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Ethopharmacological analysis of the effects of the whole plant extract of *Synedrella nodiflora* (L.) Gaertn (Asteraceae) in murine models

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ABSTRACT

The present study reports the effects of a hydro-ethanolic extract of the whole plant of Synedrella nodiflora (L) Gaertn, a plant traditionally used in Ghana for the treatment of epilepsy, in the elevated plus-maze (EPM), light/dark test (LD), the VersaMax animal activity monitor system (VAMS) and the pentobarbitone-induced sleep test. In the EPM paradigm, S. nodiflora extract (10-300 mg kg^{-1}) exhibited anxiogenic-like activity by dose-dependently decreasing the number of entries into both the open and closed arms, no significant effect on the percent number of entries into the open arms and a decrease in the time spent in the open arm in comparison to the vehicle-treated group. S. nodiflora extract (SNE) dose-dependently decreased the number of head dips, stretch-attend postures and duration of grooming. In the LD test, SNE also exhibited anxiogenic-like effect by significantly and dose-dependently reducing the number of entries into the light compartment, the number of transitions and not significantly, the time spent in the light area. In the EPM and LD test, diazepam (0.1-1.0 mg kg^{-1}), the reference anxiolytic drug, produced a directly opposite response to that exhibited by SNE. SNE (10-1000 mg kg^{-1}), in the VAMS, decreased locomotor activity of mice dose-dependently and significantly. SNE also reduced time spent at the center of the observation cage of the activity monitor indicating an anxiogenic-like effect. S. nodiflora exhibited sedation in the pentobarbitone-induced sleep test. Putting all together, S. nodiflora extract displayed an anxiogenic-like effect in mice which could be attributed to its sedative properties.

Keywords: Sedation, anxiogenic-like, elevated plus-maze, light/dark, animal activity monitor

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INTRODUCTION

Synedrella nodiflora (L.) Gaertn. (Family: Asteraceae) is an annual herb which grows to about 60-120 cm high and occur throughout the West African region. In Ghanaian traditional medicine, the whole plant is boiled and the aqueous extract drunk as required for the treatment of epilepsy. The leaves are used for the treatment of hiccup and threatened abortion [1]. The hydro-ethanolic extract of the whole plant possess anti-nociceptive effects possibly mediated via adenosinergic mechanisms [2].

Anxiety is a common psychiatric disorder inflicting more than 20 % of the world's adult population [3]. Anxiety is generally caused by a number of reasons significant of which chronic medical conditions such as diabetes; asthma and epilepsy have been implicated [4-6]. Evidence exists to indicate that the prevalence of anxiety disorders in patients with epilepsy may exceed 50 % [7-8] and the beneficial role of antiepileptic drugs with anxiolytic properties have been reported [9-16]. The main traditional use of *S. nodiflora* is for the management of epilepsy and evidence suggests that extracts of the plant possess anticonvulsant properties (Unpublished data). To date, no scientific information of the effects of plant on anxiety has been reported.

In this study we report the effect of a hydro-ethanolic extract of the whole plant of *S. nodiflora* on anxiety and related neurobehavioural effects.

MATERIALS AND METHODS

Plant collection

The whole plant of *Synedrella nodiflora* was obtained from the Botanical Gardens, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana in August 2007 and authenticated by the Department of Pharmacognosy, KNUST. A specimen voucher, FP/08/025, has been kept at the Faculty of Pharmacy Herbarium.

Preparation of extract

The plant samples collected were air-dried for seven days and powdered. Two kilograms of the powdered was cold-macerated with 70 % v/v of ethanol. The hydro-ethanolic extract was then evaporated to a syrupy mass under reduced pressure, air-dried and kept in a desiccator. A 7 % w/w yield was obtained. This is subsequently referred to as the extract or SNE.

Animals

Sprague-Dawley rats (150-200 g) and ICR mice (20-30 g) were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon and maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The animals were housed in groups of six in stainless steel cages (34 cm × 47 cm × 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 (temperature 25±2 °C, relative humidity 60-70%, and 12 hour light-dark cycle). In other experiments conducted at the Health Science Center (HSC), Kuwait University, Balb/c and MF-1 mice of either sex (20-30 g) were obtained from the Animal Resource Center (ARC), HSC, Kuwait University, Kuwait. All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services publication No. 83-23, revised 1985). The protocols for the study were approved by the Departmental Ethics Committee.

