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NEUROPHARMACOLOGICAL ASSESSMENT OF AN AQUEOUS BARK EXTRACT OF *ANTIARIS TOXICARIA* (PERS.) LESCH. (MORACEAE) IN RODENTS

**Priscilla K. Mante, Donatus W. Adongo,
Kennedy K.E. Kukuia, Elvis O. Ameyaw and Eric Woode**

Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences,
College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

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ABSTRACT

Antiaris toxicaria is a plant traditionally used in Ghana for the treatment of various neurological conditions such as epilepsy and pain. This present study therefore seeks to screen for the neuropharmacological activities of the aqueous extract of *Antiaris toxicaria* (AAE) stem bark. The effect of *Antiaris* extract on pentobarbital-induced sleeping time, tail immersion test, spontaneous locomotor activity, motor coordination, PTZ-induced convulsions as well as the Irwin test was investigated. The extract produced analgesia and Straub tail at (300-3000 mg kg^{-1}) in the Irwin test suggestive of a morphine-like action. These effects were absent after 24 h. No deaths were recorded in the test estimating the LD_{50} to be above 3000 mg kg^{-1}. Spontaneous locomotor activity of the mice in the activity meter test was decreased significantly ($p<0.01$, $F_{4, 20} = 26.61$) by the extract at 100 mg kg^{-1} but increased at 300-3000 mg kg^{-1}. It however showed no impairment on motor coordination in the beam traversal test. The extract potentiated duration of sleeping time in the pentobarbitone interaction test and showed susceptibility to metabolism by hepatic enzymes. Analgesic properties were also further confirmed in the tail withdrawal test while it inhibited PTZ-induced convulsions. Thus, *Antiaris* may be a potential source for novel drug discovery in the field of neuropsychiatric research.

Keywords: *Antiaris Toxicaria* (AAE), Pentobarbitone (PBT), Phenobarbitone (PHE), Morphine Hydrochloride (MOR), International Conference on Harmonization (ICH)

1. INTRODUCTION

Antiaris toxicaria (Moraceae), commonly known as the Bark Cloth tree, is found in forests of Africa. The bark of this plant is used as an antiepileptic traditionally and seeds as an antipyretic and treatment for pain (Mshana *et al*., 2000). In Africa, the latex produced by the bark of *A. toxicaria* (Moraceae) is applied to cuts, wounds and skin conditions such as eczema and leprosy. It is also taken internally as a purgative (Bosu and Krampah, 2008). The latex serves as a component of most dart and arrow poisons in South East Asia. *Antiaris* is known to produce mainly prenylphenols (Hano *et al*., 1990; 1991) and cardiac glycosides (Kiliani, 1910; Muhlradt *et al*., 1964; Carter *et al*., 1997a; 1997b). The species has been previously investigated and shown to be active against various cancer cell lines (Levrier *et al*., 2012; Li *et al*., 2012). However, there is no report on the psychopharmacological activity of this plant, although the decoctions of *A. toxicaria* are extensively used traditionally.

The present study was undertaken to investigate the CNS activity of *Antiaris toxicaria* extract in rodents. Methods employed in this study were adapted from the core battery of assessment of the central nervous system

Corresponding Author: Eric Woode, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

as proposed by the International Conference on Harmonization (ICH) S7A Guideline for Safety Pharmacology (Anon, 2000). The guideline recommends the testing of novel compounds on the central and peripheral nervous system and on the cardiovascular system as part of the "core battery" of assessment (Williams *et al*., 2007). Rodents are mainly the species of choice for detecting behavioural and neurological effects. The mouse shares many anatomical, cellular, biochemical and molecular features with man. Other functions, such as memory, sexual behaviour and emotional responses are also similar. Based on these similarities, murine models are therefore employed to approximate human behavioural responses in disease states (Meer and Raber, 2005). Hence, this study involved the use of *in vivo* methods in freely moving conscious animals.

General behavioural observation, measures of spontaneous activity, locomotor activity, pain and convulsive thresholds in addition to interaction with hypnotics were assessed.

2. MATERIALS AND METHODS

2.1. Plant Material

The bark of *Antiaris toxicaria* was collected from the KNUST campus, Kumasi (6° 41'6.4"N, 1° 33'42.8"W), Ghana in March, 2010 where a voucher specimen (KNUST/HM1/011/S007) has been retained. Authentication was done at the Department of Herbal Medicine, KNUST.

