

Screening of Central Effects of the Leaves of *Mallotus oppositifolius* (Geiseler) Mull. Arg. in Mice

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Abstract: Objective: Central Nervous System (CNS) effects of the hydroalcoholic extract of the leaves of *Mallotus oppositifolius* (MOE), a plant for managing epilepsy and convulsions, was investigated using the core CNS battery tests (Irwin test, activity meter test, rotarod test, pentylenetetrazol (PTZ) seizure test, pentobarbital interaction test and tail immersion test). **Results:** In the Irwin test, the extract caused sedation and motor incoordination at higher doses (300-6000 mg kg⁻¹). Convulsions were observed at the dose of 6000 mg kg⁻¹ (two mice out of seven). After 24 h, 3 animals died at the dose of 6000 mg kg⁻¹. LD₅₀ was approximately 6000 mg kg⁻¹. At doses of 3000-6000 mg kg⁻¹ MOE significantly decreased spontaneous locomotor activity in the activity meter test ($F_{7,40} = 55.78$; $p < 0.0001$) and impaired motor coordination in the rotarod test ($F_{7,48} = 37.27$; $p < 0.0001$). In the pentylenetetrazol convulsive threshold test, the extract significantly delayed the onset of both clonic ($F_{8,54} = 651.2$; $p < 0.0001$) and tonic convulsions ($F_{8,54} = 625.1$; $p < 0.0001$). It reduced the frequency of clonic ($F_{8,54} = 33.28$; $p < 0.0001$) and tonic convulsions ($F_{8,54} = 26.70$; $p < 0.0001$) and duration of clonic ($F_{8,54} = 26.74$; $p < 0.0001$) and tonic convulsions ($F_{8,54} = 52.27$; $p < 0.0001$). The extract also exhibited potent analgesic properties in the tail immersion test. MOE induced hepatic liver enzymes by decreasing duration of pentobarbitone induced sleeping time. It was also metabolized by hepatic enzymes. These results will contribute to the quest to isolate and develop effective therapeutic agents for the management of psychiatric and neurological disorders like epilepsy, anxiety and pain.

Key words: Mice, analgesia, epilepsy, pentylenetetrazol, anxiety, Irwin

INTRODUCTION

In the drug discovery process, it is important to assess and monitor the efficacy and safety of drugs especially in the preclinical phase (Kinter and Valentin, 2002). In 2000, the International Conference on Harmonization (ICH), determined guidelines for safety pharmacology. The Conference recommended the monitoring of the functions and effects of test drugs on the cardiovascular, respiratory and central nervous system.

The Core CNS battery procedures are applied at the initial stages of the drug discovery process to help eliminate substances with a potential for CNS risk. These protocols include measuring general behavioural signs induced by test substance (Irwin test), effects on spontaneous locomotion (Activity meter test), effects on neuromuscular coordination (Rotarod test), effects on the convulsive threshold (PTZ seizure test), interaction with hypnotics (Pentobarbital interaction test) and effects on the pain threshold (Tail immersion test) (Porsolt *et al.*, 2002).

Mallotus oppositifolius is a common shrub that is used traditionally for treating convulsion, epilepsy, treatment of parasitic eye and kidney infections, as diuretic, pain killer and treatment of paralysis, spasm, headache and swellings. Decoction of the root is used for anaemia, pneumonia and as aphrodisiac and the stick is chewed for oral hygiene and teeth cleaning (Burkill, 1985). Anti-inflammatory (Farombi *et al.*, 2001; Nwaeujor *et al.*, 2012) and antimicrobial effects (Adekunle and Ikumapayi, 2006; Gbedema *et al.*, 2010) of the plant have been reported. Alkaloids, saponins, sterols and glycosides have been detected in the plant (Farombi *et al.*, 2001; Adekunle and Ikumapayi, 2006). Despite its usefulness there is no available scientific data on its CNS effects. Thus these tests were carried out on the leaves to assess the neuropharmacological effects of the leaves of the plant.

MATERIALS AND METHODS

Plant collection: Leaves of the plant *M. oppositifolius* were collected from the campus of Kwame Nkrumah

University of Science and Technology (KNUST), Kumasi, Ghana (6°41'6.4"N, 1°33'42.8"W), in September, 2009. The leaves were authenticated at the Department of Herbal Medicine of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi.

Extraction: The leaves were air-dried under shade for a week and pulverized with a hammer-mill. Four hundred and fifty grams of the powder was extracted by cold maceration using 70% (v/v) ethanol in water over a period of 72 h. The resulting extract was concentrated under low temperature (60°C) and pressure to a syrupy mass in a rotary evaporator. The syrupy mass was then dried to a dark brown solid mass on a water bath and kept in a desiccator till it was ready to be used. The final yield was 9.5% (w/w). This is subsequently referred to as *Mallotus oppositifolius* Extract (MOE) or extract.

Animals: Male ICR mice were obtained from the Noguchi Memorial Institute for Medical Research, Accra, Ghana and kept at the animal facility of the Department of Pharmacology, KNUST, Kumasi, Ghana. The animals were housed in groups of five in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions. All animals used in these studies were treated according to the Guide for the Care and Use of Laboratory Animals (NRC, 1996) and were approved by the College Ethics Committee.

Drugs and chemicals: Diazepam (DZP), Pentylentetrazol (PTZ), Caffeine (CFN), Pentobarbitone (PBT) and Phenobarbitone (PHE) were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA. Morphine hydrochloride (MOR) was obtained from phyto-riker, Accra, Ghana.

Phytochemical tests: Freshly prepared aqueous extract was analyzed for phytochemical constituents as described by Trease and Evans (1989) for the detection of alkaloids, saponins, reducing sugars, sterols, tannins and terpenoids.

Irwin test: Assessment of behaviour and physiological function was carried out similar to the primary observation procedure originally described by Irwin (1968). ICR mice (20-25 g) were randomly divided into seven groups (n = 7) and kept in the experimental environment for 7 days to acclimatize. Animals were fasted overnight but had access to water *ad libitum* and then treated orally with *M. oppositifolius* extract in doses of 30, 100, 300, 1000,

3000 and 6000 mg kg⁻¹ of b.wt. Mice in the control group received 10 mL kg⁻¹, p.o. of saline (0.9% w/v). The mice were observed at 0, 15, 30, 60, 120 and 180 min, up to 24 h after treatment for general changes in behaviour and physiological function as well as mortality. The animals were assessed for behaviours related to neurotoxicity and for behaviours related to CNS depression. Effects on autonomic functions were also noted, as was the lethality of the test agent.

