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Psychopharmacological studies of *Mammea africana* stem bark extract

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Abstract

Herbal medicine is an integral part of the health care system in most developed and developing countries of the world. **Objective:** The stem bark of *Mammea africana* used traditionally in the treatment of mental disorders was evaluated for depressive effect on the central nervous system (CNS). **Materials and Method:** The stem bark extract was investigated for depressive activity in the open field, force swimming and tail suspension tests as well as its anticonvulsant potential against pentylene tetrazol and aminophylline-induced convulsions. The effect of the extract on phenobarbitone induced sleeping time was also evaluated. **Results:** The extract was found to significantly ($p<0.001$) decrease the frequency of line crossing, rearing and walling activities of the rats in open field test as well as increased the immobility time in both tail suspension and force swimming tests. The stem bark extract also significantly ($p<0.001$) shortened the onset time of sleep and prolonged the duration of sleep induced by phenobarbitone sodium. The stem bark extract and fractions (30 – 90 mg/kg), could not offered significant protection against PTZ- and aminophylline induced convulsion, but were found to delay significantly ($p<0.05 – 0.001$) the onset of tonic/clonic convulsion and also prolonged the time of death of the treated mice. **Conclusion:** The stem bark of *M. africana* has depressant, sedating and anticonvulsant properties.

Keywords: *Mammea africana*, Depressant, Sedation, Anticonvulsant, Convulsion.

Introduction

Mammea africana sabine (Guttiferae) (syn. *Ochrocarpus africana* Oliv.) is a large forest tree of 50 to 100 feet high with bark often yellow with pale scales and resinous yellow sap.[1] The plant is widely distributed in tropical Africa. The stem bark of the plant is used traditionally by the Ibibios of Niger Delta region of Nigeria in the treatment of malaria related fever, diabetes, microbial infections, convulsion and mental disorders (insanity) according to the ethno pharmacological survey. The stem bark is also used traditionally to treat stomach pains, rheumatism pains, scabies, cough and hypertension.[2, 3] The stem bark extract has been reported to possess cytotoxic activity in cell culture.[4, 5] Ouahouo *et al.*,[6] reported cytotoxic coumarins with antimicrobial activity against *Staphylococcus aureus* from the plant stem bark. The stem bark has been reported to possess antiplasmodial[7], cardioprotective[8], antidiabetic and hypolipidaemic[9], vasorelaxant[10], antihypertensive[11], anti-inflammatory and analgesic[12], antioxidant[13], antidiarrheal and antiulcer activities[14] as well as immunomodulatory and antilesihmanial activities[5]. The stem bark has been reported to contain 5,-7-dihydroxy-8-(12-methyl-butryl) –4- N - Pentyl coumarins[15-17], and Mesuxanthone B[17]. Alkaloids have been reported to be absent in the entire plant parts.[18]

Materials and Methods

Plant collection

The plant material *Mammea africana* (stem bark) were collected in a forest in Uruan area, Akwa Ibom State, Nigeria in April, 2013. The plant was identified and authenticated by Dr. Magaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

Extraction procedure

The stem bark materials were washed and shade-dried for two weeks. The dried plant materials were further chopped into small pieces and reduced to powder. The powdered material was soaked in 70% ethanol. The liquid filtrate was concentrated and evaporated to dryness in vacuo at 40ºC using a rotary evaporator. The crude ethanolic extract (10 g) was partitioned with a 50:50 mixture of distilled water and chloroform. The aqueous fraction was evaporated to dryness in a water bath at 60^{O}C and the chloroform fraction air-dried. The ethanolic extract, the aqueous and chloroform fractions were stored at -4^{O}C until used.

Experimental animals

The animals (Swiss albino rats and mice of either sex) that were used for these experiments were obtained from the University of Uyo animal house. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*. Permission and approval for animal studies were obtained from College of Health Sciences, Animal Ethics Committee, University of Uyo, Uyo.

Evaluation of Depressant activity

Open field test

Rats were randomly divided into groups of 5 rats each and treated as follows in 5 days before the open field test; control (normal saline, 2 ml/kg *p.o.*), imipramine (5.0 mg/kg, *p.o.*) and ethanolic stem bark extract of *Mammea africana* (30, 60 and 90 mg/kg, *p.o.*). The open-field arena was made of acrylic (transparent walls and black floor, 30 × 30 × 15 cm), divided into nine squares of equal areas. The open field was used to evaluate the exploratory activity of the animal. [19] The observed parameters were the number of squares crossed (with the four paws) and number of grooming and rearing, recorded for 5 min testing period.

