REPORT

Anxiolytic, sedative and toxicological effect of hydromethanolic stem bark extract of *Maerua angolensis* DC. in Wister rats

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Abstract: *Maerua angolensis* DC is traditionally used for the treatment of epilepsy and insomnia. The present study was designed to investigate the anxiolytic, sedative and toxicological effect of hydromethanolic stem bark extract of *M. angolensis* using animal model. Sub-chronic doses of the plant extract on liver and kidney function test were investigated. Elevated plus maze (EPM) and diazepam-induced sleeping time test was used in this investigation. The possible involvement of *M. angolensis* with $GABA_A$ receptor was also investigated using flumazenil. The results of acute toxicity studies showed LD_{50} to be greater than 5000mg/kg body weight. The test extract (40 and 80mg/kg) significantly ($p<0.05$) increased the number of open arm entries and time spent in the open arm entries. However, flumazenil with 80mg/kg plant extract showed no significant ($p>0.01$) difference in the number of entries into open arm when compared to control. The stem bark extract of *M. angolensis* significantly ($p<0.01$) increased the duration of sleep induced by diazepam in a dose-dependent manner. However, flumazenil with 80mg/kg extract showed no significant ($p>0.01$) sedative effect when compared to normal control. In conclusion, the result of our present findings revealed that *M. angolensis* may apparently be safe and non toxic at therapeutic dose. However, the plant may possess anxiolytic and sedative properties, which exert their effect on $GABA_A$ receptors.

Keywords: Anxiolytic, sedative, *Maerua angolensis*, $GABA_A$-receptor, benzodiazepines.

INTRODUCTION

Anxiety disorders have become the most prevalent challenging behavioral disorders in most developed countries. However, stress, anxiety and depression could trigger insomnia. Insomnia is one of the most common sleep disorders that affect people of all ages around the world (Guzmán-Gutiérrez *et al*., 2009). Stress and sleeping difficulties are now becoming part of our modern life, which forms the major symptoms of insomnia. These different behavioral disorders are associated with impairment of γ-aminobutyric acid (GABA). $GABA_A$ receptors are the major inhibitory neurotransmitter in the brain and the site of action of a variety of pharmacologically and clinically important drugs such as benzodiazepines, barbiturates, neuro active steroid, anesthetics and convulsants (Richter *et al*., 2012).

Natural products of plant origin represent a rich diversity in chemical structures that has led to the discovery of important therapeutic agents. There is now an impressive array of natural products that are known to influence the function of ionotropic receptors for GABA, the major inhibitory neurotransmitter in the brain (Johnston *et al*., 2006). These natural products exert their anxiolytic properties by allosterically enhancing the action of GABA at $GABA_A$ receptor via their benzodiazepines-binding site (Richter *et al*., 2012). However, the mode (s) of action of these natural products are still poorly elucidated.

Maerua angolensis DC belonging to the family Capparaceae is a medium to big self-planted tree up to 20 m height. The plant grows in bush and rocky areas and it is widespread in the savannah area of tropical Africa (Magaji *et al*., 2009; Mohammed *et al*., 2008). The common vernacular names of the plant include *ciciwa* (Hausa) and *baguhi* (Fulfulde). The leaves are eaten by races in Senegal and elsewhere in soups but perhaps in some areas only in the time death, for example in northern Nigeria (Burkill, 1985). The stem bark of the plant has various applications in traditional medicine particularly in the management of psychosis, ecthyma, epilepsy, diarrhea and dysentery. The decoction of the stem bark in combination with other herbs is used in the treatment of epilepsy and as tranquilizer in North-Western region of Nigeria (Magaji *et al*., 2009).

Despite the frequent uses of this medicinal plant to treat behavioral disorders, there are currently no scientific reports on the anxiolytic properties as well as details on the toxicological profiles of *M. angolensis*. However, this

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study was aimed to investigate the anxiolytic, sedative and toxicological effects of stem bark extract of *M. angolensis* and also to determine the possible involvement with $GABA_A$-receptor in animal model.