Activity meter test: This test is employed to measure spontaneous locomotor activity with an activity cage (Ugo Basile model 7401, Comerio, VA, Italy). ICR mice weighing (20-25 g), each group had 7 mice, were given the extract (30-3000 mg kg⁻¹), caffeine (2.5 mg kg⁻¹) or diazepam (8 mg kg⁻¹) by the oral route. After 60 min, the animals were placed in the activity cage individually and their activities were scored every 5 minutes for 30 min.

Rotarod test: The effect of the extract on neuromuscular coordination was determined using the rotarod (Ugo Basile, model 7600, Comerio, VA, Italy). ICR mice were divided into 8 groups of 7 animals each after an initial screening to obtain a baseline. The extract (30, 100, 300, 1000 and 3000 mg kg⁻¹, p.o.) was administered to groups A, B, C, D, E while the reference drugs d-tubocurarine (0.1 mg kg⁻¹, i.p.) and diazepam (8 mg kg⁻¹, p.o.) were administered to groups F and G, respectively. Group H received distilled water (10 mL kg⁻¹, i.p.) and served as the control group. The animals were placed on the rotarod bar prior to treatment and at 0.5, 1, 1.5 and 2 h after treatment. The time in seconds for the mouse to fall off within the cut off time of 180 sec was noted.

Convulsive threshold test (PTZ seizure test): ICR mice weighing 20-25 g, were divided into 7 groups (n = 7). Group 1 was given distilled water (10 mL kg⁻¹); groups 2-6 received MOE (30-3000 mg kg⁻¹, p.o.) and group 7, received diazepam (8 mg kg⁻¹, p.o.). Pentylentetrazol (100 mg kg⁻¹, s.c.) was administered to the mice 30 min after distilled water or 1 h after diazepam or MOE. The mice were then placed in a plastic observation cage and observed for 60 min for latency to clonic and tonic convulsions, frequency and duration of clonic and tonic convulsions for 1 h.

Pentobarbitone interaction test: The effect of MOE on pentobarbitone-induced sleeping time was studied in mice as described previously (De-Paris *et al.*, 2000). Fifty-six ICR mice were randomly divided into eight groups (n = 7). The first group served as control and received distilled water (10 mL kg⁻¹). The remaining seven groups received

either MOE (30, 100, 300, 1000, 3000 mg kg⁻¹, p.o.), diazepam (8 mg kg⁻¹, p.o.) or caffeine (16 mg kg⁻¹, p.o.). Sodium pentobarbitone (50 mg kg⁻¹) was intraperitoneally administered 1 h after diazepam, caffeine or MOE. In the case of the control group, animals received only the pentobarbitone.

In a separate experiment, the effect of hepatic enzyme induction on pentobarbitone sleeping time was assessed. Briefly eight groups of mice (n = 7) were pretreated with phenobarbitone (25 mg kg⁻¹, i.p.) for two days and on the third day, the first five groups were given extract (30-3000 mg kg⁻¹, p.o.) and pentobarbitone (50 mg kg⁻¹, i.p.), then the rest of the groups took either, diazepam (8 mg kg⁻¹, p.o.) and pentobarbitone (50 mg kg⁻¹, i.p.); caffeine (16 mg kg⁻¹, p.o.) and pentobarbitone (50 mg kg⁻¹, i.p.) or pentobarbitone alone (50 mg kg⁻¹, i.p.). In another experiment, the effect of extract (30-3000 mg kg⁻¹) on hepatic enzyme induction or inhibition was investigated. Five groups of mice (n = 7) were pretreated with extract for 2 consecutive days and on the third day, they received pentobarbitone (50 mg kg⁻¹, i.p.).

Two parameters were recorded: time elapsed since the application of pentobarbitone until the loss of the righting reflex (latency/onset of action) and the time elapsed from the loss to regaining of the righting reflex (duration of sleep).

Tail immersion test: Tail-immersion test was carried out as described by Sewell and Spencer (1976) and Luttinger (1985) with modifications. The tail of the mouse was immersed in a water bath containing water at a temperature of 48±0.5°C. The mouse reacts by withdrawing the tail (Bohn *et al.*, 2000). The reaction time was recorded with a stop watch and a cut-off time of 10 sec imposed on this measure. Mice were randomly divided into one of the following study groups (seven per group): control, morphine (10 mg kg⁻¹, i.p.) and MOE (30, 100, 300, 1000 and 3000 mg kg⁻¹, p.o.). The reaction time (T) for the study groups was taken at 0.5, 1 and 2 h intervals after a latency period of 30 min (i.p.) or 1 h (p.o.) following the administration of the morphine and extract. The percentage maximal possible effect (%MPE) was calculated from the reaction times by the formula:

$$\text{MPE (\%)} = \frac{\text{Post - Drug latency} - \text{Pre - Drug latency}}{\text{Cut - Off latency} - \text{Pre - Drug latency}} \times 100$$

where, 10 sec is the cut-off latency time.

Analysis of data: All data are presented as Mean±SEM. Data were analyzed using one-way analysis of variance

(ANOVA) with drug treatment as a between-subjects factor. Whenever ANOVA was significant, further comparisons between vehicle- and drug-treated groups were performed using the Newman-Keuls' *post hoc* test. GraphPad Prism for Windows Version 5 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. p<0.05 was considered significant.

RESULTS

Phytochemical test: The phytochemical screening revealed the presence of alkaloids, tannins, saponins, reducing sugars and sterols. Terpenoids were absent (Table 1).

Irwin test: Convulsions were observed in two mice in the highest dose (6000 mg kg⁻¹) after 15 min. During the 24 h period, no other toxic signs were observed except for sedation and loss of muscle coordination from the dose of 300-6000 mg kg⁻¹. Two mice died on the first day and after 24 h, there was one more death in the highest dose (Table 2).

