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Anti-nociceptive effects of the hydroethanolic extract of *Alysicarpus ovalifolius* in rodents

Lucy B. John-Africa[1]*, Angela M. Danborno[2], Miriam G. Tikinade[2] and Nazizi Kayinu[2]

[1]Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.
[2]Department of Human Physiology, Bingham University, Karu, Nasarawa State, Nigeria.

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Alysicarpus ovalifolius **commonly known as gadagi in Northern Nigeria is claimed to possess tonic effects. Substances that show stimulant properties have demonstrated analgesic effects. This study therefore aimed at investigating the anti-nociceptive effects of the hydroethanolic extract of *A. ovalifolius* (HEAo) in mice and rats. The activities of the extract on acetic acid induced abdominal writhing, formalin induced pain and tail immersion test were carried out in mice. Effect on carrageenan induced pain was also determined in rats. The extract at the tested doses (100 – 400 mg/kg) significantly and dose dependently decreased the number of abdominal writhing in treated mice. Similarly, extract treated mice exhibited a decrease in the pain reaction in both phases of formalin induced algesia. Carrageenan caused hyperalgesia in rats which was ameliorated in rats treated with HEAo (400 mg/kg) when administered before and after exposure to carrageenan. Reaction time in the tail immersion test was increased in extract pre-treated mice. Results obtained from this study show that the hydro-ethanolic extract of *A. ovalifolius* may contain phytoconstituent probably acting on peripheral and centrally mediated pain receptors to produce anti-nociceptive actions.**

Key words: Anti-nociceptive effects, hydro-ethanolic extract, *Alysicarpus ovalifolius*, rodents.

INTRODUCTION

The increased acceptability of herbs as having medicinal value is due to the belief that they are from natural sources thus are more likely to be safer than synthesized medicines. Several plant-derived preparations are widely available as infusions, tablets, extracts and are acclaimed to have pharmacological effects on different systems of the human body, thus these products are consumed as part of healthy life style (Hoseeinzadeh et al., 2015).

Alrashedy and Molina (2016) reported that psychoactive compounds such as caffeine, atropine and nicotine were produced in plants as a defense mechanism against predators. These compounds act on the central nervous system and produce effects such as stimulation, sedation, anxiolysis, stimulant effects that affect mental processes and behaviour (Cadar et al., 2015; Anderson and Brunzell, 2015). However, human beings developed alternate uses for plants with psychoactive constituent for relief of hunger, fatigue and pain in order to facilitate survival (Lemieux et al., 2015; Balkrishna and Misra, 2017).

*Corresponding author. E-mail: lbjafrica@yahoo.com Tel: +2348058577557.

A. ovalifolius (Schumach. & Thonn.) J. Leónard 'gadagi' is a straight or spreading annual herb (10 – 100 cm) that is widespread in West and East Africa. Although originally native to Africa, it now has a pantropic distribution. *A. ovalifolius* is a forage plant that can be found in open, well illuminated areas and has been reported as a wound medicine (Mannetje, 2002; Hassemer et al*.,* 2017). Previous studies showed that the plant demonstrated stimulant actions (Banwo, 2013) and reversed hyperglycaemia in alloxan-induced diabetic rats (Abdulrazak et al*.,* 2016). Some nutritional constituents including proteins, vitamins and minerals have been identified and quantified in *A. ovalifolius* (Ndiaye et al*.,* 2016). In Northern Nigeria, *A. ovalifolius* is commonly known as 'gadagi', where, a tea prepared by brewing of the plant alone or along with other ingredients is taken to increase endurance, for extra energy to stay alert and active (Dukku, 2017).