2.2. Preparation of Aqueous Extracts

The Antiaris bark was air-dried at room temperature (28°C) and powdered. Four hundred and thirty-one (431) gram powder was macerated with cold distilled water for five days. The filterate was concentrated under reduced temperature (60°C) and pressure in a rotary evaporator. It was then oven-dried to obtain *Antiaris* Aqueous Extract (AAE). A yield of 23.40% w/w was obtained.

2.3. Animals

ICR mice (20-25 g) were obtained from the Noguchi Memorial Institute for Medical Research. Animals were kept in the departmental Animal House and allowed to acclimatize to laboratory conditions before the study. All animals were treated according to the Guide for the Care and Use of Laboratory Animals (ILAR, 1996) and experiments were approved by the Faculty Ethics Committee.

2.4. Drugs and Chemicals

Caffeine (CFN), Diazepam (DZP), Pentobarbitone (PBT), Pentylenetetrazole (PTZ) and Phenobarbitone (PHE) were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA. Morphine hydrochloride (MOR) was obtained from Phyto-Riker Pharmaceuticals Limited, Accra, Ghana.

2.5. Phytochemical Screening

Antiaris toxicaria aqueous extract was tested for the presence of tannins, alkaloids, triterpenoids, flavonoids, general test for glycosides (reducing sugars), anthracene glycosides, steroids and saponins by simple quantitative and qualitative methods (Harborne, 1998; Trease and Evans, 1972).

2.6. Irwin Test

AAE was administered orally to male ICR mice at doses of 100-3000 mg kg^{-1} body weight in groups of seven. Behavioural, neurological and autonomic statuses were evaluated in each animal at 0, 15, 30, 60, 120 and 180 min, up to 48 h after treatment (Irwin, 1968; Williams *et al*., 2007).

2.7. Activity Meter Test

The Ugo Basile mouse activity cage (model 7401, Comerio, VA, Italy) was used in this test. Animals were first pre-treated with either AAE (100-3000 mg kg^{-1}, *p.o*) or diazepam (6 mg kg^{-1}, *p.o*) or caffeine (18 mg kg^{-1}, *p.o.*). ICR mice were placed individually in the activity cage 60 min after AAE, diazepam or caffeine treatment. Activity was observed every 5-30 min.

2.8. Beam Traversal Test

Animals were randomly divided into seven groups consisting of five mice each and treated with extract (300-3000 mg kg^{-1}, *p.o.*) or diazepam (0.1, 0.3 and 1.0 mg kg^{-1}, i.p). Animals were trained to traverse the beam (three consecutive trials each day for three days) to the goal box in less than 30 sec. Mice that could not achieve the goal were excluded from the study. During the test, mice were placed at the start end of the beam and allowed 60 sec to traverse the beam. Test sessions were recorded with a video camera and analysed for total number of steps, time to traverse and stepping errors. A score of 60 sec was awarded to animals which could not cross the beam or fell off.

2.9. Pentobarbitone Interaction Test

Animals were randomly divided into eighteen groups comprising five mice each and treated with extract (300-3000 mg kg^{-1}, *p.o*), diazepam (8 mg kg^{-1}, i.p) or caffeine (16 mg kg^{-1}, i.p). Thirty minutes later, pentobarbitone (50 mg kg^{-1}, i.p) was administered to each mouse to induce sleep. Mice were observed for the latency to sleep (time between pentobarbitone administration to loss of righting reflex) and duration of sleep (time between loss and recovery of righting reflex). Other groups of animals were pre-treated with phenobarbitone (25 mg kg^{-1}, i.p) for two days prior to the testing day to investigate the effect of hepatic enzyme induction on sleeping time. Those animals were treated in the same manner as the naïve animals during testing.

2.10. Tail Withdrawal Test

The test was carried out according to the method described by (Janssen *et al*., 1963; Steinmiller and Young, 2008) with slight modifications. Tail withdrawal latency was defined as the time (in seconds) to withdraw the tail from hot water maintained at 50.0±1.0°C. A cut-off latency of 10 s was set to avoid tissue damage. Increase in tail withdrawal latency was the measure of anti-nociception. It was calculated as:

$$\%\,\text{Maximal Possible Effect (MPE)} = \frac{[(T_1 - T_0)]}{[(T_2 - T_0)]} \times 100$$

where, T_0 and T_1 are defined as the latencies obtained before and after drug treatment respectively and T_2 is the cut-off latency.

The maximum possible anti-nociceptive effect was awarded to animals that did not show a tail withdrawal reaction within 10 s. Animals were tested at single time points of 60 min after administration of AAE (100-1000 mg kg^{-1}, *p.o*) and morphine (32-128 mg kg^{-1}, *p.o*).