Activity meter test: MOE affected spontaneous activity only at 3000 mg kg⁻¹ (F_{7,47} = 55.78, p<0.0001) (Fig. 1). Diazepam, (8 mg kg⁻¹, p.o.), significantly decreased spontaneous activity in contrast to caffeine (16 mg kg⁻¹, p.o.) which is a CNS stimulant.

Rotarod: From the time course curve, MOE caused a slight decrease in the time spent on the rotarod at lower doses but significantly decreased it at higher doses (Fig. 2a). From the Area under the Curve (AUC),

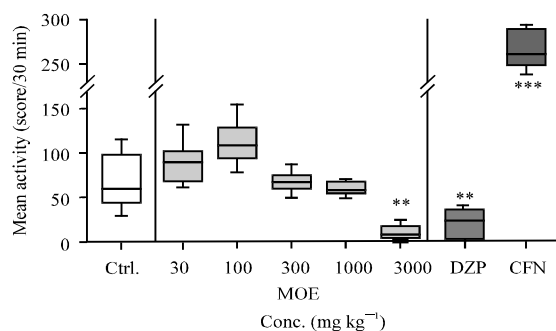


Fig. 1: Effect of different concentrations of *Mallotus oppositifolius* extract (MOE) and administration of diazepam (DZP) and caffeine (CFN) on spontaneous activity of mice. Data are Mean±SEM, (n = 7), ***p<0.001 and **p<0.01 when compared to vehicle-treated group

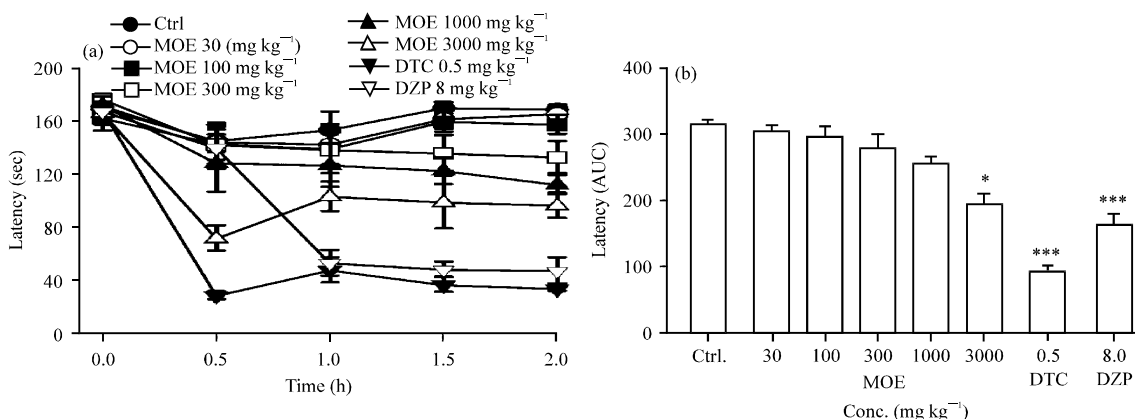


Fig. 2(a-b): Effect of different concentrations of *Mallotus oppositifolius* extract (MOE) and supplementation of diazepam (DZP) and d-tubocurarine on the time course curve of the (a) Rotarod test and (b) The area under the curve (AUC) in mice, Data are Mean±SEM (n = 7), ***p<0.001 and *p<0.05 when compared to vehicle-treated group

Table 1: Phytochemical constituents of the hydroalcoholic extract from the leaves of *M. oppositifolius*

Constituent	Results
Saponins	+
Tannins	++
Alkaloids	++
Triterpenes	-
Reducing sugars	++
Sterols	++

-: Not detected, +: Present in low concentration, ++: Present in moderate concentration

Table 2: Observations in the acute toxicity test after oral administration of *Mallotus oppositifolius* (MOE) in mice

MOE dose (mg kg ⁻¹)	Mortality		
	D/T	Latency (h)	Toxicity
0	0/7	-	-
30	0/7	-	-
100	0/7	-	-
300	0/7	-	Sedation, motor incoordination
1000	0/7	-	Sedation, motor incoordination
3000	0/7	-	Sedation, motor incoordination
6000	3/7	24	Convulsion, sedation, motor incoordination, death

D/T: Dead/treated mice, -: No toxic symptoms were seen during the observation period, Latency: Time to death (h) after the dose

MOE caused significant motor impairment from 1000-3000 mg kg⁻¹ (F_{7,48} = 37.27, p<0.0001) (Fig. 2b). Both diazepam (8 mg kg⁻¹) and d-tubocurarine (0.5 mg kg⁻¹) also caused significant motor impairment (Fig. 2a-b).

PTZ seizure test: MOE (30-3000 mg kg⁻¹), administered orally, significantly delayed both onset of clonic (F_{8,54} = 651.2, p<0.0001) (Fig. 3a) and tonic convulsions (F_{8,54} = 625.1, p<0.0001) (Fig. 3c) in mice. The frequency of both clonic (F_{8,54} = 33.28, p<0.0001) (Fig. 3a) and tonic convulsions (F_{8,54} = 26.70, p<0.0001) (Fig. 3c)

was reduced. Furthermore, duration of clonic convulsions (F_{8,54} = 26.74, p<0.0001) (Fig. 3b) and tonic convulsions (F_{8,54} = 52.27, p<0.0001) (Fig. 3d) were also reduced significantly by the extract. Diazepam (0.3-3 mg kg⁻¹), the reference drug also demonstrated similar effects as the extract (Fig. 3a-d).

Pentobarbital interaction test: In the barbiturate induced sleeping time, the extract demonstrated a significant CNS depressant effect by causing a dose dependent increase in the duration of sleep (F_{7,48} = 22.02, p<0.0001) (Fig. 4) at all doses tested except at 30 mg kg⁻¹. It however did not affect the onset of sleep compared to the control group (Fig. 4a). Diazepam (8 mg kg⁻¹, p.o.), a reference CNS depressant caused a similar effect as the extract while caffeine (16 mg kg⁻¹, p.o.), the CNS stimulant, delayed onset of sleep and significantly reduced the duration of the sleeping time (Fig. 4). MOE and diazepam had no effect on latency to sleep in phenobarbitone pretreated mice (Fig. 5a). On the contrary caffeine reduced the latency to sleep in phenobarbitone pretreated mice (Fig. 5a). MOE (F_{7,48} = 22.02, p<0.0001) and diazepam significantly reduced the duration of sleep induced in mice pretreated with phenobarbitone for 2 days (Fig. 5b). Decrease in sleep latency was reversed by MOE at doses of 1000 and 3000 mg kg⁻¹ (Fig. 6a). Pretreating mice with various doses of extract for 2 days reversed the increase in sleep duration (F_{7,48} = 22.02, p<0.0001) (Fig. 6b).