The use of drugs with stimulant properties to boost mental and physical functions have been previously reported (Juarez-Portilla et al*.,* 2018). Some of these drugs such as caffeine have shown pain relieving effects and have also been used as adjuvants for the actions of other analgesics (Shapiro, 2008; Derry et al*.,* 2012). However chronic repetitive use of these substances may lead to dependence. This analgesic property probably makes these classes of drugs susceptible to misuse as drugs with analgesic properties have been used to influence physical activity (Holgado et al*.,* 2018). *A. ovalifolius* is used locally to influence physical and cognitive actions; thus, this study was designed to investigate any potential analgesic effects of the hydroethanolic extract of this plant.

MATERIALS AND METHODS

Collection and preparation of plant material

The aerial part of the plant was collected in Chaza, Niger State, Nigeria in June 2018 and identified by the Curators in the herbarium from the National Institute for Pharmaceutical Research and Development, where a voucher specimen (NIPRD/H/6919) was prepared and deposited. The plant was air-dried to a constant weight, after which it was pulverized with a mortar and pestle. Forty grams of the powdered material were subjected to Soxhlet extraction using 70% aqueous ethanol. The filtrated fraction was evaporated to dryness on a water bath regulated at 40°C to obtain hydroethanolic extract of *A. ovalifolius* (HEAo) with a yield of 9.60 ± 1.65. This procedure was repeated and the extracts pooled. It was transferred to an air tight bottle and placed in a refrigerator until needed.

Animals

Adult Swiss albino mice (28 – 34 g) and Wistar rats (150 – 180 g) were obtained from the Animal Facility Centre (AFC) of the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja. They were maintained on standard rodent feed with free access to clean drinking water sourced from the Municipal water system (NIPRD SOP No 05:003).

Acute toxicity tests

This test was carried out in mice using the limit test. Briefly, 3 female mice received each a single oral dose of 2000 mg/kg of HEAo after which they were observed for signs of toxicity and mortality for the first 30 min, then periodically over the following 24 h, after which the animals were monitored daily over a duration of 14 days (OECD, 2001).

Acetic acid induced abdominal writhing

Adult mice were weighed and randomly divided into 6 groups of 6 animals each and treated orally as follows:

Group 1: Control group received distilled water
Group 2: Aspirin 150 mg/kg
Group 3: Morphine 10 mg/kg
Group 4: HEAo 100 mg/kg
Group 5: HEAo 200 mg/kg
Group 6: HEAo 400 mg/kg

Sixty minutes after treatment, 10 ml/kg of 0.65% $^{v}/_{v}$ acetic acid was administered intraperitoneally to each mouse to induce nociception. After a latency period of 5 min, the number of abdominal contortions or stretching exhibited by each mouse was counted over a period of 5 min (Koster et al*.,* 1959). The percentage protection was calculated using the formula:

$$\% \text{ Protection} = \frac{Nc-Nt}{Nc} X\ 100$$

Where Nc = Number of writhes in control group; Nt = Number of writhes in treated group.

Formalin pain test

This test was carried out using the method of Hunskaar and Hole (1987) as described by Holanda et al. (2015). In this test, mice were treated as earlier indicated. The animals were weighed, labelled and randomized into 6 animals per group. The formalin tests were performed in clear plastic containers to keep the injected limb in unobstructed view. Sixty minutes after HEAo or drug administration, mice were injected with 25 µl of 2% formalin. Formalin was administered into the sub-plantar surface of the right hind paw using a 26 G needle. Each mouse was immediately returned to the clear observation box. A mirror was placed beneath the box at an angle of 45° to allow an unobstructed view of the injected paw. The time each mouse spent licking or biting the injected paw was recorded using a stop watch. Recording of nociceptive behaviors (paw licking or shaking) were recorded at 0 – 5 (Phase 1) and 15 – 30 min (phase 2) post formalin injection. Percentage inhibition of pain was calculated using the formula:

$$\% \text{ Inhibition} = \frac{Tc-Tt}{Tc} X\ 100$$

where Tc = Reaction time of control group; Tt = Reaction time of test group

Carrageenan-induced hyperalgesia

Rats were weighed, labelled, randomized and placed into 8 groups of 6 rats each and were treated thus:

Group 1: Control group received distilled water
Group 2: Aspirin 150 mg/kg

Table 1. Rats treatment for carrageenan-induced pain test.