Drugs and chemicals

Diazepam and pentobarbitone were purchased from Pharm-Inter, Brussels, Belgium and Sigma, St. Louis, MO, USA respectively.

Elevated plus-maze test (EPM)

The method used was as described for rats [17] with some modifications. The elevated plus maze was made from opaque Plexiglas. It consisted of two opposite open arms (15×5 cm^2) without side walls and two enclosed arms (15×5×30 cm^3), extending from a central square platform (5×5

cm^2). A rim of Plexiglas (0.5 cm in height) surrounded the perimeter of the open arms to provide additional grip and thus prevent the mice falling off [18]. The maze was elevated to the height of 80 cm from the floor and placed in a lit room (~750 lux). The animals were divided into eight groups of five animals each and received the extracts (10, 30, 100 or 300 mg kg^{-1}, *p.o.*), the vehicle or diazepam (0.1, 0.3 or 1 mg kg^{-1}, i.p.). Animals were placed individually in the central platform of the EPM and their behavior recorded (5 min) with a camcorder (Everio^{TM} model GZ-MG 130U, JVC, Tokyo, Japan) placed 100 cm above the maze. Behavioral parameters were later scored from the videos as follows: 1) number of closed and open arm entries (absolute value and percentage of the total number); 2) time spent in exploring the open and closed arms of the maze (absolute time and percentage of the total time of testing); 3) number of head-dips (absolute value and percentage of the total number) protruding the head over the edge of either an open (unprotected) or closed (protected) arm and down toward the floor; 4) number of stretch-attend postures (absolute value and percentage of the total number) the mouse stretches forward and retracts to original position from a closed (protected) or an open (unprotected) arm. An arm entry was counted only when all four limbs of the mouse were within a given arm.

Light-dark box test

The light-dark exploration test is typically used to more directly assess anxiety-related responses. This apparatus is based on the initial model described by Crawley [19] and as modified by other workers [20-21]. It consists of wooden boxes (45 cm long × 30 cm wide × 30 cm deep), which are divided into two equal compartments by a wooden board with a 7×7 cm^2 opening located centrally at the floor level, connecting the compartments. One compartment was painted black and covered with a wooden lid. The other box (not covered) was painted white and lit by a 60 W light bulb set 30 cm above the box. Mice were grouped and treated with drugs as described for the elevated plus-maze test above. At the beginning of the experiment, mice were placed individually in the centre of the illuminated box, facing the opening away from the dark compartment. Behaviors of the animals were recorded for 5 minutes with a digital camera placed 1 m above the box. Videos were later scored for the following parameters: 1) frequency of compartmental entries; 2) total time spent by mice in each compartment and 3) the number of inter-compartmental transitions.

Open field test

Open field activity was assessed with the Versamax Animal Activity Monitoring System (AccuScan Instrument Inc, USA). It comprises four animal monitor chambers (16 in × 16 in × 12 in) covered by transparent lids with perforations, an analyzer and a computer. The base of each monitor chamber is lined with vertical and horizontal sensors. The behaviors to be measured are configured into the system. During the test period, any behavior exhibited by the test animals through beam interruptions are transmitted to the analyzer recorded on a computer. The data is later generated by software and exported into an Excel version. During an experiment, the system records the entire procedure as primary, secondary and auxiliary in succession thus providing the researcher the opportunity to test the same animal under three different experimental conditions.

In the experiment conducted, a primary followed by a secondary session was done for 60 and 120 minutes respectively. The test animals were made to acclimatize by going through a two day procedure in the system without any drug administration. On the third day, after an initial 60 min primary session, the mice were treated with SNE (10-1000 mg kg^{-1}, *p.o.*) or distilled water (i.p.) and tested for a 120 min secondary session. The data obtained provided forty parameters out of which the following were analyzed: Horizontal Activity (HACTV), Vertical Activity (VACTV), Total Distance (TOTDIST), Margin distance and duration (Thigmotaxis), Centre distance and

duration, and time spent by the animal in left-front corner (LR), right-front corner (RF), left-rear corner (LR) and right-rear corner (RR) of the cage.