Tail immersion test: MOE exhibited significant analgesic activity by increasing the (%) Maximal Possible Effect (MPE) in the tail immersion test. From the time-course curve, the analgesic activity peaked at 1 h after drug

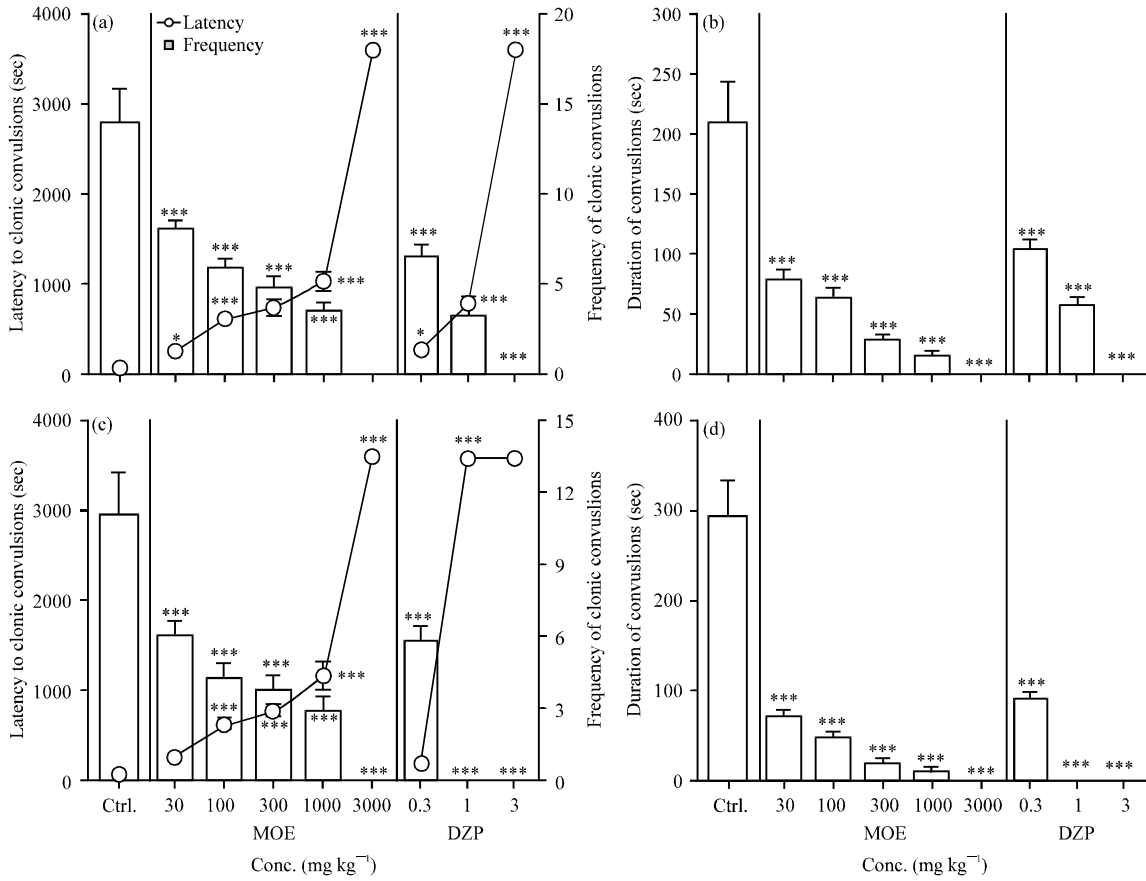


Fig. 3(a-d): (a) Effect of different concentration of *Mallotus oppositifolius* extract (MOE) and diazepam (DZP) on the (a) Latency and frequency of clonic convulsions (b) Duration of clonic convulsions, (c) Latency and frequency of tonic convulsions and (d) Duration of tonic convulsions in pentylenetetrazol induced seizure test in mice, Data are Mean±SEM (n = 7), ***p<0.001 and *p<0.05 when compared to vehicle-treated group

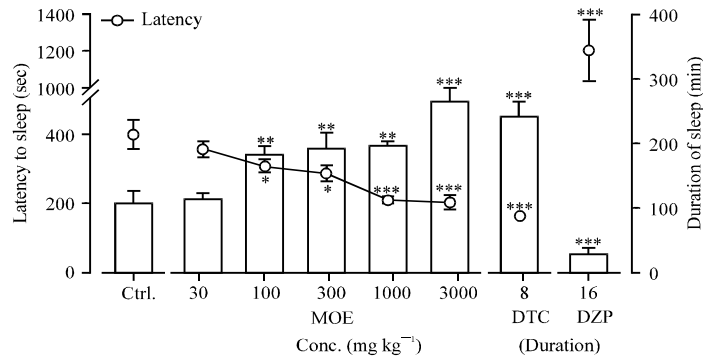


Fig. 4: Effect of different concentrations of *Mallotus oppositifolius* extract (MOE) and administration of diazepam (DZP) and caffeine (CFN) on the latency to sleep and duration of sleep, in the pentobarbitone induced sleeping test, Data are Mean±SEM (n = 7); ***p<0.001 and **p<0.01 when compared to vehicle-treated group

treatment and reduced by the second hour (Fig. 7a). One-way ANOVA followed by Newman-Keuls' *post hoc* test of the AUC indicated that MOE