Group 1	Measurement of basal paw withdrawal threshold	Administration of 1.5% carrageenan suspension	After 18 h, treat with distilled water	After 1 h, measure withdrawal threshold, repeat every 60 min for 4 h
Group 2	Measurement of basal paw withdrawal threshold	Treatment with HEAo 400 mg/kg	After 1 h, administration of 1.5% carrageenan suspension	After 18 h, measure withdrawal threshold, repeat every 60 min for 4 h
Group 3	Measurement of basal paw withdrawal threshold	Administration of 1.5% carrageenan suspension	After 18 h, treat with HEAo 400 mg/kg	After 1 h, measure withdrawal threshold, repeat every 60 min for 4 h
Group 4	Measurement of basal paw withdrawal threshold	Treatment with HEAo 400 mg/kg	After 1 h, induce pain with 1.5% carrageenan suspension	After 18 h, treat with HEAo 400 mg/kg | 1 h later, measure withdrawal threshold, every 60 min for 4 h
Group 5	Measurement of basal paw withdrawal threshold	Treatment with aspirin 150 mg/kg	After 1 h, administration of 1.5% carageenaan suspension	After 18 h, measure withdrawal threshold, repeat every 60 min for 4 h
Group 6	Measurement of basal paw withdrawal threshold	Treatment with morphine 10 mg/kg	After 1 h, administration of 1.5% carrageenan suspension	After 18 h, measure withdrawal threshold, repeat every 60 min for 4 h
Group 7	Measurement of basal paw withdrawal threshold	Administration of 1.5% carrageenan suspension	After 18 h, treat with aspirin 150 mg/kg	After 1 h, measure withdrawal threshold, repeat every 60 min for 4 h
Group 8	Measurement of basal paw withdrawal threshold	Administration of 1.5% carrageenan suspension	After 18 h, treat with morphine 10 mg/kg	After 1 h, measure withdrawal threshold, repeat every 60 min for 4 h

Group 3: Morphine 10 mg/kg
Group 4: HEAo 100 mg/kg
Group 5: HEAo 200 mg/kg
Group 6: HEAo 400 mg/kg
Group 7: Aspirin 150 mg/kg (administered post inflammation)
Group 8: Morphine 10 mg/kg (administered post inflammation)

The test was carried out using the modified method of Buczynski et al., (2010). Briefly, each rat was placed in a clear acrylic cubicle with a metal grid floor that allows access to the underside of their paws. The animals were allowed 30 min acclimation period in the cages before the tests were conducted. A volume of 0.1 ml of 1.5% carrageenan suspension was used to induce pain. A mechanical stimulus was applied to the plantar surface of the hind paw by a stainless steel filament (0.5 mm in diameter) exerting a linearly increasing pressure. A cut-off force of 50 g was pre-set to prevent tissue damage. The force (g) and reaction time (s) at which paw withdrawal occurred was automatically recorded. Each rat paw withdrawal threshold was calculated as the average of three consecutive tests performed at 5-min intervals. This was regarded as the pain threshold. Pain withdrawal threshold to mechanical stimulation of each rat was measured after which rats were subjected to treatment as shown in Table 1. Reaction to pain was assessed as time and threshold of withdrawal of the rat hind paw. This was measured as response to mechanical stimulation using a Dynamic Plantar Aesthesiometer (Ugo Basile, Italy).