2.11. Convulsive Threshold Test (PTZ-Induced Seizures)

Mice were divided into 7 groups (n = 7). The AAE was administered orally at 30-3000 mg kg^{-1}. Other animals received diazepam (0.3-3.0 mg kg^{-1}, i. p) while the control group received distilled water (10 mL kg^{-1}). Seizures were induced with pentylenetetrazole (85 mg kg^{-1}, s.c.) 30 min after distilled water or diazepam and 1 h after AAE. The mice were then observed via video recording for the frequency, duration of and latency to clonic convulsions for 1 h.

2.12. Data Analysis

Significant differences between means were determined by Analysis of Variance (ANOVA) with Newman-Keuls' post hoc test. In the pentobarbitone interaction test, effect of treatment was determined by two-way ANOVA (Dose × treatment) followed by Bonferroni test. Graph Pad Prism® Version 5.0 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Values were presented as Mean ± S.E.M. and in all cases, $p<0.05$ was considered significant.

3. RESULTS

3.1. Phytochemical Tests

Phytochemical analysis of the stem bark of *Antiaris toxicaria* revealed the presence of the anthracene glycosides, tannins, flavonoids, alkaloids, saponins, reducing sugars and triterpenoids. Steroids were absent (**Table 1**).

3.2. Irwin Test

Acute dosing between 300-3000 mg kg^{-1} produced straub tail effect in the mice. Only the 1000 and 3000 mg kg^{-1} doses parameters showed no changes and there were no deaths recorded in the Irwin test (**Table 2**).

3.3. Activity Meter Test

AAE showed significant ($p<0.01$, $F_{4, 20} = 26.61$; **Fig. 1b**) decrease in locomotor activity at 100 mg kg^{-1} and a paradoxical increase at all other doses used mimicking effects of both diazepam and caffeine. Diazepam, (6 mg kg^{-1}, p.o.), significantly ($p<0.001$, $F_{2, 12} = 136.3$; **Fig. 1b**) decreased spontaneous activity while caffeine, (18 mg kg^{-1}, p.o.) increased it.

3.4. Beam Traversal Test

No stepping errors were observed for all treatment groups in this test. The extract exhibited no significant change in the time taken to traverse the beam as well as the total number of steps as compared to the control.

Table 1. Phytochemical analysis of *A. toxicaria* aqueous extract

TEST	Results
Anthracene glycosides	Present
Tannins	Present
Alkaloids	Present
Triterpenoids	Present
Flavonoids	Present
Saponins	Present
rrSteroids	Absent