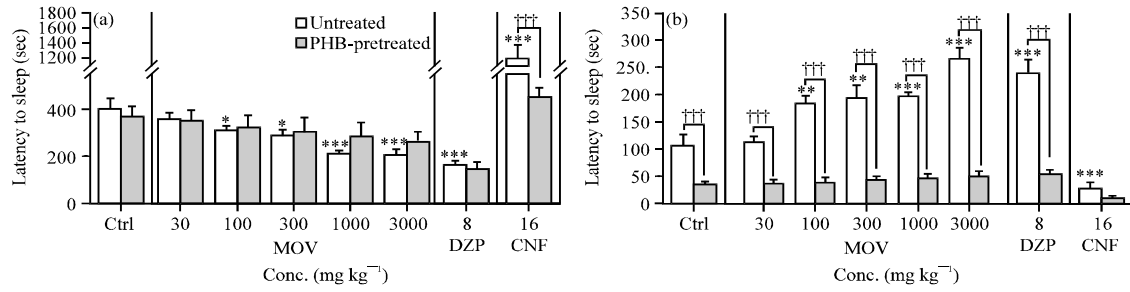


Fig. 5(a-b): Effect of different concentrations of *Mallotus oppositifolius* extract (MOE) and administration of diazepam (DZP) and caffeine (CFN) on (a) Latency to sleep and (b) Duration of sleep, after pre-treating with phenobarbitone in the pentobarbitone induced sleeping test, Data are Mean±SEM (n = 7), ***p<0.001 and *p<0.05 when compared to vehicle-treated group, †††p<0.001 when dose and treatments are compared

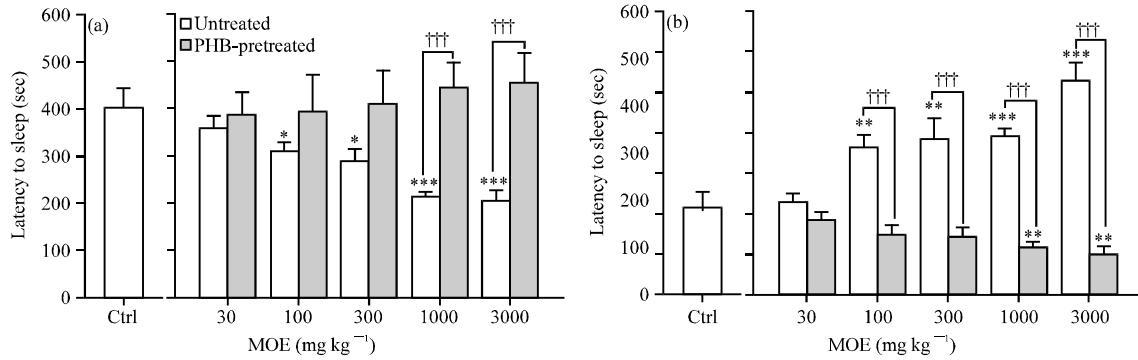


Fig. 6(a-b): Effect of phenobarbitone on (a) The latency to sleep and (b) Duration of sleep after pre-treatment with extract in the pentobarbitone induced sleeping test, Data are Mean±SEM (n = 7), ***p<0.001 and *p<0.05 when compared to vehicle-treated group, †††p<0.001 when dose and treatments are compared

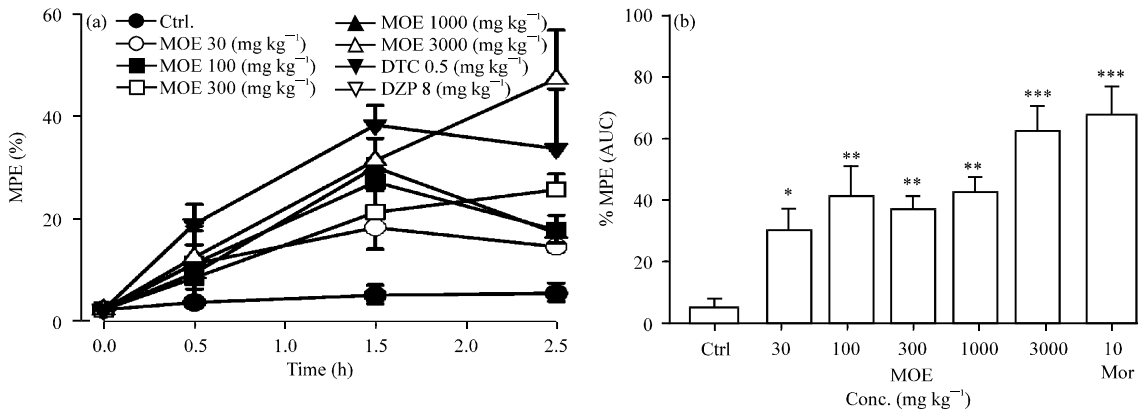


Fig. 7(a-b): Effect of different concentrations of *Mallotus oppositifolius* extract (MOE) and administration of morphine (Mor) on (a) Time course curve of the tail immersion test and (b) The area under the curve (AUC) in mice, Data are Mean±SEM (n = 7), *p<0.05, **p<0.01 and ***p<0.001 when compared to vehicle-treated group

exhibited analgesic effect ($F_{6,42} = 9.069$, $p < 0.0001$) (Fig. 7b). Similar result was obtained for morphine (10 mg kg⁻¹), the reference analgesic drug used (Fig. 7a-b).

DISCUSSION

Central effects of the hydroalcoholic extract of the leaves of *Mallotus oppositifolius* was investigated in the

core CNS battery test. The extract caused sedation and reduced spontaneous locomotor activity. It showed analgesic, anticonvulsant properties and induces hepatic enzymes. Furthermore the extract was metabolized by hepatic enzymes.

In the Irwin test, MOE exhibited sedation and motor impairment at higher doses without impairing respiration. This is indicative of possible CNS depressant activity. The Irwin test, one of the core battery tests, is used to estimate the minimum lethal dose of a test substance, the dose range for CNS responses and the primary effects on behavior and physiological functions (Irwin, 1968; Porsolt *et al.*, 2002; Roux *et al.*, 2005; Lynch *et al.*, 2011). The results of this test are used to predict potential therapeutic activity and to select doses for subsequent tests of efficacy. Data from the Irwin test are also used to assess the risks associated with the use of this agent (Irwin, 1968; Jezhitsu *et al.*, 2002). It is possible that MOE may have potential use in CNS conditions where there is CNS excitation such as in convulsions, epilepsy or anxiety. In fact the plant is used traditionally for managing these conditions. To corroborate this, the extract showed anticonvulsant activity in the PTZ convulsive threshold test. At all doses, the extract delayed the onset of clonic and tonic convulsions; and also reduced the frequency and duration of the clonic and tonic convulsions. Any substance that delays the onset and the duration of clonic or tonic clonic convulsion induced by pentylenetetrazol is described as an anticonvulsant (Vellucci and Webster, 1984; De Sarro *et al.*, 1999; Sayyah *et al.*, 2004). Caution should however be exercised since at the highest dose of 6000 mg kg⁻¹ of body weight, there were visible signs of convulsion and death in the Irwin test. Out of seven mice used, three died after 24 h at the highest dose, suggesting that the Lethal Dose-50 (LD₅₀) is approximately 6000 mg kg⁻¹.

