Research In Pharmaceutical Biotechnology Vol. 1, pp. 001-008, November, 2009
Available online at http://www.academicjournals.org/rpb

Full Length Research Paper

Neuropharmacological effects of *Sorghum bicolor* leaf base extract

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Accepted 28 September, 2009

The neuropharmacological effects of aqueous methanolic extract of leaf base of *Sorghum bicolor* were studied on Wistar rats and Swiss albino mice; evaluations were done on spontaneous motor activity, exploratory behaviour, apomorphine-induced stereotypic behaviour, pentobarbitone sleeping time and rota-rod performance for motor coordination. The results showed a significant ($P < 0.05$) reduction in the spontaneous motor activity. The treated animals exhibited: (i) A reduction in the exploratory behaviour as did diazepam (1 mg/kg i.p.); (ii) No change in Apomorphine-induced stereotypic behaviour; (iii) Prolonged pentobarbitone-induced sleep as did diazepam (1 mg/kg i.p.) and cimetidine (100 mg/kg p.o) and no significant ($P < 0.05$) effect on rota-rod performance for motor coordination. These findings suggest that leaf base extracts of *S. bicolor* contains sedative substances that act via centrally-mediated actions rather than peripheral neuromuscular blockade and may also be microsomal enzyme inhibitor like cimetidine.

Key words: *Sorghum bicolor*, spontaneous motor activity, exploratory behaviour, stereotype behaviour, pentobarbitone sleep, motor coordination.

INTRODUCTION

Medicinal herbs are used as traditional medicine worldwide as these are cheaper, easily available and their use depends on ancestral experience (Marin-Bettolo, 1980). *Sorghum bicolor* (Linn.) Pers. (Family: Poaceae) is a cultivated annual plant with widely reported ethnomedicinal uses in different parts of the world (Grieve, 1931; Watt and Breyer-Brandwijk, 1962; Perry, 1980; Duke and Wain, 1981; Morton, 1981; Chiej, 1984; Grieve, 1984; Okokoh, 1999). This plant is used as a remedy for epilepsy (a central nervous system related condition). The World Health Organisation encourages the inclusion of herbal medicines proven safe and efficacious in the health care programs of developing countries because of the great potential they have in combating various diseases. Evaluation of the effects of medicines on different body systems constitutes safety evaluation of these medicines. Presently, there is paucity of information about the neuropharmacological effects of this widely used plant.

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The present study was therefore aimed at evaluation of the neuropharmacological effect of *S. bicolor* leaf base extract.

MATERIALS AND METHODS

Plant preparation and extraction

The dry mature leaves of *S. bicolor* were collected from Maganawa town, Sokoto State, Nigeria between November and January, 2006. The plant was authenticated by a plant taxonomist, Mr. Ibrahim Muazzam of Herbarium Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The specimen was deposited at NIPRD's Herbarium with voucher specimen number 3815. The dark red portions of the leaves attached to the suckers of the plants were cut out from the entire leaves (the portion of the leaves especially claimed to be used ethnomedicinally). They were then pulverized in a mortar. Two hundred grams (200 g) of the pulverized sample was cold macerated successively in 5 litres of 70% v/v methanol over 96 h period on a shaker (GFL D 3006 mgH, Germany) to ensure maximum extraction. The extract was then filtered using clean cotton wool. The filtrate was placed on water bath to allow evaporation of the solvents and consequent concentration of the extract for subsequent studies. A yield of 23.6% w/w extract was obtained.

The aqueous-methanolic extract was further partitioned into non-

polar, medium polar and polar components using the solvents; hexane, ethylacetate and water (aqueous). 10.15 g of the 70% methanolic extract was dissolved in distilled water and then gently mixed separately with each of the solvents in a separating funnel and allowed to stand for about 30 min to produce two immiscible layers that were then separated. The process was repeated until the upper partitioning solvent became clear. All the portions (hexane, ethylacetate and aqueous portions) were concentrated to small volumes in a rota vapour and finally concentrated on water bath for subsequent use.