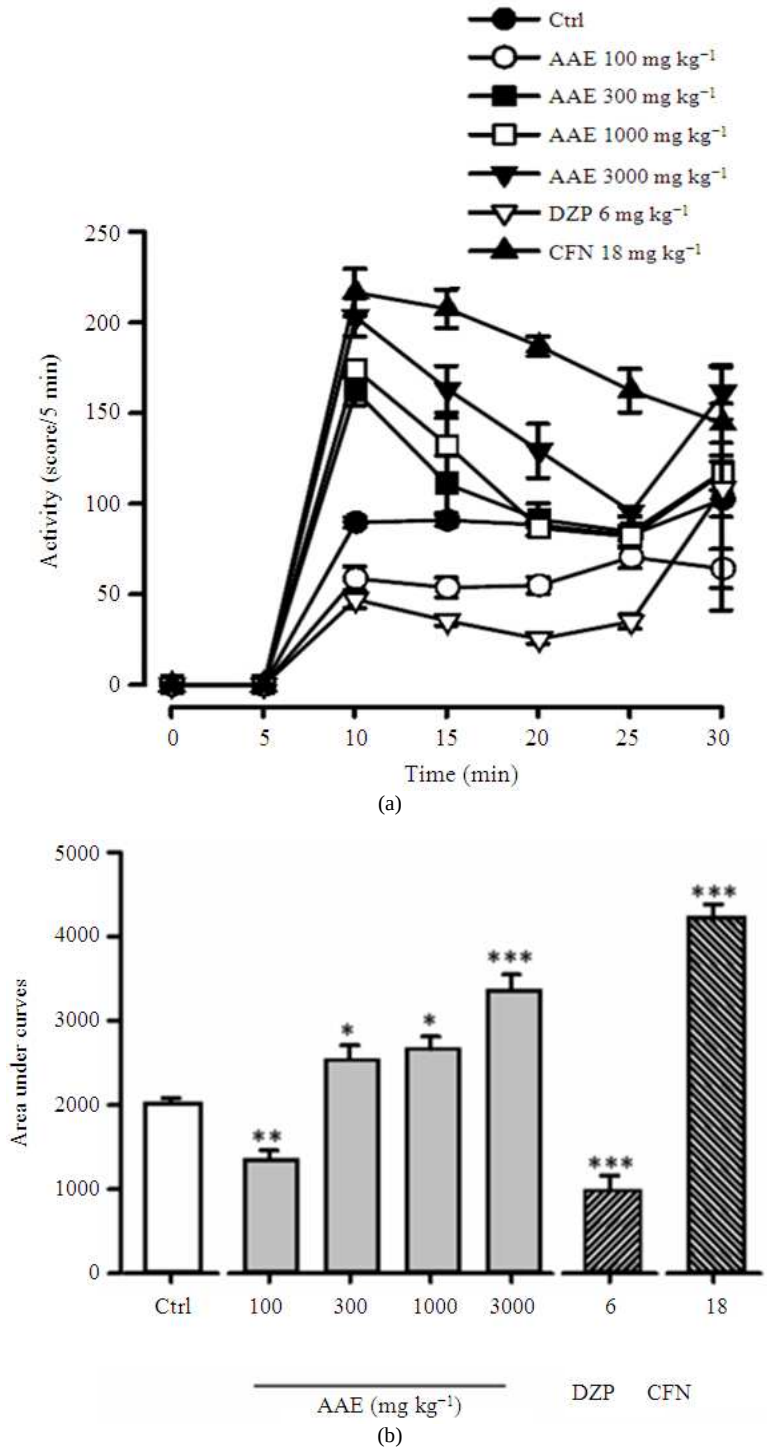


Fig. 1. Effects of acute AAE (100-3000 mg kg^{-1}, p.o), diazepam (6 mg kg^{-1}, p.o) and caffeine (18 mg kg^{-1}, p.o) treatment in the activity meter test. Data are presented as group means (±SEM). Analysis by one-way ANOVA followed by Newman-Keuls' post hoc test. *p<0.05, **p<0.01, ***p<0.001

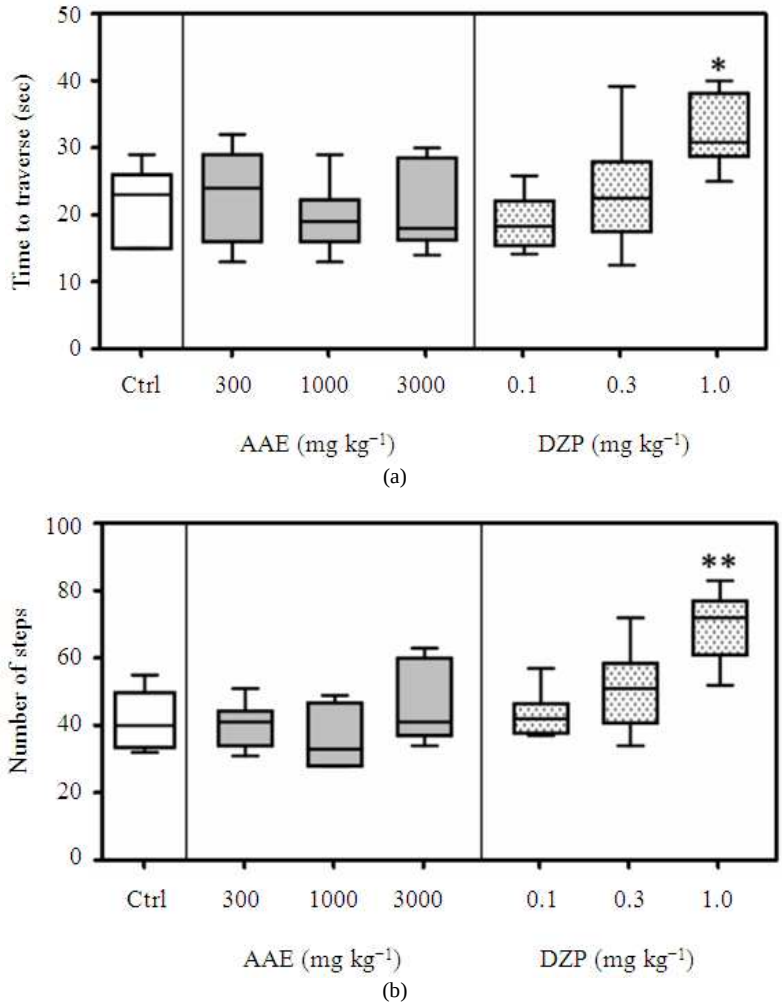


Fig. 2. Effects of AAE (300, 1000 and 3000 mg kg^{-1}, p.o) and diazepam (0.1, 0.3and 1.0 mg kg^{-1}, i.p) on the traversal time (a) and total number of steps taken (b) in the beam traversal test. Data are presented as group means (±SEM).The lower and upper margins of the boxes represent the 25 and 75th percentiles, with the extended arms representing the 10th and 90th percentiles respectively. The median is shown as a horizontal line within the box. Analysis was done by one-way analysis of variance followed by Newman-Keuls' post hocTest. Significantly different from control: *p<0.05, **p<0.01

