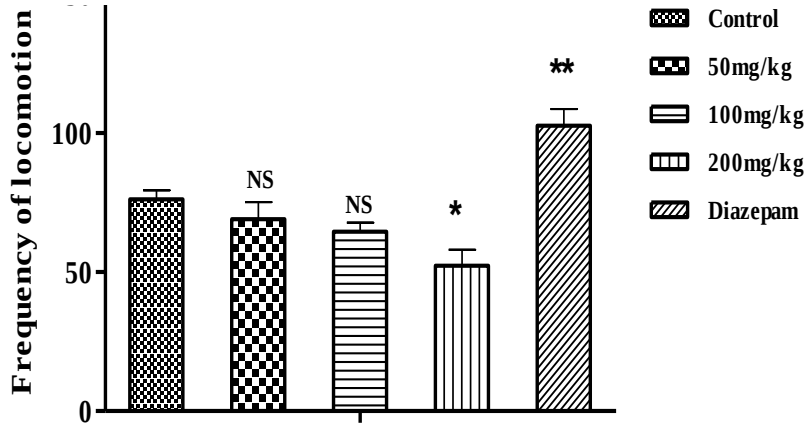

Figure 7
Effect of graded doses of kolaviron on locomotion frequency in the open field test. Values are expressed in means ± SEM (n=6). *p<0.05, **p<0.01, NS= not significant compared with the control.

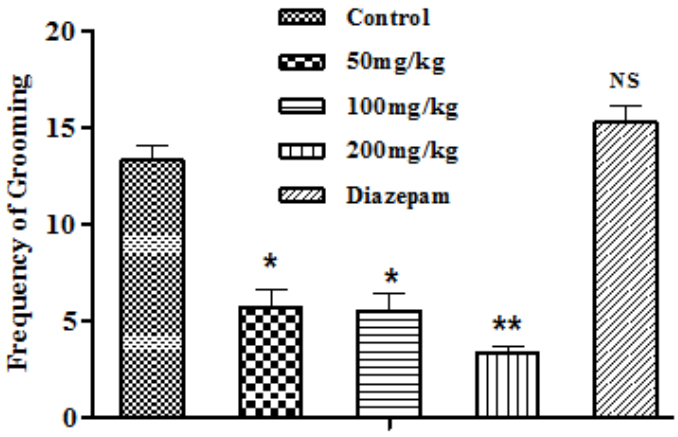

Figure 8
Effect of graded doses of kolaviron on the grooming frequency in the open field test. Values are expressed in means ± SEM (n=6). *p<0.05, **p<0.01, NS=not significant compared with the control

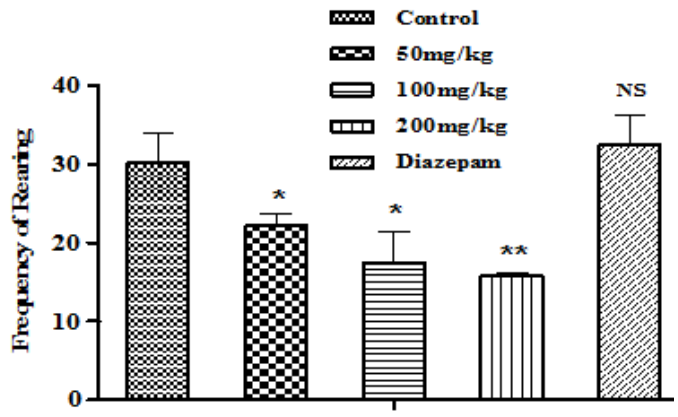

Figure 9
Effect of graded doses of kolaviron on the rearing frequency in the open field test. Values are expressed in means ± SEM (n=6). *p<0.05, **p<0.01, NS=not significant compared with the control.

DISCUSSION

This study provided evidence for the analgesic and central nervous system depressant properties of kolaviron .The findings on the analgesic activities of the extract agree with previous reports (Olaleye *et al*, 2000) on kolaviron antinociception though these authors used only one thermal test of nociception. In this study, we employed two thermal tests (tail withdrawal and hot plate) and the chemical (acetic acid induced –writhing) algesiometric tests.