The hexane portion of the crude extract was greenish, fatty/oily and very small with a yield of 0.5% w/w (a probable indication of presence of only very small quantities of non-polar components in the crude extract). Ethylacetate portion appeared shinny, deep brownish-black in colour, clumped up but not sticky. It had a yield of 95.9% w/w (constituting the major component) while the aqueous component appeared deep brownish, clumped up but sticky. It gave a yield of 3.6% w/w.

Animals

Wistar rats (81.0 - 172.3 g), Swiss albino mice (15.3 - 35.6 g) of both sexes were used for the studies. They were obtained from the Animal Facility Centre, Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The experimental animals were separated for two weeks in the experimental room for acclimatization. They were housed in appropriately designed cages. The animals were maintained under normal environmental temperature (26 - 28^{o}C), approximately normal 12 h day and night illumination cycle. The animals were fed *ad libitum* with standard NIPRD formulated feed and had free access to water from Abuja Municipal water supply. Cleanliness was ensured throughout the study.

In the experimental grouping of the animals, the body weights and sex of the animals were taken into consideration to achieve approximately equal conditions among the groups. The animals were identified using small cards stuck to different cages indicating the study number, group number, animal number and dose levels. The 'principles of laboratory animal care' (NIH Publication # 85 - 23, 1985) were followed in the study.

Drugs and Chemicals

Some of the drugs and chemicals used for carrying out the studies included: Diazepam (Calmpose®; Ranbaxy, India), Apomorphine (Sigma, USA), Pentobarbitone sodium (Sigma, USA), Phenobarbitone (Vitabiotics, England), Cimetidine (Smithkline and French, England), Methanol (Fluka Chemie, Switzerland), Hexane (BDH Chemicals Ltd., Poole, England), Ethylacetate (BDH Chemicals Ltd., Poole, England).

Acute toxicity study (LD_{50})

The modified method of Lorke (1983) was adopted for the studies. The estimation of the median lethal dose (LD_{50}) values for the aqueous-methanolic extract, its ethylacetate and aqueous fractions was done using adult Swiss albino mice and Wistar rats of both sexes. The test routes were intraperitoneal (i.p.) and oral (p.o.) for the aqueous-methanolic extract and only intraperitoneal route for the fractions. The extract administration was done in biphasic manner using doses ranging from 100 - 2000 mg/kg. The animals were observed for 72 h for behavioural effects such as nervousness, ataxia, excitement, alertness, dullness and death.

The LD_{50} was calculated as the geometric mean of the dose that caused 100% mortality and that which caused 0% mortality.

Studies on spontaneous motor activity (SMA)

The spontaneous motor activity of mice were recorded using ventilated activity cages (LE 886) connected to multi-counter (LE 3806) obtained from LETICA (Spain) and by employing the procedure described by Gamaniel et al. (1998).

Adult Swiss albino mice of both sexes were divided into four groups (n = 6). Normal saline (20 ml/kg i.p.) was administered to the first group to serve as the control. Graded doses of the aqueous methanolic extract (100, 200 and 400 mg/kg i.p.) were administered to mice in groups two, three and four, respectively. After 30 min of treatment, the animals were transferred individually into the LETICA activity cages. The activity counts were recorded for 6 min after 1 min latency period, at intervals of 30 min for 2 h (120 min). Baseline activity counts were recorded prior to the treatment.

Test for exploratory behaviour in mice

The hole-board test of Perez et al. (1998) was adopted in this test. The LETICA board (Signo 720; Printer LE 3333) of 60 cm × 30 cm with 16 evenly spaced holes with in-built infra-red sensors was used for the study. Adult Swiss albino mice of either sex used for the investigation were placed individually in the arena of the LETICA hole board. The number of times an animal dipped its head into the holes during a 5 min period was automatically counted and recorded by the instrument (Wolfman et al., 1994). A baseline count was taken for each mouse. The mice were then divided into five groups (of 5 mice each). Mice in group one received normal saline (20 ml/kg i.p.) to serve as the negative control. The aqueous methanolic extract (100, 200 and 400 mg/kg) was given intraperitoneally to mice in groups two, three and four, respectively while diazepam (1 mg/kg i.p.) was given to mice in group five to serve as a reference standard. Recording was repeated as described above at 30, 60 and 90 min post treatment.