MATERIALS AND METHODS

Plant material, animals, reagents and drugs

The plant material was collected from Galadima road, Zaria, Kaduna state, on 20th July, 2012. The plant was taxonomically identified by comparing with the existed voucher specimen (No 918) by staff of the Herbarium section of the Biological Sciences, Ahmadu Bello University Zaria, Nigeria.

Wister albino rats of either sex (70-83g and 140-180g) were obtained from Department of Pharmacology and Toxicology, Faculty of Pharmaceutical sciences, Usmanu Danfodiyo University and were kept at the animal house of the same faculty under controlled environment at 22 ± 2^0C. Twelve hours light and Twelve hours dark cycle was ensured during which they were allowed to acclimatized under optimum feeds (Excel feed, Ilorin, Nigeria) and water access for a period of 2 weeks before the commencement of the experiment. All procedure followed were in accordance with the ethical standard of the European Union Guidelines for Animals Experimentation and approved by the Institutional Animal Care Committee.

All the chemicals used were of analytical grade and were obtained from BDH Chemicals Ltd, England. Standard assay kits ware obtained from Randox Laboratory Ltd, UK, while standard rutin was obtained from Sigma-Aldrich Company Ltd.

The stem bark extract of *Maerua angolensis* was prepared by simple maceration technique in aqueous and methanol ratio (30: 70) for 48h and concentrated by evaporation at reduced temperature. The solid extract obtained was dissolved in distilled water for pharmacological and toxicological studies. Diazepam and flumazenil was supplied from Sigma chemicals Co. st. Louis, Missouri, U.S.A.

Experimental procedures

Acute toxicity studies

The limit test procedure described by Organization for Economic Cooperation and Development (OECD) guideline (OECD, 2001) was adopted. Test extract of the plant was administered to five Wister rats (5000mg/kg b.wt, p.o.) and kept under observation for the first 8 h and within the period of 48 h. The rats were further observed for weakness, difficulty in movement, reduced response to sound, hair loss, and loss of appetite, mortality and other abnormalities for a period of 14 days.

Sub-chronic toxicity studies

Animals were randomly divided in to five groups of five animals each ($n=5$) and each animal received the drug by oral feeding with cannula. One control group received normal saline (1ml/kg bwt) once daily for 28 days. Four other groups received the test extract (1000, 2000, 3000 and 4000mg/kg bwt) once daily for a period of 28 days. All the animals were sacrificed under diethyl ether anesthesia and blood sample were collected from each rat withdrawn from carotid artery at the neck as described Ahsan (2009).

Liver function test

The serum was obtained from the blood samples by centrifugation at 3000 rpm for 5 min for biochemical evaluation. Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) (Reitman and Frankel, 1957), Alkaline Phosphatase (ALP) (Kind and King, 1954), total bilirubin (Jendrasick and Grof, 1938), total protein and albumin were estimated.

Kidney function test

Serum creatinine, urea (Cheesbrough, 1991), and uric acid (Jung and Parekh, 1970) were measured. Serum electrolytes Na^+ and K^+ were measured using flame photometry, while bicarbonate ion was estimated by back titration (Cheesbrough, 1991).

Estimation of total flavonoid content

Total flavonoid content was estimated by UV-Vis spectrophotometry at 500 nm as described by Chen (2010). Total flavonoid content was calculated as rutin equivalent (mg/g) using the equation of the calibration curve: $y=12.\ 121x+0.005$, $R^2=1.0$.

Elevated plus maze test (EPM)

Elevated plus maze was used in this experiment to evaluate animal anxiety. Rat EMP described by Walf and Frye (2007) was constructed consisting of four arms (two open without a wall and two closed by 30 cm high walls) 50 cm long and 10 cm wide. The floor and the walls of each arm were wooden and painted black. The maze was elevated to a height of 50cm above floor level. An observer was recording animal's behavior two meters away from the EPM in a quite room illuminated by light. Each animal was placed in the centre of the EPM facing one of the open arms. An entry into an arm was defined as the animals placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed arms were recorded during a 5 min test period. The percentages of open arm entries (100 x Open/Total entries) were calculated for each animal (Nogueira and Vassilieff, 1996).