Drugs that act on the CNS may enhance, inhibit or not affect locomotor activity in mice (Kimmel *et al.*, 2000; Wiley and Martin, 2003; El-Mas and Abdel-Rahman, 2004; Kawaura *et al.*, 2010). MOE reduced spontaneous activity in mice in the activity meter test. Locomotion can be decreased as a result of sedation, drug-induced motor impairment or debilitation by test substance (Brooks *et al.*, 1999; Betarbet *et al.*, 2002; Henry *et al.*, 2002; Porsolt *et al.*, 2002). In order to ascertain whether reduction in spontaneous activity was by drug-induced motor impairment or sedation, the rotarod test was performed. From the results, it was clear that there was significant motor impairment at the dose that reduced spontaneous activity in the activity meter test. Since, sedation was also observed at the dose of 3000 mg kg⁻¹ in the Irwin test, it is possible that sedation may also

contribute to the reduction in activity. Diazepam, a CNS depressant, reduced spontaneous activity and impaired motor coordination at the dose used while caffeine, a CNS stimulant increased the locomotor activity (El Yacoubi *et al.*, 2000; Solinas *et al.*, 2002; Wiley *et al.*, 2002; Dunne *et al.*, 2007). Many CNS depressant compounds can cause reduction in spontaneous locomotor activity in laboratory animals. Nearly all the neuroleptic agents used in psychiatry diminish spontaneous locomotor activity in all species including man (Baldessarini *et al.*, 1990; Simon *et al.*, 2000; Kinkead and Nemeroff, 2002).

Sleep-enhancing effect of substances can readily be detected in the barbiturate induced sleeping time test by substances which do not induce sleep even at high doses when administered alone (Montalto de Mecca *et al.*, 2000; Porsolt *et al.*, 2002; Nayak *et al.*, 2004). There is also a high correlation between the effects observed in this procedure and those observed in more complex tests and in man (Andre *et al.*, 1984; Renton, 1985; Zhao *et al.*, 2006). In the present study albeit MOE enhanced the duration of sleep in mice profoundly, this was not apparent in the Irwin test. The prolongation of pentobarbitone induced sleep further supports the central depressant activity of the extract.

Pretreatment of mice with phenobarbitone for two days reduced the increase in sleep duration induced by the extract or diazepam. This suggests that the extract is metabolized by the hepatic enzymes (Conney, 1967; Kushikata *et al.*, 2003). Some drugs which are metabolized by the liver can either induce or inhibit hepatic enzyme metabolism (Conney, 1967; Brockmoller and Roots, 1994; Back *et al.*, 2003; Park *et al.*, 2005). Hence, the possible effect of the extract on hepatic enzymes was assessed by pre-treating mice with the extract for two days before administering pentobarbitone. It is evident that the extract was a hepatic enzyme inducer since it reduced the sleep duration when compared to the group that received only pentobarbitone or extract and pentobarbitone. Drugs that are either metabolized by liver enzymes or induce hepatic enzymes are potential targets for drug-drug interactions. This pharmacokinetic effect of substances may result in toxicity or reduced therapeutic effect as a result of decrease in the effective concentration of drug in the plasma. On the other hand, beneficial synergistic effects can be observed through these interactions (George *et al.*, 1995; Park *et al.*, 2005).

Analgesic activity was observed from 100-3000 mg kg⁻¹ in the Irwin test which also supports the traditional use of the plant in managing headache and allodynia. This observation in the Irwin test was further confirmed in the tail immersion test, an acute thermal

pain model (Le Bars *et al.*, 2001; Simmons *et al.*, 2002; Jones *et al.*, 2005). Morphine, the reference analgesic, also gave similar results. Thermal nociception models such as hot plate and the tail immersion tests are used to evaluate central analgesic activity. The tail immersion model is sensitive to drugs that act spinally or supraspinally (Ramabadran *et al.*, 1989; Pal *et al.*, 1999; Muhammad *et al.*, 2012). It is possible that the analgesic effect of MOE may be due to spinal or supraspinal pathways. That MOE has sedative properties may confound the test because most pain models are based on behavioural measures which involve motor coordination (Jarvis *et al.*, 2002; Kehl *et al.*, 2003; Nassar *et al.*, 2005; McGowan *et al.*, 2009; Zhao *et al.*, 2006). However, in this study, doses required for analgesia were much lower than those that caused motor impairment.

CONCLUSION

From the core CNS battery tests, MOE exhibited significant CNS depressant, analgesic and anticonvulsant effect. The LD₅₀ is estimated to be approximately 6000 mg kg⁻¹. The extract reduced spontaneous activity and impaired motor coordination at high doses. MOE is a hepatic enzyme inducer and also metabolized by liver enzymes.