Studies on apomorphine-induced stereotypic behaviour

The effect of the aqueous methanolic extract on apomorphine-induced stereotypic behaviour was investigated as described by Kenneth and Kenneth (1984). Adult Swiss albino mice of both sexes were divided into four groups (n = 5). Normal saline (20 ml/kg i.p.) was administered to mice in group one to serve as the control while graded doses of the extract (100, 200 and 400 mg/kg i.p.) were given to mice in groups two, three and four, respectively. 30 min post treatment, apomorphine (0.1 mg/kg i.p.) was administered to each mouse. Signs of stereotypic behaviours, which included mainly sniffing and gnawing were observed and rated. The stereotypic episodes were scored as follows: absence of stereotype (0); occasional sniffing (1); occasional sniffing with occasional gnawing (2); frequent gnawing (3); intense and continuous gnawing (4); intense gnawing and jumping (5). The stereotypic behaviour was measured 1 min post apomorphine administration. They were scored after every minute over 5 min period. The mean of the 5 min period was calculated and recorded.

Studies on pentobarbitone sleeping time

The procedure described by Wambebe (1985) was adopted for the study. The test was carried out on adult Swiss albino mice of either sex divided into five groups (of five mice each). The first group of mice received normal saline (20 ml/kg i.p.) to serve as the control. Groups two, three and four received the aqueous methanolic extract (100, 200, 400 mg/kg i.p.), respectively while the fifth group

was given diazepam (1 mg/kg i.p.) to serve as the reference standard. 30 min post treatment, pentobarbitone sodium (30 mg/kg i.p.) was administered to each mouse to induce sleep. The time of onset and duration of sleep observed for each mouse was recorded. The criterion for sleep was loss of righting reflex (Miya et al., 1973; Wambebe, 1985; Ramirez et al., 1998). The interval between loss and recovery of righting reflex was used as the index of hypnotic effect (Fujimori, 1965). This model/test was also adopted as one of the bioassay guides for evaluation of ethylacetate and aqueous fractions of the extract. The study was repeated using 100, 200 and 400 mg/kg i.p. doses of ethylacetate fraction as well as 100, 200 and 400 mg/kg i.p. doses of the aqueous fraction. Diazepam (1 mg/kg i.p.) was used as the reference drug while normal saline (20 ml/kg i.p.) was used as the negative control.

Use of pentobarbitone-induced sleep as a model to test effect of *S. bicolor* on microsomal enzyme of mice and rats

The study was carried out on adult Swiss albino mice and adult Wistar rats of both sexes. They were divided into six groups of mice and six groups of rats (n = 5). Group one received normal saline (20 ml/kg p.o.) to serve as the control. Phenobarbitone (1 mg/kg p.o.) was given to group two to serve as a reference microsomal enzyme inducer drug. Group three was given cimetidine (100 mg/kg p.o.) to serve as reference microsomal enzyme inhibitory drug. Graded doses of the aqueous methanolic extract (100, 200 and 400 mg/kg p.o.) were administered to groups four, five and six, respectively. The treatment was administered for six (6) consecutive days. 30 min post 6th day treatment, pentobarbitone (30 and 40 mg/kg i.p.) was administered to all the groups of mice and rats respectively to induce sleep. Each experimental animal was observed for the onset and duration of sleep, with the criterion for sleep being loss of righting reflex (Miya et al., 1973; Wambebe, 1985; Ramirez et al., 1998). The index of microsomal enzyme effect was taken as the duration of hypnosis observed in the experimental animals to which it is inversely related.

Test for motor coordination (rota-rod performance)

The test was conducted following the procedure of Ozturk et al. (1996). A rota-rod treadmill device (Ugo Basile No. 7650, Varies, Italy) was used to assess the locomotor activity of mice. Adult Swiss albino mice of either sex were placed on a horizontal rotating rod with diameter of 5 cm set at 16 revolutions per min. Mice that were able to continuously walk on the rotating rod for 3 min (180 s) were selected and grouped into four (of five mice each). Normal saline (20 ml/kg i.p.) was given to group one mice to serve as the control. Graded doses of the aqueous methanolic extract (100, 200 and 400 mg/kg i.p.) were administered to mice in groups two, three and four respectively. After 30 min post-treatment, each mouse was placed back on the rotating rod for 3 min (180 s) at intervals of 30 min up to a period of 3 h (180 min). The time an animal fell from the rod within the 180 s was recorded. Failing of an animal more than once within 180 seconds indicated lack of motor coordination (Fujimori and Cobb, 1965).