Forced swimming test

Mice were randomly divided into groups of 5 mice each and treated as follows in 5 days before the behavioral test; control (normal saline, 2 ml/kg *p.o.*), imipramine (5.0 mg/kg, *p.o.*) and ethanolic stem bark extract of *Mammea africana* (30, 60 and 90 mg/kg, *p.o.*). For assessing antidepressant activities, we employed the method described by Porsolt *et al*.[20, 21] The development of immobility when mice were placed inside an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. Briefly, mice was individually placed in a circular tank (46 cm tall × 20 cm in diameter) filled with tap water (25 ± 1°C) to a depth of 20 cm and left there for 5 min. During this period, the behavior of the animals was recorded by an observer. Mice were considered immobile when remained floating without struggling and making only slight movements necessary to maintain the head above the water.

Tail suspension test (TST)

Mice of either sex were randomly divided into groups of 5 mice each and treated as follows in 5 days before the open field test; control (normal saline, 2 ml/kg *p.o.*), imipramine (5.0 mg/kg, *p.o.*) and ethanolic stem bark extract of *Mammea africana* (30, 60 and 90 mg/kg, *p.o.*). The total duration of immobility induced by tail suspension was measured according to the methods described by Steru *et al*.[22] Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered immobile only when they hung passively and were motionless.

Evaluation of Anticonvulsant activity

Pentylene tetrazol induced convulsion

The anticonvulsant effect of the extract was assessed using a modified method of Vellucci and Webster[23] on overnight fasted mice. The mice were divided into seven groups of six animals each and treated with 30, 60 and 90 mg/kg of the extract, 60 mg/kg of chloroform and aqueous respectively, phenytoin, 40 mg/kg one hour before induction of convulsion. The seizure was induced in each

set of mice with PTZ (70 mg/kg IP). The control group received normal saline. The onset of Clonic/tonic convulsion and the mortality rate was recorded and compared with the respective control group. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity.[24]

Aminophylline-induced Convulsion

The extract and fractions were evaluated for aminophylline –induced convulsion using the method of Juliet.[25] The mice were divided into seven groups of six animals each and treated with 30, 60 and 90 mg/kg of the extract 60 mg/kg of chloroform and aqueous respectively, phenytoin, 40 mg/kg one hour before induction of convulsion. The seizure was induced using aminophylline (280 mg/kg, i.p). The animals were observed for 120 mins after the administration of AMPH and the following parameters were noted:

1. Time to onset of myoclonic jerks in mins.
2. Time to onset of tonic convulsions in mins.
3. Time to death during experimental time of 120 mins.
4. Number of mice dead/alive at 24 hours.

Effect on phenobarbitone –induced sleeping time of rats

The crude ethanolic extract was evaluated for effect at the phenobarbitone sodium sleeping time of rats. The rats were divided into five groups of five rats each (n=5). The extract (30, 60 and 90 mg/kg) was administered to various groups of rats, diazepam (2 mg/kg, i.p) was given to the reference group and the control group was given distilled water (10 ml/kg). After 30 min the groups were treated with phenobarbitone sodium (40 mg/kg, i.p). The onset and the duration of sleep were noted and recorded in the minutes. The animals were observed for the latent period (time between phenobarbitone administration to loss of righting reflex) and the duration of sleep (the time between the loss and recovery of righting reflex).

Statistical analysis and Data evaluation

Data obtained from this work were analyzed statistically using Students' t-test and ANOVA (One- way) followed by a post test (Turkey-Kramer multiple comparison test). Differences between means was considered significant at 1% and 5% level of significance, that is $P \leq 0.05$ and 0.01

Results

Evaluation of Depressant activity

Open Field Test

Administration of the stem bark extract of *Mammea africana* caused prominent dose dependent decreases in the number of lines crossing of the extract-treated rats. The highest dose of the extract (90 mg/kg) produced a decrease which was significant ($p<0.001$) when compared to control. The standard drug produced an insignificant increase in the crossing frequency. (Figure 1).