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REFERENCES

- Adekunle, A.A. and A.M. Ikumapayi, 2006. Antifungal property and phytochemical screening of the crude extracts of *Funtumia elastica* and *Mallotus oppositifolius*. West Indian Med. J., 55: 219-223.
- Andre, P., J. Descotes, R. Simonet and J.C. Evreux, 1984. Reappraisal of the barbiturate sleeping time in mice as predictive tool for the detection of liver enzymes inhibiting drugs. Methods Find. Exp. Clin. Pharmacol., 6: 695-699.
- Back, D., S. Gibbons and S. Khoo, 2003. Pharmacokinetic drug interactions with nevirapine. J. Acquir. Immune. Defic. Syndr., 34: 8-14.
- Baldessarini, R.J., E.R. Marsh, N.S. Kula, R.S. Zong, Y.G. Gao and J.L. Neumeyer, 1990. Effects of isomers of hydroxyaporphines on dopamine metabolism in rat brain regions. Biochem. Pharmacol., 40: 417-423.
- Betarbet, R., T.B. Sherer and J.T. Greenamyre, 2002. Animal models of Parkinson's disease. Bioessays, 24: 308-318.
- Bohn, L.M., F. Xu, R.R. Gainetdinov and M.G. Caron, 2000. Potentiated opioid analgesia in norepinephrine transporter knock-out mice. J. Neurosci., 20: 9040-9045.
- Brockmoller, J. and I. Roots, 1994. Assessment of liver metabolic function. Clinical implications. Clin. Pharmacokinet., 27: 216-248.
- Brooks, A.I., C.A. Chadwick, H.A. Gelbard, D.A. Cory-Slechta and H.J. Federoff, 1999. Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. Brain Res., 823: 1-10.
- Burkill, H.M., 1985. The Flora of West Tropical Africa. 2nd Edn., Kew, Royal Botanic Gardens, London, ISBN: 1-90034-713-X.
- Conney, A.H., 1967. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev., 19: 317-366.
- De Sarro, A., V. Cecchetti, V. Fravolini, F. Naccari, O. Tabarrini and G. De Sarro, 1999. Effects of novel 6-desfluoroquinolones and classic quinolones on pentylenetetrazole-induced seizures in mice. Antimicrob. Agents Chemother., 43: 1729-1736.
- De-Paris, F., G. Neves, J.B. Salgueiro, J. Quevedo, I. Izquierdo and S.M. Rates, 2000. Psychopharmacological screening of *Pfaffia glomerata* Spreng. (Amaranthaceae) in rodents. J. Ethnopharmacol., 73: 261-269.
- Dunne, F., A. O'Halloran and J.P. Kelly, 2007. Development of a home cage locomotor tracking system capable of detecting the stimulant and sedative properties of drugs in rats. Prog. Neuropsychopharmacol. Biol. Psychiatry, 31: 1456-1463.
- El Yacoubi, M., C. Ledent, J.F. Menard, M. Parmentier, J. Costentin and J.M. Vaugeois, 2000. The stimulant effects of caffeine on locomotor behaviour in mice are mediated through its blockade of adenosine A(2A) receptors. Br. J. Pharmacol., 129: 1465-1473.
- El-Mas, M.M. and A.A. Abdel-Rahman, 2004. Effects of long-term ovariectomy and estrogen replacement on clonidine-evoked reductions in blood pressure and hemodynamic variability. J. Cardiovasc. Pharmacol., 43: 607-615.
- Farombi, E.O., O. Ogundipe and J.O. Moody, 2001. Antioxidant and anti-inflammatory activities of *Mallotus oppositifolium* in model systems. Afr. J. Med. Med. Sci., 30: 213-215.

- Gbedema, S.Y., F. Adu, M.T. Bayor, K. Annan and J.S. Boateng, 2010. Enhancement of antibacterial activity of amoxicillin by some Ghanaian medical plant extracts. *Int. J. Pharma. Sci. Res.*, 1: 145-152.
- George, J., M. Murray, K. Byth and G.C. Farrell, 1995. Differential alterations of cytochrome P450 proteins in livers from patients with severe chronic liver disease. *Hepatology*, 21: 120-128.
- Henry, S.A., V. Lehmann-Masten, F. Gasparini, M.A. Geyer and A. Markou, 2002. The mGluR5 antagonist MPEP but not the mGluR2/3 agonist LY314582, augments PCP effects on prepulse inhibition and locomotor activity. *Neuropharmacology*, 43: 1199-1209.
- Iezhitsa, I.N., A.A. Spasov, L.I. Bugaeva and I.S. Morozov, 2002. Toxic effect of single treatment with bromantane on neurological status of experimental animals. *Bull. Exp. Biol. Med.*, 133: 380-383.
- Irwin, S., 1968. System analysis: A strategy for drug experimentation and evaluation. *Perspect. Biol. Med.*, 11: 654-674.
- Jarvis, M.F., E.C. Burgard, S. McGaraughty, P. Honore and K. Lynch *et al.*, 2002. A-317491, a novel potent and selective non-nucleotide antagonist of P2X3 and P2X2/3 receptors, reduces chronic inflammatory and neuropathic pain in the rat. *PNAS*, 99: 17179-17184.
- Jones, C.K., S.C. Peters and H.E. Shannon, 2005. Efficacy of duloxetine, a potent and balanced serotonergic and noradrenergic reuptake inhibitor, in inflammatory and acute pain models in rodents. *J. Pharmacol. Exp. Ther.*, 312: 726-732.
- Kawaura, K., R. Miki, E. Shima, S. Honda, F. Soeda, T. Shirasaki and K. Takahama, 2010. Antidepressant-like effect of centrally acting non-narcotic antitussive caramiphen in a forced swimming test. *Neurosci. Lett.*, 481: 188-190.
- Kehl, L.J., D.T. Hamamoto, P.W. Wacnik, D.L. Croft, B.D. Norsted, G.L. Wilcox and D.A. Simone, 2003. A cannabinoid agonist differentially attenuates deep tissue hyperalgesia in animal models of cancer and inflammatory muscle pain. *Pain*, 103: 175-186.
- Kimmel, H.L., W. Gong, S.D. Vechia, R.G. Hunter and M.J. Kuhar, 2000. Intra-ventral tegmental area injection of rat cocaine and amphetamine-regulated transcript peptide 55-102 induces locomotor activity and promotes conditioned place preference. *J. Pharmacol. Exp. Ther.*, 294: 784-792.
- Kinkead, B. and C.B. Nemeroff, 2002. Neurotensin: An endogenous antipsychotic? *Curr. Opin. Pharmacol.*, 2: 99-103.
- Kinter, L.B. and J.P. Valentin, 2002. Safety pharmacology and risk assessment. *Fundam. Clin. Pharmacol.*, 16: 175-182.
- Kushikata, T., K. Hirota, H. Yoshida, M. Kudo and D.G. Lambert *et al.*, 2003. Orexinergic neurons and barbiturate anesthesia. *Neuroscience*, 121: 855-863.
- Le Bars, D.L., M. Gozarri and S.W. Cadden, 2001. Animal models of nociception. *Pharmacol. Rev.*, 53: 597-652.
- Luttinger, D., 1985. Determination of antinociceptive efficacy of drugs in mice using different water temperatures in a tail-immersion test. *J. Pharmacol. Methods*, 13: 351-357.
- Lynch, J.J., V. Castagne, P.C. Moser and S.W. Mittelstadt, 2011. Comparison of methods for the assessment of locomotor activity in rodent safety pharmacology studies. *J. Pharmacol. Toxicol. Methods.*, 64: 74-80.
- McGowan, E., S.B. Hoyt, X. Li, K.A. Lyons and C. Abbadie, 2009. A peripherally acting Na(v)1.7 sodium channel blocker reverses hyperalgesia and allodynia on rat models of inflammatory and neuropathic pain. *Anesth. Analg.*, 109: 951-958.
- Montalto de Mecca, M., A.S. Bernacchi and J.A. Castro, 2000. Prevention of benznidazole-induced prolonging effect on the pentobarbital sleeping time of rats using different thiol-containing compounds. *Res. Commun. Mol. Pathol. Pharmacol.*, 108: 39-48.
- Muhammad, N., M. Saeed and H. Khan, 2012. Antipyretic, analgesic and anti-inflammatory activity of *Viola betonicifolia* whole plant. *BMC Complem. Altern. Med.*, 12: 59-59.
- Nassar, M.A., A. Levato, L.C. Stirling and J.N. Wood, 2005. Neuropathic pain develops normally in mice lacking both Na(v)1.7 and Na(v)1.8. *Mol. Pain*, 1: 24-24.
- Nayak, S.S., A.K. Ghosh, B. Debnath, S.P. Vishnoi and T. Jha, 2004. Synergistic effect of methanol extract of *Abies webbiana* leaves on sleeping time induced by standard sedatives in mice and anti-inflammatory activity of extracts in rats. *J. Ethnopharmacol.*, 93: 397-402.
- Nwaehujor, C.O., M.I. Ezeja, N.E. Udeh, D.N. Okoye and R.I. Udegbunam, 2012. Anti-inflammatory and anti-oxidant activities of *Mallotus oppositifolius* (Geisel) methanol leaf extracts. *Arab. J. Chem.*, 10.1016/j.arabjc.2012.03.014
- NRC, 1996. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, DC., USA., ISBN-10: 0-309-05377-3.
- Pal, S., T. Sen and A.K. Chaudhuri, 1999. Neuropsychopharmacological profile of the methanolic fraction of *Bryophyllum pinnatum* leaf extract. *J. Pharm. Pharmacol.*, 51: 313-318.