The animals were randomly divided in to seven groups of five animals each ($n=5$) and all received the drug by oral feeding by cannula. Two control groups received normal

saline (1m/kg b.wt, p.o.) only and the other received diazepan (3mg/kg bwt) only. Three groups received test extract (20, 40 and 80mg/kg bwt) respectively. Two other groups received flumazenil (1mg/kg bwt) only and flumazenil and test extract (flumazenil administered 15 min before administration of test extract 80mg/kg bwt) respectively. All the treated animals were subjected to EMP 30 min after administration of normal saline, diazepam, flumazenil or the test extract. Between each trial, the elevated plus maze was wiped with 70% ethanol to prevent olfactory cue from animals, which may affect the test (Aderibigbe *et al*., 2010).

Diazepam-induced sleeping time test

A method described by Rakotonirina (2001) was adopted to evaluate sleep-potentiating effect of the plant extract. The animals were randomly divided in to six groups of five animals each (n=5) and all received the drug by oral feeding cannula. One control group received normal saline (1m/kg bwt). Three groups received test extract (20, 40 and 80mg/kg bwt) respectively. Two other groups received flumazenil (1mg/kg bwt) only and flumazenil with test extract (flumazenil administered 15 min before administration of test extract 80mg/kg bwt) respectively. All animals were administered diazepam (3mg/kg, bwt) 30 min after administration of normal saline, diazepam, flumazenil or the test extract. The time between the loss of the straightening reflex and the regain of this reflex was measured as the sleeping time.

STATISTICAL ANALYSIS

The results were expressed as means ±SD using one-way ANOVA followed by Dunnett's test for multiple comparisons.

RESULTS

Acute toxicity

The stem bark extract of *M. angolensis* showed neither toxic nor mortality effect at a dose of 5000mg/kg for the whole experimental period. However, symptom of sedation was observed during the period of studies.

Sub-chronic toxicity

The sub-chronic effects of the test extract on the weight changes of the animals are shown in table 1. The results showed significant decreased in the body weight in animals treated with higher dose of the plant extract as compared to the normal control.

Liver function test

Serum enzymes ALP and ALT of the animals treated with sub-chronic doses of the test extract increased significantly (p<0.05) while the animals on higher doses showed significant difference in AST (p<0.05) to that of the normal control (fig. 1A). Total proteins and bilirubins significantly (p<0.05) increased at higher doses. However, albumins showed no significant increase in all the doses (fig. 1B).

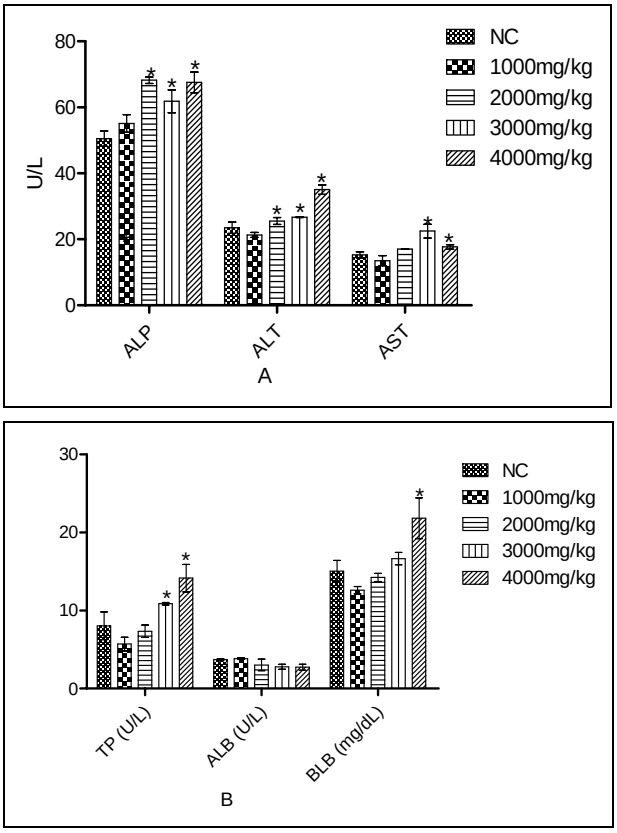

Fig. 1: Effect of *M. angolensis* on liver biochemical parameters. (A) Sub-chronic effects of test extract on serum enzymes AST, ALT, and ALP (B) Sub-chronic effects of test extract on serum TP, ALB, and BLB. Data are expressed as mean ±SD, n=5, *p<0.05 as compared to the normal control, NC: normal control.