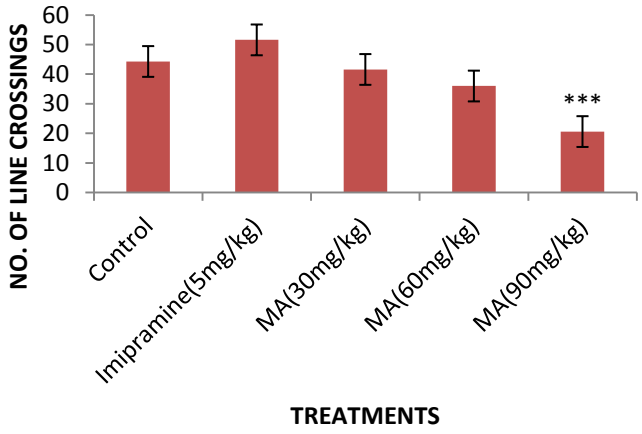

Figure 1: Bar diagram showing the effect of *Mammea africana* stembark on line crossing frequency of Rat. Results are represented as mean ± SEM with n = 5 in each group. ***P < 0.001 when compared with control group

The stem bark extract (30-90 mg/kg) exerted dose-dependent decreases in the walling activity of the extract-treated rats. These decreases were significant ($p<0.001$) when compared to control. The standard drug, imipramine (5 mg/kg) produced an insignificant decrease of walling activity of the treated animals. (Figure 2)

The rearing activities of the rats were increased following the administration of the stem bark extract of *M. africana* (30-90 mg/kg) in a dose dependent fashion. The extract (60 and 90 mg/kg) produced significantly ($p<0.01$-0.001) increases in the rearing frequency of the rats when compared to control. The standard drug, imipramine, did not exert any significant effect on the rats behavior. (Figure 3)

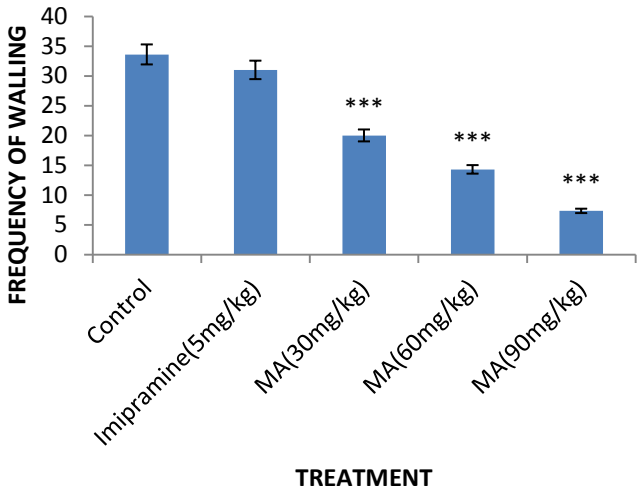

Figure 2: Bar diagram showing the effect of *Mammea africana* stembark on walling activity of rats. Results are represented as mean ± SEM with n = 5 in each group. ***P < 0.001 when compared with control group

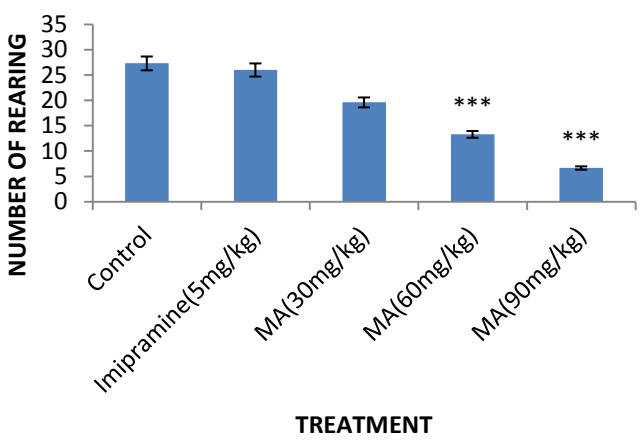

Figure 3: Bar diagram showing the effect of *Mammea africana* stembark on rearing activity of Rat. Results are represented as mean ± SEM with n = 5 in each group. ***P < 0.001 when compared with control group.

Force Swimming Test

The stem bark extracts of *M. africana* (30-90 mg/kg) exerted dose dependent increases in the immobility of rats following its administration to rats. These increases were significant (p<0.001) at higher doses (60 and 90 mg/kg) of the extract. The standard drug produced a significant (p<0.001) reduction in the immobility time in the treated rats (Figure 4).