Tail immersion test

The test was done following the method of D'Amour and Smith (1941) as reported by Relagado et al. (2017). In this test, test, mice were weighed, labelled, randomized, placed in 6 groups (n=6) and treated as earlier indicated. Two (2) cm of the distal tail portion of the animals were marked. Each mouse was placed in a perforated cylindrical holder with the tail hanging out freely. The tail was then immersed in a beaker of water maintained at 55 ± 0.5°C. The time taken for the tail to be withdrawn from the water was recorded using a stopwatch and was noted as the reaction time. A cut-off time of tail immersion was set at 10 s to avoid tissue damage. The reaction time was measured before and at 60 min after treatment. An increase in latency period between tail immersion and tail deflection was taken as indicator of anti-nociceptive action. The percentage of analgesic activity was calculated using the formula:

$$\% \text{ Inhibition} = \frac{PostTL - PreTL}{Cut{-}off\ time - PreTL}\ X\ 100$$

where PostTL = Post-treatment latency; PreTL = Pre-treatment latency.

Statistical analysis

Group data were presented as Mean ± SEM. The data (acetic acid, formalin and tail immersion test) were analyzed by one-way ANOVA followed by a post hoc Dunnet test and two-way ANOVA followed by a post hoc Bonferroni test was used to compare the change in withdrawal threshold and reaction time in carrageenan treated rats. Graphpad PRISM® 6.0 was used. The level for statistical significance was set at $p<0.05$.

Table 2. Effect of the hydro-ethanolic extract of *Alysicarpus ovalifolius* (HEAo) on acetic acid-induced pain in mice.

Treatment	Dose (mg/kg)	No of abdominal writhes	% Protection
Distilled water	-	29.20 ± 2.35	-
Aspirin	150	11.20 ± 2.04[b]	61.64
Morphine	10	0.80 ± 0.37[d]	97.26
HEAo	100	25.40 ± 5.20	13.01
HEAo	200	12.60 ± 2.11[b]	56.85
HEAo	400	9.40 ± 3.27[c]	67.81

HEAo = Hydroethanolic extract of *Alysicarpus ovalifolius*. Values are presented as mean ± SEM (n = 6), Significance: compared to control [b] $p<0.01$, [c] $p<0.001$ and [d] $p<0.0001$ groups (One-way ANOVA).

Table 3. Effect of the hydro-ethanolic extract of *Alysicarpus ovalifolius* on formalin-induced pain in mice.

Treatment (mg/kg)	Phase 1 reaction time (s)	% Inhibition	Phase 2 reaction time (s)	% Inhibition
DW	47.67 ± 4.38	-	210.50 ± 9.85	-
Aspirin 150	34.00 ± 4.05	28.68	96.00 ± 22.36[b]	54.39
Morphine 10	4.67 ± 2.09[c]	90.20	21.67 ± 9.49[c]	89.71
HEAo 100	33.00 ± 6.47	30.77	121.50 ± 12.95[b]	42.28
HEAo 200	26.17 ± 3.99[a]	45.10	116.00 ± 22.61[b]	44.89
HEAo 400	30.83 ± 3.76[a]	35.32	133.50 ± 10.16[b]	36.58

DW = Distilled water, HEAo = hydroethanolic extract of *Alysicarpus ovalifolius*. Values are presented as mean ± SEM (n = 6); Significance [a] $p<0.05$ [b] 0.01 and [c] $p<0.001$ treatment vs control groups (One-way ANOVA).

RESULTS

Acute toxicity test

No death of mice was recorded in this test. Also, no abnormal behavioural sign was observed in treated mice. The LD_{50} was therefore estimated to be greater than 2000 mg/kg.

Effects on Acetic acid induced abdominal writhing

Administration of dilute acetic acid induced abdominal writhing in mice. A dose-dependent significant ($P< 0.05$) decrease in the number of abdominal writhing was observed in extract treated mice. Treatment with aspirin and morphine reduced the number of abdominal writhes (Table 2).

Effects on formalin-induced pain

After administration of formalin, mice exhibited two phases of pain (the early and late phase). Treatment of animals with the extract caused reduction in the duration of paw licking or biting. These responses were not dose dependent but were significant ($P<0.05$) at 200 – 400 mg/kg in comparison with the distilled water group. Treatment with aspirin and morphine also caused significant reduction in pain behavior in both phases when compared to control group. However, the change for aspirin in phase 1 was not significant (Table 3).