Kidney function test

Serum urea and uric acid of the animals treated with sub-chronic doses of the test extract showed no significant difference, while creatinine at different doses differ significantly (p<0.05) as presented in fig. 2A. Serum K^+ at different doses significantly differ as compared to the normal control, while Na^+ and HCO_3^- differ significantly (p<0.05) at higher doses (fig. 2B).

Estimation of total flavonoid content

The result of the quantitative analysis of the extract obtained showed the presence flavonoid to contain 9.482 mg/g of the plant extract.

Elevated plus maze test (epm)

Both diazepam (3mg/kg, p.o) and the test extract (40 and 80mg/kg, p.o) significantly (p<0.05) increased the number of open arm entries while the time spent in the open arm entries was observed to significantly (p<0.01) increased higher than that of the diazepam (p<0.05). A

significant (p<0.05) increased in the percentage number of open arm entries was observed at higher dose of 80mg/kg which is not significantly different from that produced by diazepam (table 3 A, B and C).

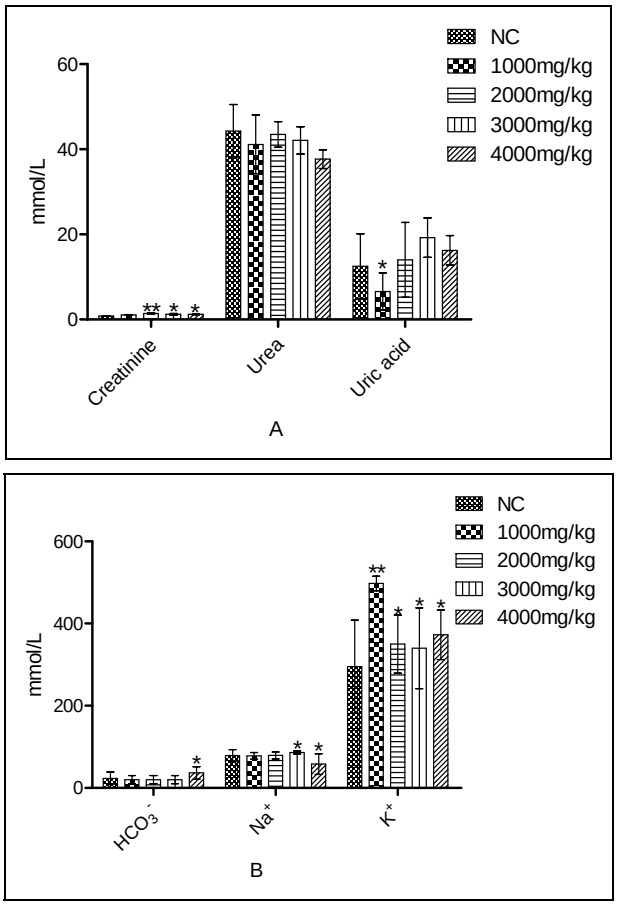

Fig. 2: Effect of *M. angolensis* on kidney biochemical parameters. (A) Sub-chronic effects of test extract on serum creatinine, urea, and uric acid (B) Sub-chronic effects of test extract on serum electrolyts. Data are expressed as mean ±SD, n=5, *p<0.05 as compared to the normal control, NC: normal control.

However, flumazenil (1mg/kg, p.o) showed no significant difference in the number of entries into open and closed arm as well as the time spent in the arms. Flumazenil in the presence of 80mg/kg showed no significant difference in the number of entries into open and closed arm but showed a significant (p<0.05) decreased in the time spent in the closed arm. Percentage open arm entries showed no significant difference when compared to normal control (fig. 4A, B and C).

Diazepam-induced sleeping time test

The stem bark extract of *M. angolensis* significantly (p<0.01) increased the duration of sleep induced by diazepam in a dose-dependent manner. *M. angolensis* at a dose of 20mg/kg showed no significant different from the normal control. However, flumazenil (1mg/kg, p.o) and flumazenil in the presence of 80mg/kg showed no significant sedative effect when compared to normal control (fig. 5A and B).