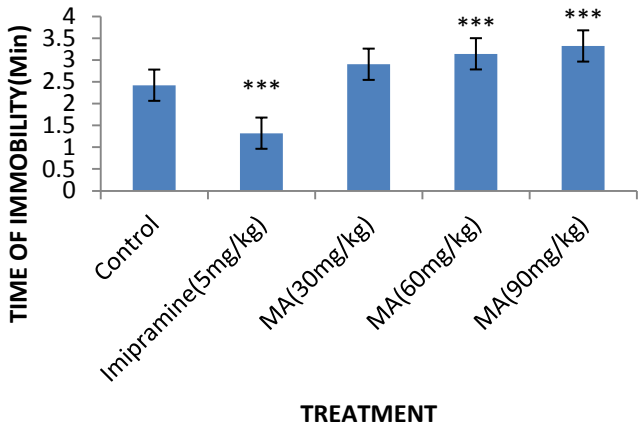

Figure 4: Bar diagram representing the immobility duration (in min) of mice in force swimming test. Results are represented as mean ± SEM with n = 5 in each group. ***P < 0.001 when compared with control group.

Tail Suspension Test

Administration of the *M. africana* stem bark extract (30-90 mg/kg) produced dose dependent increases in the immobility time of the treated rats. The increases were significant (p<0.001) only at the highest dose (90 mg/kg) of the extract when compared to control. The standard drug, imipramine (5 mg/kg) produced a significant (p<0.001) decrease of the immobility time of the treated rats (Figure 5).

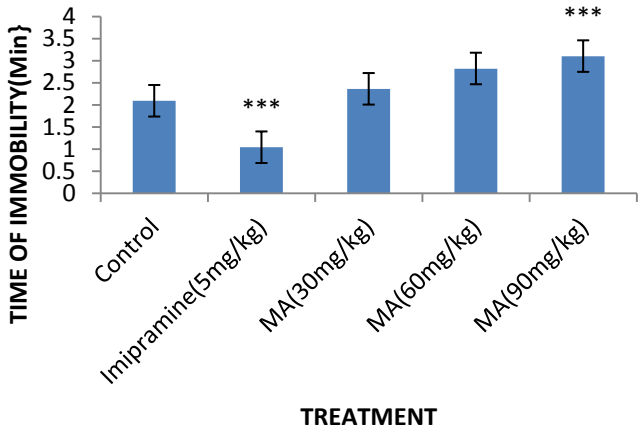

Figure 5: Bar diagram representing the immobility duration (in min) of mice in tail suspension test. Results are represented as mean ± SEM with n = 5 in each group. ***P < 0.001 when compared with control group.

Evaluation of Anticonvulsant activity

Anticonvulsant activity Mammea africana stem bark extract on aminophylline- induced convulsion

Administration of *Mammea africana* extract and fractions to mice raised the threshold of seizure induction and delayed the onset of clonic and tonic convulsion induced by aminophylline in a dose dependent fashion. The delay was only significant ($p<0.001$) at the highest dose of the extract during the onset of clonic convulsion. However, the extract and fractions were unable to offer considerable protection to the mice by preventing the onset of seizure, but were able to prolong significantly ($p<0.05$-0.001) the time of death of the animal when compared to control in a dose dependent manner. The chloroform fraction produced the highest degree of delay. The activities of the extract/fractions were lower than that of the standard drug, phenytoin (40 mg/kg) (Table 1).

Anticonvulsant Activity of Mammea africana stem bark extract on PTZ- induced convulsion

The pretreatment of mice with *Mammea africana* stem bark extract and fractions (30-90 mg/kg) caused a significant ($p<0.05$-0.001) delayed in the onset of seizure induced by pentylene tetrazol. The delay was significant ($p<0.05$-0.001) at lower doses (30 and 40 mg/kg) of the extract when compared to control. The extract/fractions further prolonged significantly ($p<0.05$-0.001) the time of death of the animals, though incomparable to that of the standard drug, Phenytoin. The chloroform fraction exerted higher activity than the aqueous fraction (Table 2).

Effect of Mammea africana stem bark on phenobarbitone induced sleeping time of rats

Administration of the stem bark extract of *M. africana* (30-90 mg/kg) to rats shortened considerably the time for the onset of sleep. The extract also prolonged the duration of sleep significantly ($p<0.001$) when compared to control. However, the effect was lower than that exerted by the standard drug. (Table 3).