Effects on carrageenan-induced hyperalgesia

After administration of carrageenan, force of paw withdrawal threshold in the rats experienced decrease thus indicating the establishment of hyperalgesia by carrageenan. Treatment of rats with 400 mg/kg of the hydroethanolic extract of *A. ovalifolius* either before or after induction of hyperalgesia with carrageenan did not produce significant change in the force of paw withdrawal threshold (Table 4). However, on administration of the extract both before and after pain induction with carrageenan, significant increase of paw withdrawal threshold was observed from 2 h post administration of the second dose of 400 mg/kg of HEAo. Administration of HEAo did not cause significant increase in reaction time when a force was applied to the inflamed paws of the rat (Table 5). Morphine showed significant increase in force of paw withdrawal threshold and reaction time in treated rats.

Effects of tail-immersion test in mice

The results obtained in this test indicated that treatment with 400 mg/kg of extract caused significant ($p<0.05$) increase in reaction time with an increased % MRT of 61.31 on comparison with the control group. The groups treated with aspirin and morphine also exhibited significantly ($p<0.05$) increased reaction time (Table 6).

Table 4. Effect of the hydroethanolic extract of *Alysicarpus ovalifolius* on withdrawal threshold (g) in carrageenan -induced hyperalgesia in Wistar rats.

Treatment	Withdrawal threshold (g)				
	0 h	1 h	2 h	3 h	4 h
DW + Car	24.54 ± 1.52	18.30 ± 2.13	17.54 ± 2.26	17.31 ± 1.53	17.47 ± 1.11
HEAo + Car	25.83 ± 2.45	19.72 ± 2.92	21.32 ± 3.21	28.84 ± 1.90	22.75 ± 2.98
Car + HEAo	26.26 ± 1.86	21.79 ± 3.32	20.41 ± 1.49	21.29 ± 3.96	20.89 ± 4.11
HEAo + Car + HEAo	26.83 ± 1.66	25.12 ± 1.25	24.02 ± 1.54[a]	24.15 ± 1.44	23.77 ± 2.44
Mor + Car	26.47± 2.53	25.77 ± 4.21[a]	28.83 ± 1.62[b]	29.84 ± 2.01[b]	30.02 ± 1.06[b]
Car + Mor	26.01 ± 2.06	27.44 ±1.43[b]	30.50 ± 1.32[c]	31.42 ± 1.81[c]	31.65 ±1.57[c]
ASA + Car	25.62 ± 1.66	22.02 ± 4.07	22.95 ± 3.61	20.16 ± 2.37	20.71 ± 2.86
Car + ASA	23.64 ± 1.38	18.29 ± 1.76	19.39 ± 1.63	19.45 ± 0.49	19.73 ± 0.37

ASA= Aspirin (150 mg/kg), MOR = Morphine (10 mg/kg), Car= carrageenan 1.5 %, DW= Distilled water (10 ml/kg), HEAo = hydroethanol extract of *Alysicarpus ovalifolius* (400 mg/kg), Values are presented as mean ± SEM (n = 6), Significance [a]p<0.05, [b]p<0.01, [c]p<0.001 compared to control group (Two-way ANOVA)

Table 5. Effect of the hydroethanolic extract of *Alysicarpus ovalifolius* on reaction time (s) in carrageenan -induced hyperalgesia in Wistar rats.