Statistical analysis

The results of the studies were expressed as mean ± SEM (standard error of mean). The difference between the control and treated means were analysed using analysis of variance (ANOVA). Student t-test and Least Significant Difference (LSD) were applied where ANOVA showed significant difference. P-values < 0.05 were taken to be statistically significant. Results were presented as Tables and Figure.

Compliance with good laboratory practice (GLP)

The studies were carried out according to Good Laboratory Practice (GLP) Regulations of Organization for Economic Cooperation and Development – OECD (UNDP/World Bank/WHO, 2001).

RESULTS

Acute toxicity studies (LD_{50})

No overt toxicity sign or death was observed in rats and mice during the 72 h post oral treatment with 100 - 2,000 mg/kg doses of *S. bicolor* leaf base extract. The oral median lethal dose (LD_{50}) of the extract in rats and mice was therefore ≥ 2000 mg/kg p.o. The rats treated intraperitoneally (i.p.) with the leaf base extract (100 - 2,000 mg/kg) showed no overt toxicity sign or death in the 24 h post treatment. However, all the rats treated with 2,000 mg/kg i.p. dose became recumbent and died within 48 h of the intraperitoneal treatment while those treated with 100 - 1,000 mg/kg i.p. doses neither showed toxicity signs nor death during 72 h post i.p. treatment. For the estimation of the intraperitoneal median lethal dose (LD_{50} i.p.) in rats, assessment based on 24 h post treatment showed a median lethal dose (LD_{50}) ≥ 2,000 mg/kg i.p. since no overt toxicity sign or death was observed in i.p.-treated rats after 24 h. However, an assessment based on 48 h post i.p. treatment observation gave a calculated median lethal dose of 1,414.2 mg/kg i.p. in rats. The mice treated with doses of the extract ≤ 1,200 mg/kg i.p. showed neither toxicity signs nor death during the 24 h post treatment. At the dose of 1,500 mg/kg i.p., the mice were calm, dull, with increased respiratory rate. At this dose, mortality of 66.7 and 100.0% occurred within 24 and 48 h of i.p. treatment respectively.

The mice treated i.p. with 2,000 mg/kg dose were calm, dull and recumbent with increased respiratory rate. A mortality of 100.0% occurred at this dose within 24 h. The calculated intraperitoneal medial lethal dose in mice was 1,248.0 and 1,341.6 mg/kg i.p. for 24 and 48 h post treatment observations, respectively. For the ethylacetate fraction of *S. bicolor* leaf base extract, 33.3 and 66.7% of 1000 and 2000 mg/kg i.p-treated mice were dull, immobilised with increased respiration within 12 min post administration. All the mice later recovered and no further toxicity signs or death was observed during the 24, 48 and 72 h post intraperitoneal administration. The intraperitoneal LD_{50} of ethylacetate fraction of *S. bicolor* leaf base extract in mice is therefore ≥ 2000 mg/kg. For the aqueous fraction of *S. bicolor* leaf base extract, only 33.3% of mice treated intraperitoneally with the dose of 2,000 mg/kg were dull, immobilized with increased respiration within 10 min of administration. The mice observed during the 24, 48 and 72 h post i.p. administration. The intraperitoneal LD_{50} of aqueous fraction of *S. bicolor* leaf base extract in mice is therefore ≥ 2000 mg/kg.