Pentobarbitone-induced sleeping time

Mice were divided into five groups and received vehicle, SNE (30-300 mg kg^{-1}, *p.o.*) or diazepam (1 mg kg^{-1}, i.p.) 30 min before after the administration of pentobarbitone (40 mg kg^{-1}, i.p.). The onset time and duration of sleep were recorded.

Data analysis

Data are presented as mean±S.E.M. (n=5-10) and analyzed by Two-way ANOVA followed by Bonferroni's test for time course curves and One-way ANOVA followed by Newman-Keuls test for column graphs. GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. $P<0.05$ was considered statistically significant in all analysis.

RESULTS

Elevated Plus-Maze test (EPM)

The effects of SNE and diazepam on the various parameters measured in the EPM paradigm are shown in figures 1-2. The administration of SNE (10-300 mg kg^{-1}) dose-dependently decreased the number of entries into both the open ($F_{4,25}$= 0.834, P= 0.51563, fig. 1a) and closed ($F_{4,25}$= 2.669, P= 0.0556, fig 1a) arms, no significant effect on the percent number of entries into the open arms (P= 0.2414, $F_{4,25}$= 1.478, fig 1c) and a decrease in the time spent in the open arm ($F_{4,23}$=0.3196, P=0.8619, fig 1e) in comparison to the vehicle-treated group. Additionally, SNE dose-dependently, though not significant, decreased the number of head dips ($F_{4,22}$= 0.4916, P=0.7398, fig 2a) and stretch attend postures ($F_{4,25}$= 0.8171, P= 0.5264, fig 2c) and significantly, decreased the duration of grooming ($F_{4,22}$= 5.0, P= 0.046, fig 2e).

Diazepam (0.1-1.0 mg kg^{-1}) dose-dependently and significantly increased the number of open arm entries ($F_{3,17}$= 5.597, P= 0.0074, fig. 1b), decreased the number of entries into the closed arm ($F_{3,19}$= 3.837, P= 0.0265, fig. 1b), increased percentage open arm entries ($F_{4,25}$= 4.664, P=0.0140, fig.1d) and percentage time spent in the open arm ($F_{3,20}$=4.815, P= 0.0141, fig. 1f) in comparison to the vehicle-treated group. Also, diazepam dose-dependently increased the number of head dips ($F_{3,20}$=1.720, P= 0.1950, fig. 2b) and significantly decreased the number of stretch attend postures ($F_{3,`18}$= 4.301, P= 0.0187, fig. 2d) and not significantly, the duration of grooming ($F_{3,20}$=1.983, P= 0.1490, fig. 2f).

Open field test (VersaMax Animal Activity Monitor System)

Effects on locomotor activity

The locomotor activity of mice were assessed by measuring the horizontal and vertical activities as well as the total distance travelled by the mice pre-treated with SNE (10-1000 mg kg^{-1}) or vehicle over a period of two hours. The results obtained indicated that the SNE (10-1000 mg kg^{-1}) decreased dose-dependently and significantly the vertical ($F_{3,68}$=18.65, P<0.0001, fig.4a, b) and horizontal ($F_{3,68}$=12.34, P<0.0001, fig.4c, d) activities and the total distance travelled ($F_{3,68}$ =17.31, P<0.0001, fig.4e, f) with respect to the vehicle-treated group. The percentage effects calculated from analysis of the AUCs revealed similar trends (fig. 4b, d, f).