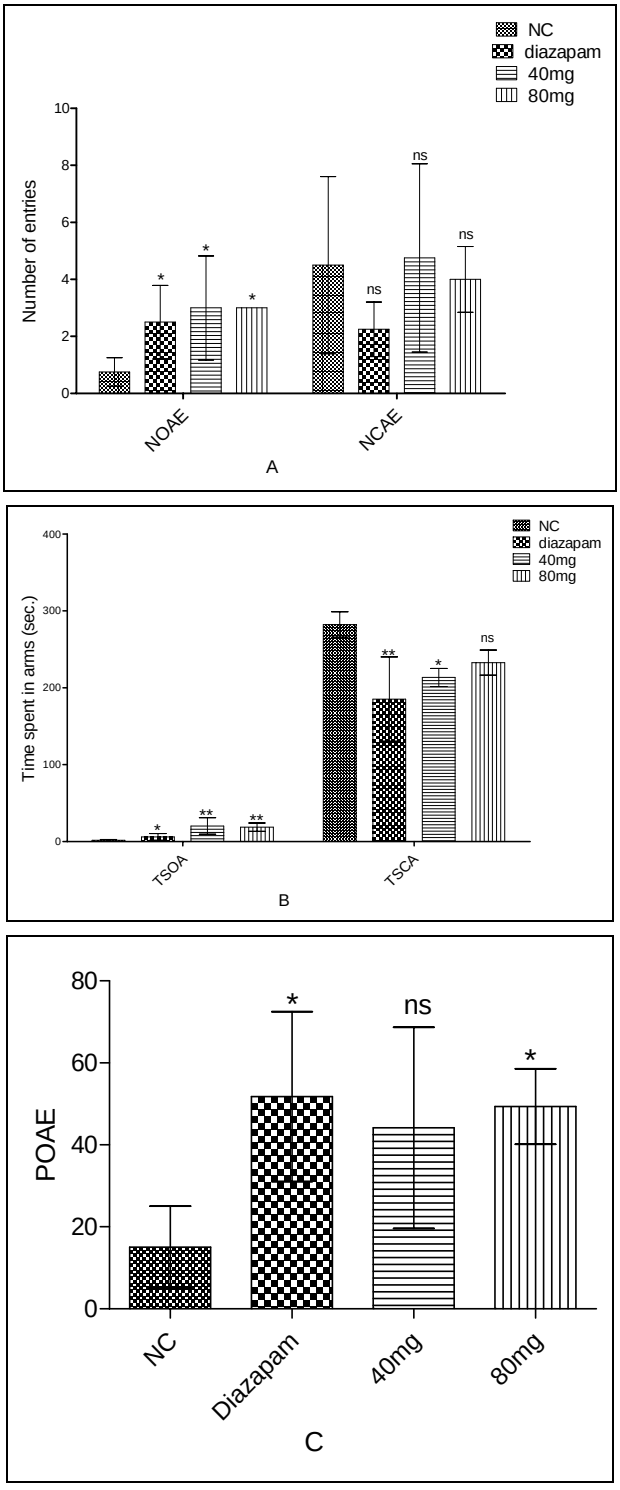

Fig. 3: (A) Effect of *M. angolensis* showing number of open arm and closed arm entries, (B) Effect of *M. angolensis* showing time spent in the open and closed arm entries, (C) Effect of *M. angolensis* showing percentage open arm entries. Data are expressed as mean ±SD, n=5,

*p<0.05, **p<0.01, ns: non significant when compared to the normal control, NC: normal control, NOAE: number of open arm entries, NCAE: number of closed arm entries, TSOA: time spent in open arm, TSCA: time spent in the closed arm, POAE: percentage open arm entries.