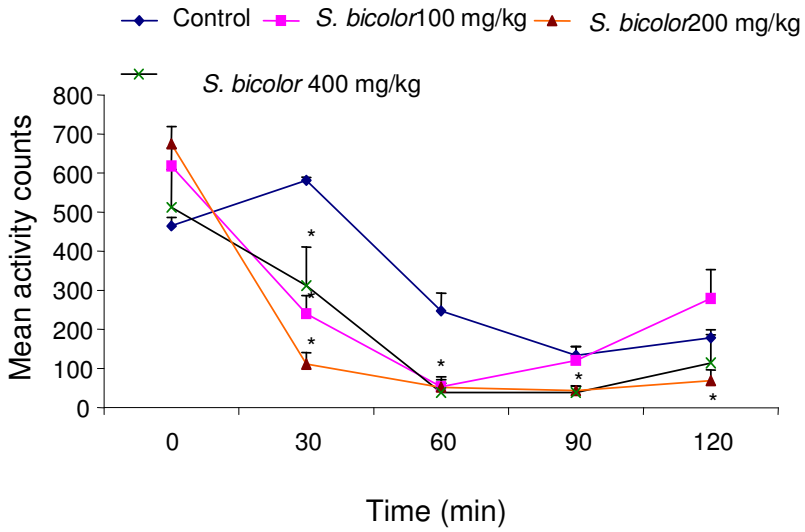

Figure 1. Effect of aqueous methanolic extract of *S. bicolor* leaf base (100, 200 and 400 mg/kg i.p.) on spontaneous motor activity in mice.

Effect on spontaneous motor activity (SMA)

S. bicolor leaf base extract (100, 200 and 400 mg/kg i.p.) produced a significant ($p < 0.05$) reduction of spontaneous motor activity in mice in relation to the control. This effect was time related. Maximum effect was observed at 60th minute for 100 mg/kg dose and at 90th minute for both 200 and 400 mg/kg doses. The SMA of the control group was also observed to have decreased also recovered and no further toxicity sign or death was from the 60th minute of the experiment. The reduction was however, not as the treated groups (Figure 1).

Effect on exploratory behaviour of mice

The study revealed a reduction in the exploratory activity of mice in all the groups. However, the observed reductions were more in the treated groups. The statistical comparisons made for every group with its zero readings showed statistical significance ($p < 0.05$) for the extract (100 mg/kg i.p) at 30, 60 and 90 min, for extract (200 mg/kg i.p.) at 90 min, the extract (400 mg/kg i.p.) at 30, 60 and 90 min and for diazepam (1 mg/kg i.p.) at 30, 60 and 90 min.

The statistical comparisons made between the treated groups and normal saline control group showed significant ($p < 0.05$) reduction for the extract (100 mg/kg i.p.) at 30 min, extract (400 mg/kg i.p. at 90 min and for diazepam (1 mg/kg i.p.) at 30 and 60 min intervals (Table 1).

Effect on apomorphine-induced stereotypic behaviour

The aqueous methanolic extract of *S. bicolor* leaf base (100 - 400 mg/kg i.p.) did not inhibit apomorphine-induced stereotypic behaviour in mice (Table 2).

Effect on pentobarbitone sleeping time

The aqueous methanolic extract of *S. bicolor* leaf base caused a reduction in the sleep onset of mice dosed once with the extract. This effect was not dose-dependent and was only significant ($p < 0.05$) at the dose of 100 mg/kg i.p. The extract also prolonged the duration of pentobarbitone sleep in a manner that was also not dose-dependent. The prolongation was only significant ($p < 0.05$) at the dose of 100 mg/kg i.p. Diazepam (1 mg/kg i.p.) on the other hand significantly ($p < 0.05$) caused a reduction in the sleep onset and prolongation of the sleep duration (Table 3). However, the aqueous (100 - 400 mg/kg i.p.) and ethylacetate (100 - 400 mg/kg i.p.) fractions of *S. bicolor* leaf extract produced no significant effect on both the onset and duration of pentobarbitone-induced sleep in mice while diazepam (1 mg/kg i.p.) caused a significant ($p < 0.05$) prolongation of the sleep duration (Table 4).

Effect on pentobarbitone-induced sleep for microsomal enzyme of mice and rats

Mice dosed cumulatively for six days with aqueous methanolic extract of *S. bicolor* leaf base had slightly increased onset of pentobarbitone (30 mg/kg i.p.)-induced sleep at 100 mg/kg p.o. dose while the doses of 200 and 400 mg/kg p.o. of the extract produced reduced sleep onset. These observations were however, non-significantly different from the control. The duration of

Table 1. Effect of aqueous methanolic extract of *S. bicolor* leaf base (100, 200, 400 mg/kg i.p.) on exploratory behaviour of mice.