Treatment	Reaction time (s)				
	0 h	1 h	2 h	3 h	4 h
DW + Car	11.1 ± 1.21	10.54 ± 1.57	9.89 ± 0.81	10.55 ± 0.56	8.99 ± 0.63
HEAo + Car	13.34 ± 0.08	11.28 ± 1.71	12.34 ± 2.00	14.57 ± 0.94	13.06 ± 1.75
Car + HEAo	14.11 ± 0.89	12.11 ± 1.77	13.36 ± 1.61	13.20 ± 1.59	12.72 ± 1.76
HEAo + Car +HEAo	14.43 ± 1.82	13.04 ± 1.92	11.21 ± 1.11	12.36 ± 2.44	11.97 ± 2.41
Mor + Car	14.69 ± 1.71	15.96 ± 3.60	15.00 ± 1.47	15.26 ± 1.85	17.32 ± 0.61[b]
Car + Mor	15.38 ± 1.15	16.28 ± 2.10	15.69 ± 1.26	16.06 ± 1.54	17.51 ± 0.44[b]
ASA + Car	12.79 ± 1.36	12.63 ± 2.38	13.36 ± 1.68	14.13 ± 2.07	11.84 ± 1.69
Car + ASA	12.79 ± 1.36	12.97 ± 2.26	15.03 ± 1.36	12.46 ± 1.43	13.83 ± 1.80

ASA= Aspirin (150 mg/kg), MOR = Morphine (10 mg/kg), Car = carrageenan 1.5 %, DW= Distilled water (10 mL/kg), HEAo = hydro ethanolic extract of *Alysicarpus ovalifolius* (400 mg/kg), Values are presented as mean ± SEM (n = 6), Significance [b]p<0.01 treatment vs control groups (Two-way ANOVA).

Table 6. Effect of the hydroethanolic extract of *Alysicarpus ovalifolius* on tail-immersion test in mice.

Treatment	Dose (mg/kg)	Reaction time(s) pre-treatment	Reaction time(s) post-treatment	% Inhibition
Distilled water	-	2.70 ± 0.21	2.72 ± 0.16	0.27
Aspirin	150	2.95 ± 0.19	3.77 ± 0.31	11.25
Morphine	10	2.96 ± 0.07	9.37 ± 0.27[d]	90.92
HEAo	100	3.09 ± 0.36	4.04 ± 0.52	13.75
HEAo	200	2.72 ± 0.17	5.02 ± 0.46	31.59
HEAo	400	2.98 ± 0.21	7.03 ± 0.72[c]	57.69

HEAo = Hydroethanolic extract of *Alysicarpus ovalifolius.* Values are presented as mean ± SEM (n = 6), Significance [c]p<0.001 and [d]p<0.0001 p<0.05 treatment vs control groups (One-way ANOVA).

DISCUSSION

This study was designed to evaluate the effect of *A. ovalifolius* hydroethanolic extract on laboratory models of analgesia. The acetic acid induced abdominal writhing, formalin, carrageenan induced pain models and tail immersion test were used with the aim of detecting analgesic effects of the extract and its possible involvement on peripheral or centrally mediated actions.

Injection of dilute acid into the mouse peritoneal cavity caused the release of inflammatory mediators that activate nociceptors causing the animals to react with twists/contortions of the abdominal region which indicate a positive reaction to pain. The acetic acid induced

abdominal writhing is a tool that is widely applied for the screening of compounds with potential analgesic and anti-inflammatory effects (Chang et al., 2011). The test is reactive for peripherally and centrally acting analgesics (Naghizadeh et al., 2016). In this study, administration of the extract caused a decrease in the number of abdominal contortions which is an indication of anti-nociceptive effects by the extract of *A. ovalifolius*. Although the test is sensitive for detection of analgesic effects, it is a non-specific model as several classes of compounds which include opioids, non-steroidal anti-inflammatory drugs (NSAIDs), antispasmodics, antihistamines respond to this test (Sarmento–Neto et al., 2016; Regalado et al., 2017).