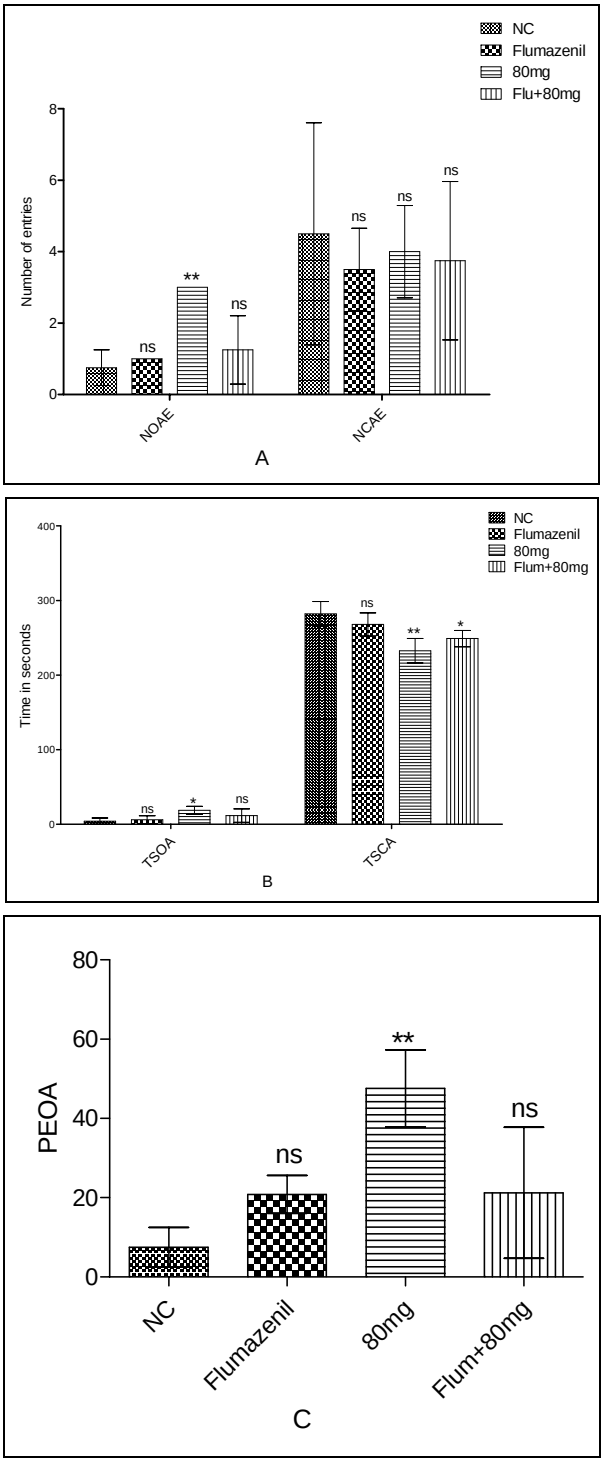

Fig. 4: (A) Effect of *M. angolensis* showing number of open arm and closed arm entries, (B) Effect of *M. angolensis* showing time spent in the open and closed arm entries, (C) Effect of *M. angolensis* showing percentage open arm entries. Data are expressed as mean ±SD, n=5, *p<0.05, **p<0.01, ns: non significant when compared to the normal control, NC: normal control, NOAE: number of open arm entries, NCAE: number of closed arm entries, TSOA: time spent in open arm, TSCA: time spent in the closed arm, POAE: percentage open arm entries.