Treatment (i.p.)	Mean Head-dips ± SEM			
	0 min	30 min	60 min	90 min
Normal saline (control; 20 ml/kg) *S. bicolor*	79.8 ± 6.3	70.6 ± 14.1	59.8 ± 13.3	43.2 ± 14.4
100 mg/kg	81.0 ± 11.3	30.4 ± 9.3*@	46.8 ± 9.2*	40.4 ± 4.6*
200 mg/kg	66.4 ± 5.1	78.4 ± 22.5	47.6 ± 10.9	25.4 ± 9.7*
400 mg/kg	91.4 ± 16.8	61.0 ± 12.6*	39.2 ± 10.5*	16.4 ± 4.4*@
Diazepam (1 mg/kg)	70.4 ± 7.6	39.4 ± 14.0*@	17.0 ± 6.3*	16.8 ± 4.7*

SEM= Standard error of mean; 0 min = reading before treatment; * = p < 0.05, statically different from zero reading of same group (2-way ANOVA; Least Significant Difference - LSD).@ = P < 0.05, statistical difference from normal saline control reading (2-way ANOVA; LSD).

Table 2. Effect of aqueous methanolic extract of *S. bicolor* leaf base (100 - 400 mg/kg i.p.) on apomorphine-induced stereotypic behaviour in mice.

Treatment	Mean score per 5 min ± SEM
Control *S. bicolor*	2.76 ± 0.9
100 mg/kg i.p.	3.10 ± 1.0
200 mg/kg i.p.	4.40 ± 0.7
400 mg/kg i.p.	4.60 ± 0.6

Table 3. Effect of aqueous methanolic extract of *S. bicolor* leaf base (100 - 400 mg/kg i.p.) on pentobarbitone-induced sleep in mice.

Treatment (i.p.)	Onset of sleep (min)	Duration of sleep (min)
Normal saline (control; 20 ml/kg) S. bicolor	5.6 ± 0.51	56.0 ± 5.1
100mg/kg	4.2 ± 0.37*	92.0 ± 9.3*
200 mg/kg	5.4 ± 0.70	69.2 ± 13.2
400 mg/kg	4.0 ± 0.71*	111.3 ± 43.1*
Diazepam (1 mg/kg)	3.2 ± 0.60*	126.0 ± 16.7*

Values are expressed as mean ± SEM (n = 5)= p < 0.05; statistical difference between treated and control group (ANOVA, Student t-test)

Table 4. Effects of aqueous and ethylacetate fractions of *S. bicolor* leaf extract (100 - 400 mg/kg i.p.) on pentobarbitone-induced sleep in mice.

Treatment (i.p.)	Onset of sleep (min)	Duration of sleep (min)
Normal saline (control; 20 ml/kg)	6.0 ± 1.5	87.0 ± 22.3
Aqueous fraction		
100 mg/kg	3.8 ± 0.5	109.0 ± 37.3
200 mg/kg	4.0 ± 1.1	94.0 ± 37.4
400 mg/kg	7.3 ± 2.0	89.8 ± 24.2
Ethylacetate fraction		
100 mg/kg	8.5 ± 0.3	80.0 ± 24.7
200 mg/kg	4.3 ± 0.8	101.5 ± 32.4
400 mg/kg	7.0 ± 1.3	88.8 ± 19.1
Diazepam (1 mg/kg)	4.0 ± 0.4	137.0 ± 6.7*

Values are expressed as mean ± SEM. * = P < 0.05; statistical difference between treated and control group (ANOVA, Student t-test)

Table 5. Effect of aqueous methanolic extract of *S. bicolor* leaf base (100 - 400 mg/kg p.o.) on microsomal enzyme of mice tested on pentobarbitone-induced sleep model.

Treatment (p.o. x 6 days)	Onset of sleep (min)	Duration of sleep (min)
Control (normal saline; 20 ml/kg)	15.25 ± 7.0	31.25 ± 5.7
S. bicolor		
100 mg/kg	18.42 ± 6.4	28.00 ± 1.2
200 mg/kg	6.83 ± 0.5	38.5 ± 0.4
400 mg/kg	13.00 ± 0.5	72.75 ± 27.0
Phenobarbitone (1 mg/kg)	11.33 ± 2.6	10.00 ± 0.6*
Cimetidine (100 mg/kg)	3.47 ± 0.46*	92.23 ± 16.0*

Values are expressed as mean ± SEM; Pentobarbitone dose = 30 mg/kg i.p. *= $p < 0.05$; statistical difference between treated and control group (ANOVA, Student t-test).