Table 1: Anticonvulsant activity *Mammea africana* stembark extract on aminophylline- induced convulsion

Drug Extract	Dose (mg/kg)	Latency of clonic convulsion (s)	Latency of Tonic convulsion (s)	Convulsion %	Mortality	Time of death
Control (normal saline)	0.2ml	197.7 ± 14.53	281.3 ± 19.93	100	100	385.4 ± 12.28
Mammea africana stembark extract	30	217.5 ± 13.86	280.0 ± 12.00	100	100	337.3 ± 34.33
	60	206.3 ± 7.83	298.0 ± 22.81	100	100	773.2 ± 78.01[b]
	90	330.5 ± 31.97[c]	354.4 ± 19.97[a]	100	100	1313.5 ± 87.55[c]
Chloroform fraction	60	231.6 ± 8.05	352.3 ± 20.14[a]	100	100	514.8 ± 53.39 [a]
Aqueous fraction	60	220.0 ± 13.93	303.6 ± 17.71	100	100	481.2 ± 11.55 [a]
Phenytoin	40	252.6 ± 10.13[a]	380.2 ± 21.66[b]	100	100	2566.6 ± 23.18[c]

Data are represented as mean ± SEM. significant at [a]$P < 0.05$, [b]$p<0.01$, [c]$p<0.001$ when compared to control. (n=6)

Table 2: Anticonvulsant activity of *Mammea africana* stembark extract on PTZ- induced convulsion

Drug Extract	Dose (mg/kg)	Latency of clonic convulsion (s)	Latency of tonic convulsion (s)	Convulsion %	Mortality %	Time of Death
Control (normal saline)	0.2ml	35.0 ± 1.73	72.2 ± 2.00	100	100	128.6 ± 12.65
Mammea africana stembark extract	30	101.4 ± 17.24[b]	155.2 ± 12.66 [b]	100	100	291.5 ± 3.12 [b]
	60	157.2 ± 11.54[c]	213.0 ± 19.59[c]	100	100	286.6 ± 34.45 [b]
	90	55.3 ± 15.50	92.0 ± 17.83	100	100	386.0 ± 48.20 [c]
Chloroform fraction	60	96.0 ± 3.51[b]	223.3 ± 20.86[c]	100	100	311.3 ± 19.83 [b]
Aqueous fraction	60	43.3 ± 5.92	87.0 ± 6.65	100	100	271.4 ± 36.34 [a]
Phenytoin	40	53.3 ± 3.84	84.5 ± 2.72	100	100	436.7 ± 18.16 [c]

Data are represented as mean ± SEM. significant at aP < 0.05, bp<0.01, cp<0.001 when compared to control. (n=6)

Table 3: Effect of *Mammea africana* stembark extract on phenobarbitone induced sleeping time of rats

Drug Extract	Dose (mg/kg)	Onset of Sleep (min)	Duration of Sleep (min
Control (normal saline)	0.2ml	8.97 ± 1.53	71.30 ± 3.93
Mammea africana stembark extract	30	6.52 ± 1.86	98.04 ± 2.00[c]
	60	6.34 ± 1.74	128.14 ± 3.81[c]
	90	5.78 ± 1.97	154.43 ± 2.97[c]
Diazepam	2	4.96 ± 1.13[a]	160.32 ± 2.66[c]

Data are represented as mean ± SEM. significant at [a]P < 0.05, [b]p<0.01, [c]p<0.001 when compared to control. (n=6)

Discussion

This investigation was carried out to assess the psychopharmacological effects of stem bark extract *Mammea africana* (30-90 mg/kg) on the central nervous system (CNS) especially to ascertain its effect on the central nervous system of the rodents. This was to establish if the extract has depressant or antidepressant effect on the CNS. Different models were employed, which included open field, force swimming test and tail suspension tests. The effect of the stem bark extract on chemical–induced convulsions and phenobarbitone-induced sleeping time was also evaluated. The extract was found to decrease the frequency of line crossing, rearing and walling activities of the rats in open field test as well as increase the immobility time in both tail suspension and force swimming tests. Monitoring of locomotor activity of animals has been an important step in assessing effects of

drugs on the CNS. The movement is a measure of the level of excitability of the CNS[26] and its decrease may be intimately related to sedation resulting from the depression of the CNS[27]. The extract significantly decreased locomotor activity and increased immobility time as revealed by the results of the aforementioned tests suggesting depression and sedating potentials. Sedation may be due to interaction with benzodiazepines-like compounds. The stem bark extract might have acted by potentiating GABAergic inhibition in the CNS by membrane hyperpolarization which diminish the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts.[27] Previous investigation on phytoconstituents and plants indicate that many flavonoids and neuroactive steroids are ligands for $GABA_A$ receptors in the central nervous system, which led to the postulation that they can act as benzodiazepine.[28]