- Park, B.K., N.R. Kitteringham, J.L. Maggs, M. Pirmohamed and D.P. Williams, 2005. The role of metabolic activation in drug-induced hepatotoxicity. *Annu. Rev. Pharmacol. Toxicol.*, 45: 177-202.
- Porsolt, R.D., M. Lemaire, N. Durmuller and S. Roux, 2002. New perspectives in CNS safety pharmacology. *Fundam. Clin. Pharmacol.*, 16: 197-207.
- Ramabadran, K., M. Bansinath, H. Turndorf and M.M. Puig, 1989. Tail immersion test for the evaluation of a nociceptive reaction in mice: Methodological consideration. *J. Pharmacol. Methods*, 21: 21-31.
- Renton, K.W., 1985. Inhibition of hepatic microsomal drug metabolism by the calcium channel blockers diltiazem and verapamil. *Biochem. Pharmacol.*, 34: 2549-2553.
- Roux, S., E. Sable and R.D. Porsolt, 2005. Primary observation (Irwin) test in rodents for assessing acute toxicity of a test agent and its effects on behavior and physiological function. *Curr. Protoc. Pharmacol.*, 10.1002/0471141755.ph1010s27.
- Sayyah, M., L. Nadjafnia and M. Kamalinejad, 2004. Anticonvulsant activity and chemical composition of *Artemisia dracunculus* L. essential oil. *J. Ethnopharmacol.*, 94: 283-287.
- Sewell, R.D. and P.S. Spencer, 1976. Antinociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail-immersion test in mice and rats. *Neuropharmacology*, 15: 683-688.
- Simmons, R.M., A.A. Webster, A.B. Kalra and S. Iyengar, 2002. Group II mGluR receptor agonists are effective in persistent and neuropathic pain models in rats. *Pharmacol. Biochem. Behav.*, 73: 419-427.
- Simon, V.M., A. Parra, J. Minarro, M.C. Arenas, C. Vinader-Caerols and M.A. Aguilar, 2000. Predicting how equipotent doses of chlorpromazine, haloperidol, sulpiride, raclopride and clozapine reduce locomotor activity in mice. *Eur. Neuropsychopharmacol.*, 10: 159-164.
- Solinas, M., S. Ferre, Z.B. You, M. Karcz-Kubicha, P. Popoli and S.R. Goldberg, 2002. Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. *J. Neurosci.*, 22: 6321-6324.
- Trease, G.E. and W.C. Evans, 1989. *Textbook of Pharmacognosy*. 12th Edn., Balliere Tindall, London, UK.
- Vellucci, S.V. and R.A. Webster, 1984. Antagonism of caffeine-induced seizures in mice by Ro 15-1788. *Eur. J. Pharmacol.*, 97: 289-293.
- Wiley, J.L. and B.R. Martin, 2003. Cannabinoid pharmacological properties common to other centrally acting drugs. *Eur. J. Pharmacol.*, 471: 185-193.
- Wiley, J.L., R.E. Fagalde, K.G. Buhler, K.L. LaVecchia and R.L. Balster, 2002. Evaluation of 1,1,1-trichloroethane and flurothyl locomotor effects following diazepam treatment in mice. *Pharmacol. Biochem. Behav.*, 71: 163-169.
- Zhao, X., X.Y. Cui, Q.P. Chu, B.Q. Chen and X.M. Wang *et al.*, 2006. Potentiating effects of L-type Ca(2+) channel blockers on pentobarbital-induced hypnosis are influenced by serotonergic system. *J. Neural. Transm.*, 113: 1395-1402.