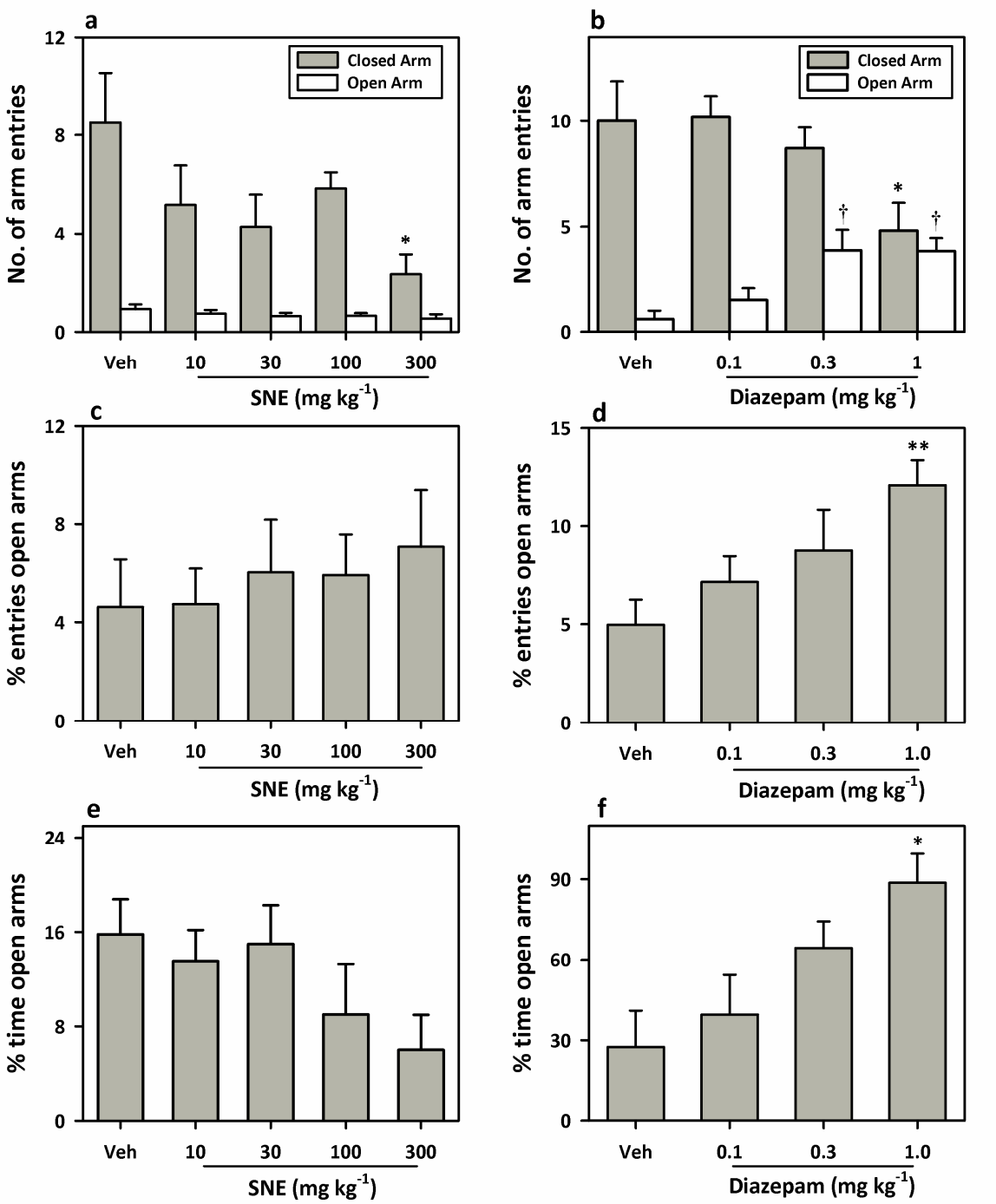

Figure 1 Effects of SNE (10-300 mg kg^{-1}, *p.o.*) and diazepam (0.1-1.0 mg kg^{-1}, i.p.) on the number entries into the open and closed arms (a, b), the percent open arm entries (c, d) and the percent time spent in the open arm (e, f) of the elevated plus-maze. Data are mean±S.E.M. (n=5). **P*<0.05; ***P*<0.01; ****P*<0.001 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls *post hoc* test).