Diazepam however at dose of 1 mg kg^{-1} showed a significant increase in both the time it took animals to traverse the beam ($p<0.05$, $F_{3,\ 16} = 5.009$, **Fig. 2a**) and the total number of steps taken ($p<0.01$, $F_{3,\ 16} = 6.350$, **Fig. 2b**).

3.5. Pentobarbitone Interaction Test

In the pentobarbitone interaction test, AAE significantly ($p<0.001$, $F_{1,\ 8} = 211.97$ [Two-way ANOVA]; (**Fig. 3a**) prolonged the duration of sleeping time in test animals as compared to control. The onset of sleep was also significantly affected as it was reduced by 43.59% compared to saline- treated animals. Pre-treatment with phenobarbitone resulted in significant ($p<0.0001$, $F_{1,\ 8} = 39.8$ [Two-way ANOVA]; **Fig. 3b**) decreases in the duration of sleep at dose levels 1000 and 3000 mg kg^{-1}. Onset of sleep was not affected significantly by pretreatment with phenobarbitone. Sleep induced by diazepam was not significantly affected by either pentobarbitone or phenobarbitone.Sleep onset of caffeine was significantly ($p<0.01$) decreased by pentobarbitone administration but phenobarbitone pre-treatment had no effect.

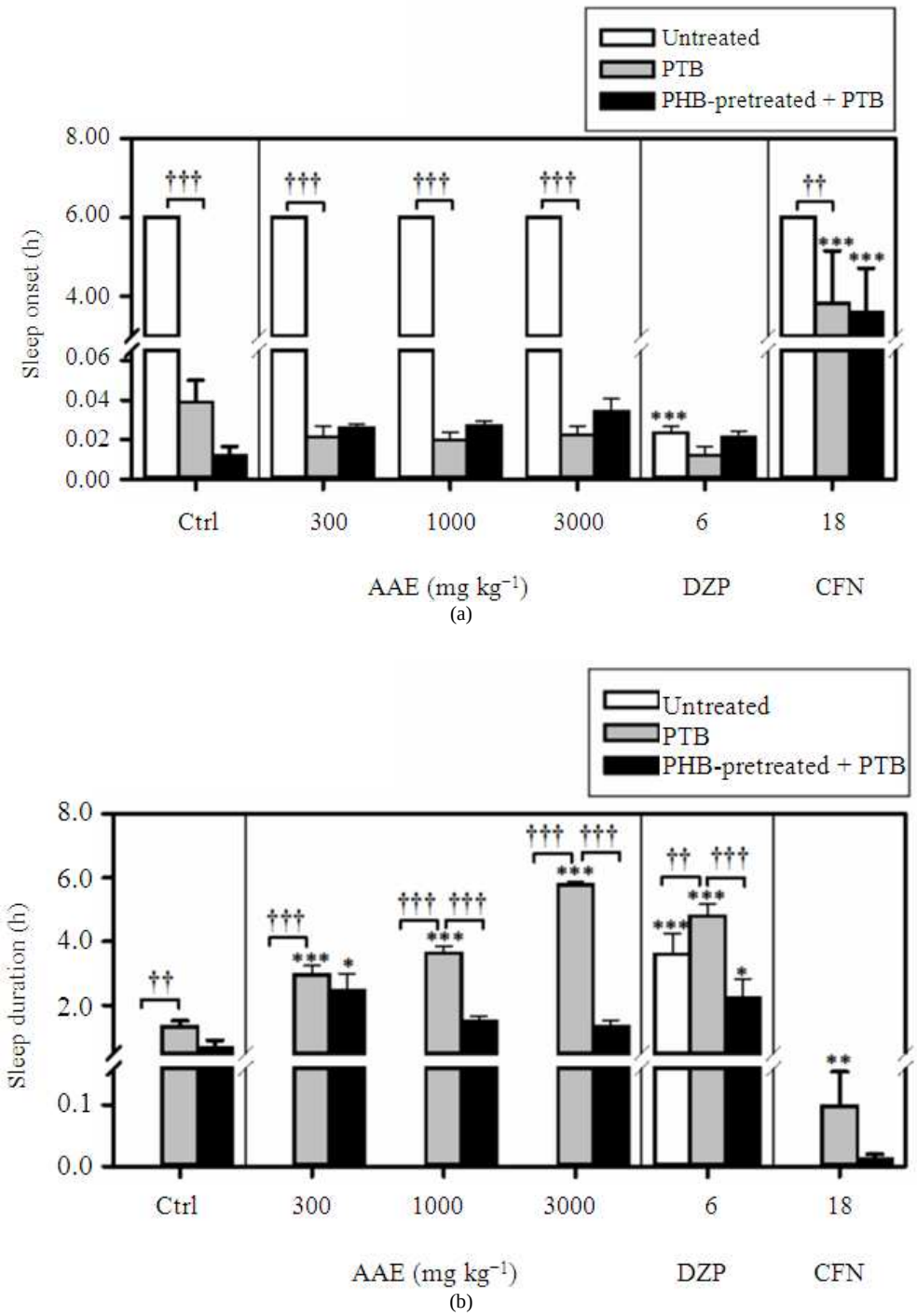
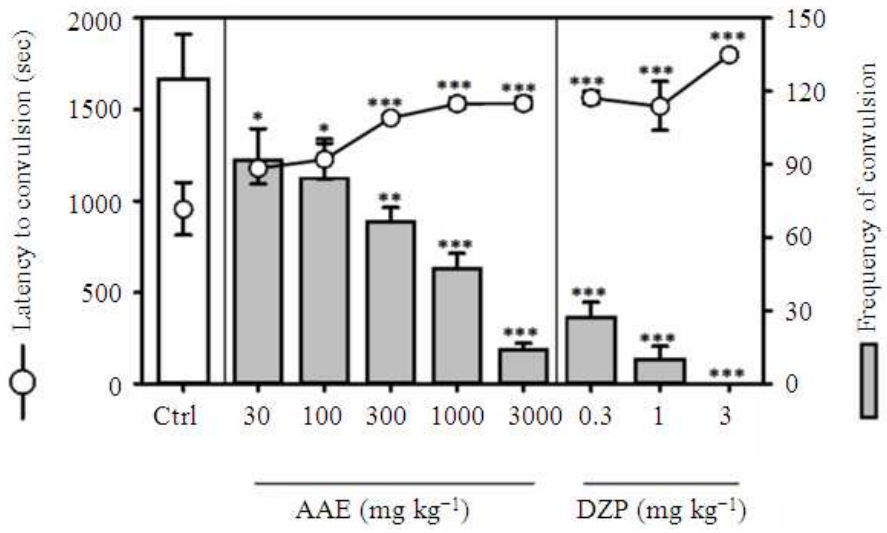
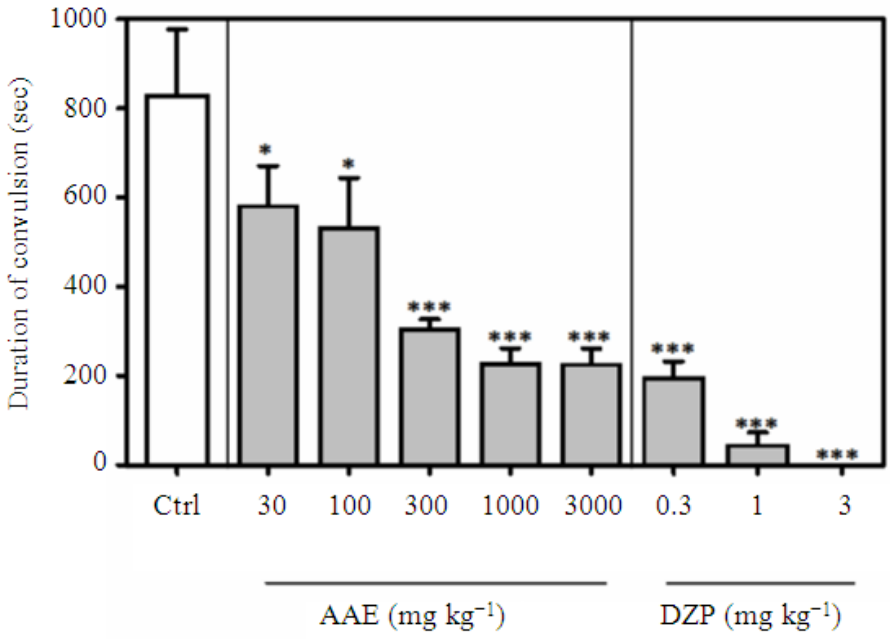