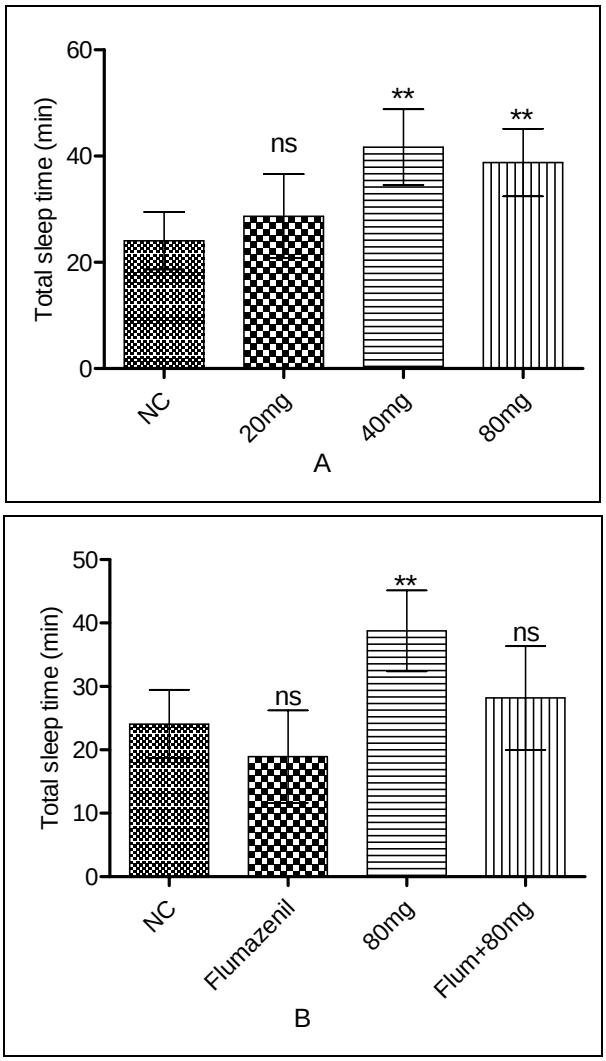

Fig. 5: (A) Effect of *M. angolensis* on diazepam-induced sleep in rats, (B) Effect of *M. angolensis* on diazepam-induced sleep in rats. Data are expressed as mean ±SD, n=5, **p<0.01, ns: non significant when compared to the normal control, NC: normal control

DISCUSSION

The major hindrance to the use of traditional herbal preparations is the lack of scientific and clinical data in support of better understanding of the efficacy and safety of the drugs (Sathya *et al*., 2012). This is largely due to the negligence of the evaluation of the toxicity and adverse drug reactions of the herbal medicines as they are considered natural and safe (Saidu *et al*., 2007) by the indigenous people of North-Western region of Nigeria.

The result of the acute toxicity studies indicate the lethality of the stem bark extract was found to be greater than 5000mg/kg, no symptoms of toxicity and mortality were observed. Therefore, the plant extract may be considered safe in accordance with the OECD guideline (OECD, 2001). The animals treated with sub-chronic doses of the plant extract showed slight progressive decrease in the body weight of the animals. The decreased in the animal body weight may be attributed to high content of tannins in the plant extract (Chivalittumrong *et al*., 1996). The rise in serum enzyme levels of AST, ALT and ALP and decreased in serum levels of total proteins and albumin has been attributed to the damaged structural integrity of the liver (Ahsan *et al*., 2009). Serum liver enzymes ALP, ALT and AST of animals treated with sub-chronic doses increased progressively at higher doses suggesting that the plant extract might have side effects on the liver at higher doses. However, increase in serum total protein at higher doses may be attributed to dehydration and increase in serum bilirubin at higher dose may possibly increase haemolytic breakdown of erythrocytes as a result of haemoglobin degredation Decrease in serum albumin is an apparent indication of liver disorder. Therefore, the insignificant effect of serum albumin suggest that the deleterious effect of the plant extract on the liver has not yet affected the metabolism of the biomolecule (Saidu *et al*., 2007; John *et al*., 2001). Renal function indices such as serum electrolytes, urea, creatinine and uric acid are the common parameters used to assess the functional state of the kidney (Yakubu *et al*., 2003; Eteng *et al*., 2009). The elevation of serum creatinine at higher doses may be attributed to glomerular dysfunction, while uric acid and urea remain unaffected. Though, serum urea is less reliable than the creatinine as an index of Glomerulus Filtration Rate (GFR), by virtue of the fact that it diffuses back into the renal tubular cells. Elevation of serum K^+ at all sub-chronic doses may indicate renal failure. Other causes include hyperglycemia or massive tissue destruction. However, Na^+ and HCO_3^- at higher sub-chronic doses of the plant extract may be due to dehydration and changes in acid-base balance respectively (Aboyade *et al*., 2010; Saidu *et al*., 2007).