Table 6. Effect of aqueous methanolic extract of *S. bicolor* leaf base (100 – 400 mg/kg p.o.) on microsomal enzyme of rats tested on pentobarbitone-induced sleep model.

Treatment (p.o. X 6 days)	Onset of sleep (min)	Duration of sleep (min)
Normal saline (control; 20 ml/kg)	3.75 ± 0.25	90.5 ± 7.27
S. bicolor		
100 mg/kg	3.33 ± 0.33	105.67 ± 4.33
200 mg/kg	4.25 ± 0.75	128.75 ± 39.1
400 mg/kg	4.50 ± 0.50	128.50 ± 10.0*
Phenobarbitone (1 mg/kg)	3.25 ± 0.25	86.25 ± 3.42
Cimetidine (100 mg/kg)	3.67 ± 0.33	222.0 ± 19.17*

Values are expressed as mean ± SEM; pentobarbitone dose = 40 mg/kg i.p.*= $p< 0.05$; statistical difference between treated and control group (ANOVA, Student t-test).

pentobarbitone sleep was slightly reduced by the leaf base extract at 100 mg/kg p.o. dose while they were prolonged at 200 and 400 mg/kg p.o. doses. These effects were also non-significantly different from the control. Phenobarbitone (1 mg/kg p.o.) produced non-significant reduction of sleep onset but a significant ($p < 0.05$) reduction in the duration of sleep while cimetidine (100 mg/kg p.o.) produced significant ($p < 0.05$) prolongation of duration of pentobarbitone-induced sleep in mice (Table 5).

Conversely, rats treated cumulatively for six days with the aqueous methanolic extract had a slightly reduced onset of pentobarbitone (40 mg/kg i.p.)-induced sleep at 100 mg/kg dose while the doses of 200 and 400 mg/kg p.o. slightly increased the sleep onset time. These effects were non-significantly different from the control. However, the duration of pentobarbitone sleep was prolonged by all the doses of the leaf base extract (100 - 400 mg/kg p.o.). These effects were not dose-dependent and was only significant at dose of 400 mg/kg p.o. Phenobarbitone (1 mg/kg p.o.) produced some reduction in onset and duration of pentobarbitone-induced sleep while cimetidine (100 mg/kg p.o.) produced a significant ($p < 0.05$) prolongation of the sleep (Table 6).

Effect on motor co-ordination (Rota-rod Performance)

The aqueous methanolic extract of *S. bicolor* leaf base (100 - 400 mg/kg i.p.) did not produce significant effect on the rota-rod performance of the mice. Most of the mice were able to stay on the rotating rod through the 3 min (180 s) cut-off time point without falling (Table 7).

DISCUSSION

The spontaneous motor activity is a model that has been used in laboratory animals to evaluate the gross behavioural effects of drugs (Hsieh et al., 1991; Carpenedo et al., 1994; File and Fernandes, 1994). The model measures the level of excitability of the central nervous system (Mansur et al., 1971) which correlates well with drug effects in humans. Agents that suppress this behaviour usually do so through central inhibition (Adzu et al., 2002). The significant ($p < 0.05$) reduction in the spontaneous motor activity by the *S. bicolor* leaf base extract therefore suggests a reduction in the excitability of the central nervous system which could be suggestive of sedative activity. Ozturk et al. (1996) reported that the decrease in the activity may be closely related to sedation

Table 7. Effect of aqueous methanolic extract of *S. bicolor* leaf base. (100 - 400 mg/kg i.p.) on motor coordination (rota-rod performance) of mice.