In the antinociceptive tests, we observed a prolongation of both the tail flick and the tail withdrawal latencies in these animals and a reduction in the number of writhes produced by acetic acid in the writhing test both of which are suggestive of reduced pain sensation. The hot plate test is commonly used to study nociception as it is sensitive to strong analgesics and tissue damage is limited because of the cut off time that limits the exposure of the animal to the hot environment .It is a supraspinal test of nociception as it has been established that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally (Le Bars *et al*,2001) ,this shows that kolaviron must be acting through a centrally mediated mechanism

The ability of the KV extract to inhibit the acetic acid –induced wrthing which is a model of visceral pain suggests that it could be used in the management of visceral pain .This test is highly sensitive and useful for the development of new analgesic drugs , however, it is not a selective pain test as it gives false positives with sedatives and muscle relaxants (Elizabetsky,1995) .The sedative nature of kolaviron as we observed in this study therefore makes it mandatory that other tests be used to really confirm the antinociceptive property of kolaviron , hence the use of the tail withdrawal and the hot plate tests in this study . Pre-treatment with L-arginine and glibenclamide , an ATP-Sodium channel antagonist resulted in a potentiation of the observed analgesic activity of kolaviron as shown by a greater reduction of the number of writhes compared with that produced by kolaviron alone .This observation clearly suggests the involvement of the nitrergic and ATP-Sodium sensitive pathways in the centrally mediated kolaviron antinociception .

This study also revealed that the extract may contain psychoactive substances that are sedative in nature . The extract significantly decreased locomotion, grooming and rearing frequencies indicating a central depressant effect . According to Masur *et al* (1971) and Morais *et al* (1998), mobile and rearing activities are functions of CNS excitability and a decrease in these parameters is highly suggestive of sedative properties (Ozturk *et al*,1996) .This assertion of its sedative property was further strengthened by a marked inhibition of the exploratory behavior in mice as observed in this study as shown by a reduction in the head dip –count (File and Pellow,1985).

The therapeutic benefits of traditional remedies are often attributed to a combination of active constituents (Amos *et al*, 2001) .For example , saponin which is one of the chemical constituents of kolaviron is known to have a sedative property apart from causing a decrease in spontaneous motor activity in experimental animals (Wegner *et al* ,1983 ,Dubois *et al*,1986) .It is therefore possible that the saponin content of kolaviron might contribute in part to the observed pharmacological activities (decrease in motor activity and the sedative nature) of the kolaviron extract .In conclusion , this study had demonstrated that both kolaviron analgesia which

involves the nitrenergic and ATP-Sodium sensitive pathways and its central nervous system depressant activitions are mediated via a central mechanism as a result of the prolongation of the hot plate latency and a reduction in mobile and rearing activities which are measures of central nervous system excitability.

REFERENCES

Adaramoye O A ,Adedara I A and Farombi E O (2012) . Possible ameliorative effects of kolaviron against reproductive toxicity in sub- lethally whole body gamma irradiation in rats .Exp Toxicol Pathol. 64 (4):379-85

Ayensu E S (1978) . Medicinal Plants of West Africa Reference Publication Inc; Algonac MI, pp162 .

Bars D L ,Gozarriu M and Gadden S W (1990) . New data concerning the interaction between cholinergic, enkephalinergic and serotoninergic systems during analgesia: In Opiate Receptors and the Neurochemical Correlates of Pain. ed. Fiirst, S.pp 171-181 . Akademiai Kiad6 , Budapest : Pergamon Press .

Dubois M A, Ilyas M and Wagner H .(1986) . Cussonosides A and B , two triterpines –saponins from Cussonia barteri . Planta Medica 56, 80-83 .

Eddy N B and Leimbach D (1953) .Synthetic analgesics II : Diethienylbutenyl

Elizabetsky E T ,Amador R R ,Albuquerque D S and Caualh A C T .(1995) . Analgesic activity of Psychotria colorata . J Pharmacol ,48:77-83 .

Farombi E O ,Adedara I A , Ajayi B O , Aiyepola O R and Egbeme E E (2013). Kolaviron, a natural antioxidant and anti-inflammatory phytochemical prevents dextran sodium –induced colitis in rats .Basic Clin Pharmacol Toxicol . p22

Farombi O E , Akanni E O and Emerole G O (2012). Antioxidant and Scavenging activities of flavonoid extract (kolaviron) of Garcinia kola seeds. Pharmaceut . Biol. 40 : 107-116 .