Administration of formalin into the sub-plantar area of rodents' hind paws causes spontaneous nociceptive response which can be separated into phases that are distinct in timing, duration and underlying mechanisms. Rodents react by licking, biting, shaking the injected paw or flinching which is considered as a positive manifestation of pain. The behavioral and electro-physiological responses to formalin consist of an acute neurogenic phase (Phase 1) with short-lasting response followed by a quiescent interphase period and subsequently a prolonged response of the inflammatory phase (Phase 2). The pain reaction in Phase 1 is attributed to direct stimulation of nociceptors and the pain response is sensitive to opiates and local anaesthetics, while the second phase of the pain reaction has been reported to involve both inflammatory mechanisms and central sensitization within the dorsal horn. This phase responds to drugs that include NSAIDS and opiates (Ellis et al., 2008; Gong et al., 2014; Da Silva et al., 2014; Holanda et al., 2015). HEAo decreased the pain reaction time in both phases of the formalin test. This suggests that HEAo extract may possess anti-nociceptive properties as demonstrated earlier in the acetic acid test. The formalin test comprises inflammatory, neurogenic and central mechanisms (Ellis et al., 2008). The neurogenic pain is caused by direct chemical stimulation of nociceptive afferent fibres (predominantly C fibres) while the inflammatory pain is caused by the release of inflammatory mediators like histamine, prostaglandins, bradykinin, serotonin in the peripheral tissues and from central sensitization within the spinal dorsal horn (Tjolson et al., 1992; Holanda et al., 2015). Pain reaction inhibition produced by the extract was similar to morphine which produced significant reduction in both phases; however, the efficacy was not comparable. Morphine is used for the treatment of severe acute and chronic pain acting on both central and peripherally mediated pain (Cunha et al., 2009). Thus, it is possible that the extract may be acting to inhibit activity at the nociceptive fibres and/or inhibiting the release and activity of inflammatory mediators.

Intra-plantar administration of carrageenan produced muscle hyperalgesia in rats. The hypersensitivity measured as tactile threshold persisted up to 24 h and returned to baseline after 72 h in the injected limb thus indicating inflammatory response. The carrageenan induced hyperalgesia is through the induction of the release of pro-inflammatory agents. Treatment with extract both before and after pain induction caused significant increased paw withdrawal threshold, thus indicating that the extract may be effective in reversing already established hyperalgesia. Morphine also caused significant increase in paw withdraw threshold and has been shown to be effective against established hyperalgesia (Cunha et al., 2009).

The tail immersion test is a model used for the detection of centrally acting anti-nociceptive agents (Mannan et al., 2017). Treatment with the extract prolonged the latency period of the tail curling reflex when immersed in hot water. Thus, indicating that the extract might be mediating its effects by central mechanisms in a similar manner to morphine.

Banwo (2013) has reported in the aerial part of *A. ovalifolius,* presence of phytochemical compounds that include steroids, terpenes, saponins, cardiac glycosides and flavonoid. These compounds have been shown to possess analgesic and anti-inflammatory effects in several experimental models of pain and inflammation (Kutama et al., 2018). Plant compounds such as flavonoids, and steroids compounds, have been demonstrated to inhibit prostaglandin synthesis (Malar and Chellaram, 2017; Awad et al., 2004). The analgesic actions of terpene isolated from other plants have been associated with mechanisms that include decreased neuronal excitability, inhibition of the release of pain mediators or interaction with receptors of the opioid, cholinergic, adrenergic and glutemateric systems (Guimaraes et al., 2013). The analgesic effect observed in this study may be attributed to synergistic actions of these compounds in the plant.

The LD_{50} obtained in this study, was estimated to be greater than 2000 mg/kg as no death was recorded at 2000 mg/kg. Based on this incidence, HEAo should be placed in category 5 in the Globally Harmonized System (GSH) of classification and labelling of chemicals. Generally, category 5 is intended to enable the identification of test substances which are of relatively low acute toxicity hazard but which, under certain circumstances may present a danger to vulnerable populations (OECD, 2001).

Conclusion

The data obtained from this study infers that the hydro-ethanolic extract of *A. ovalifolius* possesses analgesic actions against centrally and peripherally mediated pain, induced by thermal and chemical irritants. Further studies are recommended to determine the duration of the analgesic effect and the mechanism of action. The plant extract should be fractionated to isolate and identify the active components of the extract.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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