The *Mamma africana* stem bark has been reported to contain flavonoids, xanthones, coumarins and other phenolic compounds.[5,15-17] Therefore, the phytoconstituents (coumarins, flavonoids, and phenolic compounds) may be responsible for their CNS depressant activity observed in the study.

The evaluation of central nervous system depressant activities of stem bark extracts and fractions of *Mammea africana* was also carried out in this study. Pretreatment of the mice with stem bark extract and fractions (aqueous and chloroform) of *Mammea africana* (30-60 mg/kg) was found to offer significant delay in the onset of tonic/ clonic convulsions and also prolonged the time of death of the pretreated mice against convulsions induced by pentylene tetrazol and aminophylline. The chloroform fraction was found to exert the highest activity.

The exact mechanisms of seizures induced by aminophylline appear to be diverse, multiple and complex, and also unclear. Evidence suggests that seizures induced by aminophylline, could be the result of adenosine receptor antagonism or due to inhibition of cerebral nucleotidase activity [29,30] which lowered the adenosine content in the brain and eventually lead to a process of disinhibition. However, report has it that di-phenylhydantoin a potent inhibitor of adenosine uptake was ineffective in preventing these seizures.[31] Apart from non-specific adenosine receptor antagonism[32], aminophylline is thought to have inhibitory influence on adenosine synthesis. At higher dose inhibition of phosphodiesterase activity, including mobilization of intracellular calcium ions from labile stores are said to be implicated in AMPH-induced seizures. [33,34] However, a report by Ray *et al.*[35], has implicated oxidative stress due to the generation of free radicals and reactive oxygen species to be responsible for the seizures induced by aminophylline.

The stem bark extract of *Mammea africana* has been reported above to contain some phytochemical compounds like Coumarins, flavonoids and xanthones.[15-17] These compounds have been implicated in the anticonvulsant activities of many plants.[36-40] The compounds may have been responsible for the observed anticonvulsant activity. Besides, the stem bark of *Mammea africana* have been reported to antioxidant activity.[5,13] The extract and fraction may in part have exerted their anticonvulsant action by inhibiting or countering the activities of the free radicals generated by aminophylline.

According to De Sarro *et al.*,[41] pentylene tetrazol (PTZ) is suggested to exert its anticonvulsant effect by inhibiting the activity of gamma aminobutyric acid (GABA) at $GABA_A$ receptors. Gamma amino butyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion respectively.[42,43] Phenobarbitone and diazepam, standard epileptic drugs, have been shown to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain.[44,45] These drugs are reported to antagonize PTZ-induced convulsion[46] by enhancing GABA neurotransmission. Phenytoin was unable to prevent PTZ- induced seizure because it is thought to exert its antiepileptic effect by blocking sodium ions into brain cells thus inhibiting generation of repetitive action potential[44]. Since the stem bark extract of *Mammea africana* was able to delay PTZ – induced convulsion it is probable that they may be interfering with gabaergic mechanism(s) to exert its effect. Their anticonvulsant activities are due to their phytochemical components as reported above.

In our study, an ethanolic stem bark extract of *Mammea africana* significantly enhanced duration of the phenobarbitone sodium -induced hypnotic effect, which was observed in the shortening of time of onset of sleep and prolonging the duration of sleep following their administration suggesting a depressing activity on the CNS. Substances which possess CNS depressant activity either decrease the time for the onset of sleep or prolong the duration of sleep or both.[47,48] A prolongation of the

phenobarbitone effect could involve a facilitation of GABA mediated postsynaptic inhibition through allosteric modification of $GABA_A$ receptors.

Conclusion

From the results of this study, it can be concluded that the stem bark extract of *Mammea africana* has depressant activity on the central nervous system as can be seen its depressant and anticonvulsant activities. This result justifies its use traditionally in the treatment of mental disorders.

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