The elevated plus maze is a widely used as behavioral assay for rodents and it has been validated to assess the anti-anxiety effects of pharmacological agents and to define brain regions and mechanisms underlying anxiety-related behavior (Walf and Frye, 2007). Therefore, our present findings showed that *M. angolensis* increases the number of open arm entries; time spent in the open arm as well as the percentage entries into open arms. Several reports have shown to used diazepam as a standard anxiolytic drug to compare the potential effect of medicinal plant extract as source of anxiolytic agents (Rabbani *et al*., 2008; Akindele and Adeyemi, 2010; Gupta *et al*., 2010; Aderibigbe *et al*., 2010). As we expect to have the same effect diazepam produced anxiolytic effect by significantly increasing the number of open arm entries and time spent in the open arm. The percentage entries into open arm also increased in the presence of diazepam. This effect is in agreement with other studies reported to use diazepam as a standard anxiolytic drug to compare the potential effect of anxiolytic agents (Rabbani *et al*., 2008; Aderibigbe *et al*., 2010; Akindele and Adeyemi, 2010). In order to support the possible involvement of *M. angolensis* acting like benzodiazepines on $GABA_A$ receptors, a specific benzodiazepine antagonist was used in this investigation. It was observed that flumazenil was able to inhibit the anxiolytic effect produced by *M. angolensis*. This inhibitory effect of flumazenil is in agreement with the studies reported by Aderibigbe (2010). Therefore, our present findings suggest that *M. angolensis* may possibly act on $GABA_A$ receptor via their benzodiazepines-binding site thereby enhancing anxiolytic effect.

Diazepam-induced sleeping time test model is considered to be a valid animal model to test for sedative effects of drugs. Studies has shown that stem bark extract of *M. angolensis* potentiate the sedative property of diazepam thereby prolonging the duration of sleep at the dose of 800mg/kg (Magaji *et al*., 2009). Our present findings showed that *M. angolensis* increased the duration of sleep induced by diazepam at the dose of 40 and 80mg/kg. The sedative effects suggest that *M. angolensis* may possibly act by interacting with $GABA_A$ receptor via their benzodiazepines-binding site. Supporting this hypothesis, a specific benzodiazepine antagonist (flumazenil) was used in this investigation. It was observed that flumazenil was able to inhibit the sedative property of *M. angolensis*,

Table 1: Weekly weight changes of rats treated with sub-chronic doses of hydromethanolic stem bark extract of *M. angolensis*

Dose (mg/kg b.wt)	Initial weight	1st week	2nd week	3rd week	4th week
NC	197.86±12.68	183.38±18.14^{ns}	175.37±19.85^{ns}	168.14±10.56*	174.23±13.04^{ns}
1000	198.25±16.12	181.75±19.68^{ns}	173.50±10.52*	165.23±12.32**	172.00±11.50^{ns}
2000	195.70±16.04	179.75±19.68^{ns}	171.00±19.87^{ns}	163.00±12.85*	154.25±12.05**
3000	193.25±16.12	177.00±19.35^{ns}	167.75±10.75^{ns}	160.25±15.55*	136.50±13.74**
4000	191.25±16.60	164.25±14.65*	156.50±14.65**	147.00±18.73**	143.00±10.40**

Data are expressed as mean ±SD, n=5, *p<0.05 as compared to the normal control, ns: not significant as compared to the control, NC: normal control.

thus, decreased the duration of sleep induced by diazepam. Therefore, these findings suggest that *M. angolensis* may possibly act by interacting with $GABA_A$ receptor via their benzodiazepines-binding site.

Maeru angolensis has been reported to contain flavonoids, tannins, cardiac glycosides, and steroids/ terpenoids (Magaji *et al*., 2009). Flavonoids detected in the plant extract may be responsible for the pharmacological effects observed in this investigation. Studies have shown that flavonoids are able to modulate $GABA_A$ receptors (Yang *et al*., 2011).

CONCLUSION

In conclusion, the results of our present study have shown that *Maerua angolensis* possess good anxiolytic and sedative properties and it is safe and relatively non-toxic at therapeutic dose. We suggest that the anxiolytic and sedative activity of this plant is due to the presence of flavonoids constituents which exert their effect on $GABA_A$ receptors. Research is ongoing in our laboratory to isolate the bioactive principles and elucidate their biochemical mechanism of action.

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