Treatment (i.p.)	Time (second)					
	30	60	90	120	150	180
Normal saline (control; 20ml/kg) *S. bicolor*	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0
100 mg/kg	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0
200 mg/kg	180.0 ± 0.0	176.6 ± 3.4	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0
400 mg/kg	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0	180.0 ± 0.0

Values are expressed as mean ± SEM (n = 5).

resulting from central nervous system (CNS) depression. Similar effect was seen in the significant ($p < 0.05$) reduction of the number of head dips in the hole board test by the leaf base extract. This test is a measure of exploratory behaviour (File and Wardill, 1975; Crawley, 1985) that reveals sedative activity of agents (File and Pellow 1985; Amos et al., 2001). The test has also been accepted as a parameter for the evaluation of anxiety conditions in animals (Crawley, 1985). The extract therefore possibly has l sedative property. Apomorphine acts directly on the post-synaptic dopamine D-2 receptors to induce hyperactivity and stereotypic behaviour. Inhibition of apomorphine-induced climbing behaviour in mice is suggestive of D2 receptor inhibition (Moore and Axton, 1988). The ability of a drug to antagonise apomorphine-induced climbing behaviour has been correlated to central depressant activity with potential neuroleptic effect (Protais et al., 1976; Costal et al., 1978). The inability of *S. bicolor* leaf base extract to inhibit apomorphine-induced stereotypic behaviour possibly suggests non-inhibition of the D-2 receptors and also indicates that the extract may not be a potential neuroleptic. Potentiation of pentobarbitone-induced hypnosis may be attributed to an action on the central mechanisms involved in the regulation of sleep (Chindo et al., 2003) or an inhibition of pentobarbitone metabolism (Kaul and Kulkarni, 1978). Endogenous neurotransmitters in the brain especially dopamine and gamma-aminobutyric acid (GABA) are implicated in the mechanism of sleep (Osuide and Wambebe, 1980). It is generally accepted that the sedative effects of drugs can be evaluated by measurement of pentobarbitone sleeping time in laboratory animals (Ming-Chin Lu, 1998; Carpenedo, 1994; Gamaniel et al., 1998). Prolongation of pentobarbitone-induced hypnosis is suggestive of centra depressant activity of a compound (Perez et al., 1998). The present study showed that the aqueous methanolic extract of *S. bicolor* leaf base administered once prolonged pentobarbitone-induced hypnosis. This indicates that the extract may not have acted via dopaminergic pathway as indicated by apomorphine-induced stereotypic behaviour test but possibly by enhancing the central inhibitory effect of GABA or by inhibiting pentobarbitone metabolism or via other mechanisms that may be remotely involved in the mechanism of sleep. These effects were not seen in the aqueous and ethylacetate fractions of the leaf base extract indicating that the pharmacological constituents of the plant responsible for this effect may have been lost or distorted due to the fractionation.

This result was further buttressed by the microsomal enzyme test result that also showed prolongation of pentobarbitone-induced hypnosis after six (6) days treatment with the leaf base extract. This observation was similar to that of cimetidine (100 mg/kg p.o.), which is a known microsomal enzyme inhibitory drug. Microsomal enzymes are associated with a number of drug metabolisms. Administration of microsomal enzyme inhibitor reduces metabolic effect of the microsomal enzyme, producing effects of the drug with longer duration (Grant, 2001). The leaf base extract may have therefore inhibited the metabolic effect of microsomal enzymes on pentobarbitone thereby prolonging its hypnotic effects as did cimetidine.

The test for motor coordination (rota rod performance) was adopted to evaluate the effect of the extract on the physical performance, endurance and possible neuromuscular inhibition. The study revealed that the extract did not produce any effect on motor coordination. This therefore suggests that the extract has centrally-mediated actions (based on the inhibitory effects observed in the previous studies) and not through peripheral neuromuscular blockade (Perez et al., 1998).

In conclusion, the study has shown that the leaf base extract of *S. bicolor* has sedative activity which is central nervous system related effect. This sedative effect can be taken advantage of therapeutically. The knowledge of this sedative effect is also useful in listing the precautionary measures to be taken when *S. bicolor* extract is being indicated for use as medicine.

ACKNOWLEDGEMENTS

The authors are grateful to U.S. Inyang, the Director General, National Institute for Pharmaceutical Research and Development (NIPRD) and his Management team for

funding this investigation. They are also grateful to Ibrahim Muazzam, a plant Taxanomist with NIPRD's herbarium for the ethnobotanical information he provided on the study plant. The technical assistance offered by Sunday Dzarma is also appreciated.

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