Fig. 3. Effects of acute AAE (300, 1000 and 3000 mg kg^{-1}, p.o), diazepam (8 mg kg^{-1}, i.p) and caffeine (16 mg kg^{-1}, i.p) in the Pentobarbital Interaction Test. Data are presented as group means (±SEM). Analysis was done by one-way analysis of variance followed by Newman-Keuls' post hocTest. Significantly different from control: *p<0.05**,** **p<0.01. ***p<0.001 and two-way ANOVA followed by Bonferroni test. †† p<0.01, ††† p<0.001



(a)



(b)

Fig. 4. Effects of AAE (30-3000 mg kg^{-1}, p.o.) and diazepam (0.3, 1.0 and 3.0 mg kg^{-1}, i.p.) on the latency and frequency of seizures (a) and duration of convulsions (b) in PTZ - induced seizures. Data are presented as mean (±SEM). Analysis was done by one-way analysis of variance followed by Newman-Keuls' post hocTest. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001

3.6. Tail Withdrawal Test

AAE increased tail withdrawal latency significantly ($p<0.01$, $F_{3, 28} = 5.440$) at all doses in a non-dose dependent anner. Similarly, morphine also showed marked increases ($p<0.001$, $F_{3, 28} = 38.38$) in latency at all doses; effects which were greater than that of the extract (**Table 3**).

Table 2. Effects of *Antiaris toxicaria* aqueous extract in the Irwin test

DOSE (mg kg^{-1})	Mortality D/T	Effects
0	0/7	No change
100	0/7	No change
300	0/7	Straub tail at 15'→120'
1000	0/7	Straub tail and Analgesia at 15'→120'
3000	0/7	Straub tail and Analgesia at 15'→120'

Table 3. Effect of *Antiaris toxicaria* aqueous extract in the tail withdrawal test

Control	Dose (mg kg^{-1})	Percentage of maximal possible effect
		-20.27±15.29
AAE	100	48.52±14.53*****
	300	48.80±15.49******
	1000	44.27±12.44******
Morphine	32	31.89±10.81*******
	64	99.40±00.60*******
	128	99.39±00.61*******

*; p<0.05, **; p<0.01,***; p<0.001 by Newman-Keuls post hoc test

3.7. Convulsive Threshold Test (PTZ-Induced Seizures)

Onset of clonic convulsions were delayed by AAE significantly ($p<0.0001$, $F_{5, 36} = 7.664$; **Fig. 4a**) in mice. The frequency of convulsions likewise was reduced significantly ($p<0.0001$, $F_{5, 36} = 11.46$; **Fig. 4a**) in addition to the duration of clonic convulsions ($p<0.0001$, $F_{5, 36} = 8.043$; **Fig. 4b**). The standard drug diazepam (0.3- 3.0 mg kg^{-1}) also significantly reduced and abolished convulsions.

4. DISCUSSION

Investigations carried out on the aqueous extract of *Antiaris toxicaria* show that it possesses CNS depressant activity and analgesia at high doses without muscle relaxant properties. It is also metabolized by hepatic enzymes and inhibits PTZ-induced convulsions.

In the Irwin test the extract exhibited potential analgesic properties to tail pinch responses as well as a Straub tail effect. The Irwin test involves systematic observational methods for assessing effects of drugs on the behaviour and physiology of rodents. It is a component of the basic protocols satisfying International Committee for Harmonization (ICH) recommendations for safety pharmacology studies. It was first described by Irwin (1968). It helps one detect potential adverse effects of drugs on the Central Nervous System (CNS) prior to clinical testing and may also be helpful in revealing novel therapeutic effects (Irwin, 1968; Porsolt *et al*., 2002; Williams *et al*., 2007). The Straub effect is often measured in response to opioids and has been shown to be mediated by the μ_2-receptor (Nath *et al*., 1994; Houshyar *et al*., 2000). Other agonists have also been shown to be able to produce the said response by other mechanisms such as nicotinic and serotoninergic receptor activation (Duffard *et al*., 1995; Zarrindast *et al*., 2001; Fonck *et al*., 2003; Diaz and Maroteaux, 2011). This suggests possible opioidergic, serotoninergic or even nicotinic mechanisms of action. Since no deaths were recorded, the LD_{50} may be estimated to be above 3000 mg kg^{-1}.