File S and Pellow S (1985) .The effect of triazolobenzodiazepines in two animal

Ibironke G F , Saba O J , Olopade F O (2004) . Glycemic control and pain threshold in alloxan diabetic rat . Afr. J. Biomed.Res. 147-151 .

Ijomone O M and Obi A V (2013). Kolaviron isolated from Garcinia kola inhibits acetylcholinesterase activities in the hippocampus and striatum of Wistar rats .Ann of Neurosciences (2013) vol 20 (2). P 42 .

Iwu M M and Igboko O (1982) . Flavonoids of Garcinia kola seeds, J. Natural Prod ; 45 :650-651

Konster R, Anderson M and M de Beer E J (1959) . Acetic acid for analgesic screening . Federation Proceedings . 18: 412-413 .

Lichem W , Hsiu –Wen H , Yung –Chen C ,Yu –In L and Janan H H (2006) . Antioxidant and antiproliferative activities of red pitaya .Food Chem 95:319-327

Masur J , Martz R M W and Carlimi E A (1971) . Effects of acute and chronic administration of Canabis sativa and transtetrahydrocannabinol on the behavior of rats in an open-field arena .Psychopharmacology 19,338-392 .

Morais L C S L , Barbosa – Filho J M and Almeida R N (1998) . Central depressant effects of reticuline extract from Octa dukei in rats and mice . Journal of Ethnopharmacology 62, 67-7.

Samson Amos, B. Adzu, L. Binda, C. Wambebe, K. Gamaniel (2001): Neuropharmacological effects of aqueous extracts of *Spharerantus senegalensis* in mice. Journal of Ethnopharmacology 78 , 33-37.

Nwaneri- Chidozie V O , Anyanwu K C , Adaramoye A O and Emrole G O (2014) . Cardioprotective effect of kolaviron (Garcinia kola seed extract) in cholesterol fed rats. Int .Journal of Pharmaceutical Sciences vol 5 (03) p95- 98.

Okereke C, Elekwa I, Osuocha U , Kelechi U and Chukwuma S (2014) . Preliminary phytochemical screening and gas chromatographic FID evaluation of Garcinia kola seed extracts .Journal of Pharmacognosy and Phytochemistry; (6) :115-119

Olaleye S B (2005) . Gastroprotective effects of a methanol extract from the seeds of *Garcinia kola* (Heckel). Afr J. Biomed . Res . 2005; 8: 207-212

Olaleye SB and Farombi E O (2006) .Attenuation of HCI/ ethanol –induced oxidative gastric mucosa damage in rats by kolaviron a natural bioflavonoid of G. kola seeds .Phytotherapy Research . 2006; Vol 20 (1) pp14-20

Olaleye SB, Farombi E O , Adewoye A ,Owoyele B V, Onasanwo S A and Elegbe R A .(2000) .Analgesic and anti-inflammatory effects of kolaviron . Afri .J. Biomed . Res. (2000) vol 3 : 171-174.

Olayinka E T , Ore A (2014) . Kolaviron and L-ascorbic acid attenuate chlorambucil induced oxidative stress in in rats . J . Toxicol http://dx doi.org/10.1155

Ozturk Y , Aydinie S , Baser K H C and Berberoglu H (1996) .Effects of Hypericum perforatum L and Hypericum calcinum L extracts on the central nervous system in mice . Phytomedicine 3 , 139-146 .

Siegmund E , Cadmus R and and Lu G (1957) .A method for evaluating both narcotic and non-narcotic analgesics . Proceedings of the Society for experimental Biology and Medicine .95 :729-731 .

tests of anxiety and on the hole - board . British Journal of Pharmacology 86,729-735 .

Ukponmwan O E, Rupreht J and Dzoljic M R (1984) . REM sleep deprivation decreased the antinociceptive property of enkephalinase- inhibition, morphine and cold water swim .Gen. Pharmacol, 15, 255-258 .

Wagner H , Ott S ,Jurcic K, Morton J and Neszmelyi A (1983) . Chemistry, 12C NMR study and pharmacology of two saponins from Colubrina asiatica . Planta Medica 48, 136-141.