Pentobarbitone is a hypnotic at appropriate doses. Its sedation or hypnosis is by potentiation of GABA-mediated postsynaptic inhibition at GABA receptors (Ffrench-Mullen *et al*., 1993; Brust, 2004). Potentiation of pentobarbitone-induced hypnosis is an indication of central depressant activity (Fujimori, 1965). Such substances either decrease the time for onset of sleep and/or prolong the duration of sleep. Diazepam was used as the positive control. It is a hypnotic belonging to the benzodiazepine group. The extract most likely possesses depressant action on the CNS similar to that of diazepam. Pretreatment with phenobarbitone for two days prior to testing induced liver metabolising enzymes (Ioannides and Parke, 1975; Whysner *et al*., 1996; Kushikata *et al*., 2003). Results indicate that the duration of sleep produced by the extract is shortened in the presence of metabolising enzymes. It is a strong indication that the extract might be broken down by cytochrome-P450 enzymes. This brings to the fore the possibility of drug interactions with other drugs that may be metabolised in the same manner since 60% of drugs are known to be metabolised by cytochrome-P450 enzymes (Zhou *et al*., 2005; Sweeney and Bromilow, 2006).

Testing for drug effect on motor coordination is a vital step in CNS drug evaluation. The beam traversal test helps to measure skilled walking, fine motor balance as well as coordination skills (Carter *et al*., 1999; Meredith and Kang, 2006). As such, increased errors in experiments indicate impaired motor coordination (Meredith and Kang, 2006). The extract showed no significant effect on motor coordination as shown by the results. Benzodiazepines at high doses have muscle relaxant effects (Woods and Winger, 1995; Charney *et al*., 2001) hence, the effect of diazepam on motor coordination.

AAE however reduced spontaneous locomotor activity in mice in the activity meter test at 100 mg kg^{-1}. Motor impairment that is drug- induced can result in decreased locomotor activity (Porsolt *et al*., 2002). Since motor impairment was absent in the beam traversal it can be ruled out as the cause of the reduction in locomotor activity observed. Another possible cause of reduced locomotion may be sedation which was not observed at any of the doses in the Irwin test. On the other hand, since the extract exhibited CNS depressant activity in the pentobarbitone interaction test, it may be safe to conclude on potential sedative effects since it is generally accepted that the sedative effects of drugs can be evaluated by measurement of pentobarbital sleeping time in laboratory animals (Brown, 1961; Carpenedo *et al*., 1994). Locomotion tests estimate whether a substance is psychostimulant or sedative. Substances with marked psychostimulant properties are expected to increase locomotor activity (Riviere *et al*., 1999; Kafkafi *et al*., 2001; Gentry *et al*., 2004). But can cause a decrease due to animals rotating rapidly in a small space or showing stereotyped behaviours (Morita *et al*., 2000; Quinn *et al*., 2003). The increase in locomotor activity at higher doses may be due to some increased exploratory behaviour that may be evident with anxiolytic compounds such as these benzodiazepines (Turski *et al*., 1982). But this is in contrast to benzodiazepines that produce increased spontaneous activities at low doses and sedation at much higher doses. Diazepam, a CNS depressant, reduced spontaneous activity (Savic *et al*., 2003) and impaired motor coordination at the dose used while caffeine, a CNS stimulant, increased the locomotor activity (Kafkafi *et al*., 2001; Gentry *et al*., 2004).

The extract also had a significant effect in the tail withdrawal test. This test is considered to model somatosensory pain (Bjorkman, 1995; Shannon *et al*., 1997; Kalra *et al*., 2001) and known to be more sensitive to centrally acting analgesics (Prado *et al*., 1990; Gupta *et al*., 2005; Santos *et al*., 2005). Such agents act to elevate pain threshold of animals towards heat and pressure. Central activity involves spinal and supra spinal mechanisms which can be conferred on the extract since it exhibited significant activity in this model (Jain *et al*., 2001; Muhammad *et al*., 2012).

Finally, the extract produced significant inhibition of PTZ-induced seizures which helps to confirm its traditional use in epilepsy management. One of the generally accepted mechanisms by which pentylenetetrazole exerts its action is by acting as an antagonist at the $GABA_A$ receptor complex (Ramanjaneyulu and Ticku, 1984; Katzung, 2004). GABA is a major inhibitory neurotransmitter in the mammalian central nervous system (Katzung, 2004). Inhibition of pentylenetetrazole-induced seizures is an indication that the effects of *Antiaris toxicaria* may be associated with modulation of GABA activity in the central nervous system. This may not be surprising as it has demonstrated significant central depressant properties.

5. CONCLUSION

The overall results from this study show that AAE possesses CNS depressant, analgesic and anticonvulsant activity. It also can increase spontaneous activity without motor impairment and its LD_{50} may be above 3000 mg kg^{-1}.

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