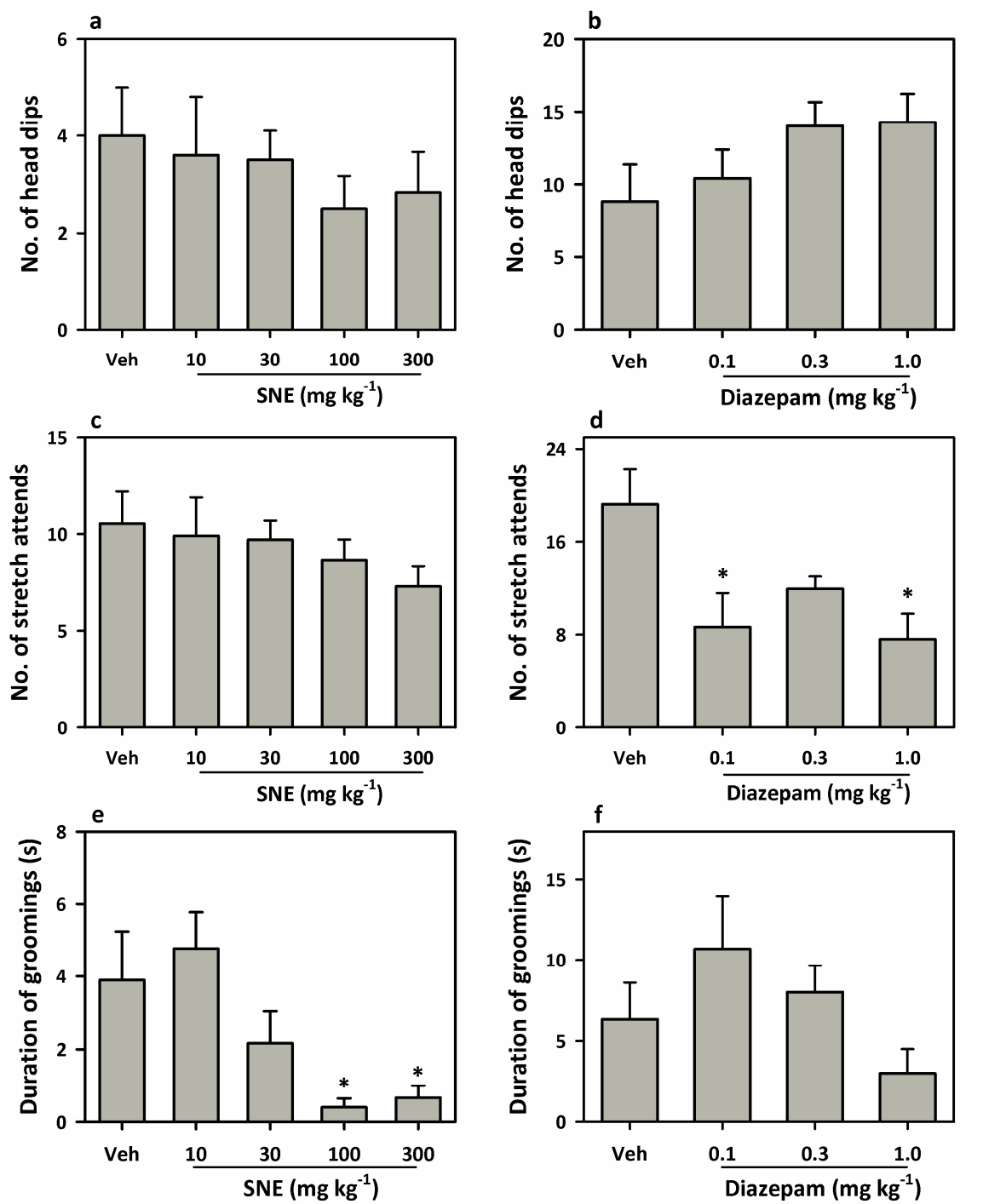

Figure 2 Effects of SNE (10-300 mg kg^{-1}, *p.o.*) and diazepam (0.1-1.0 mg kg^{-1}, i.p.) on the number of head dips (a, b), number of stretch-attend postures (c, d) and duration of grooming (e, f) in the EPM. Data are mean±S.E.M. (n=5). **P*<0.05; ***P*<0.01; ****P*<0.001 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls *post hoc* test).

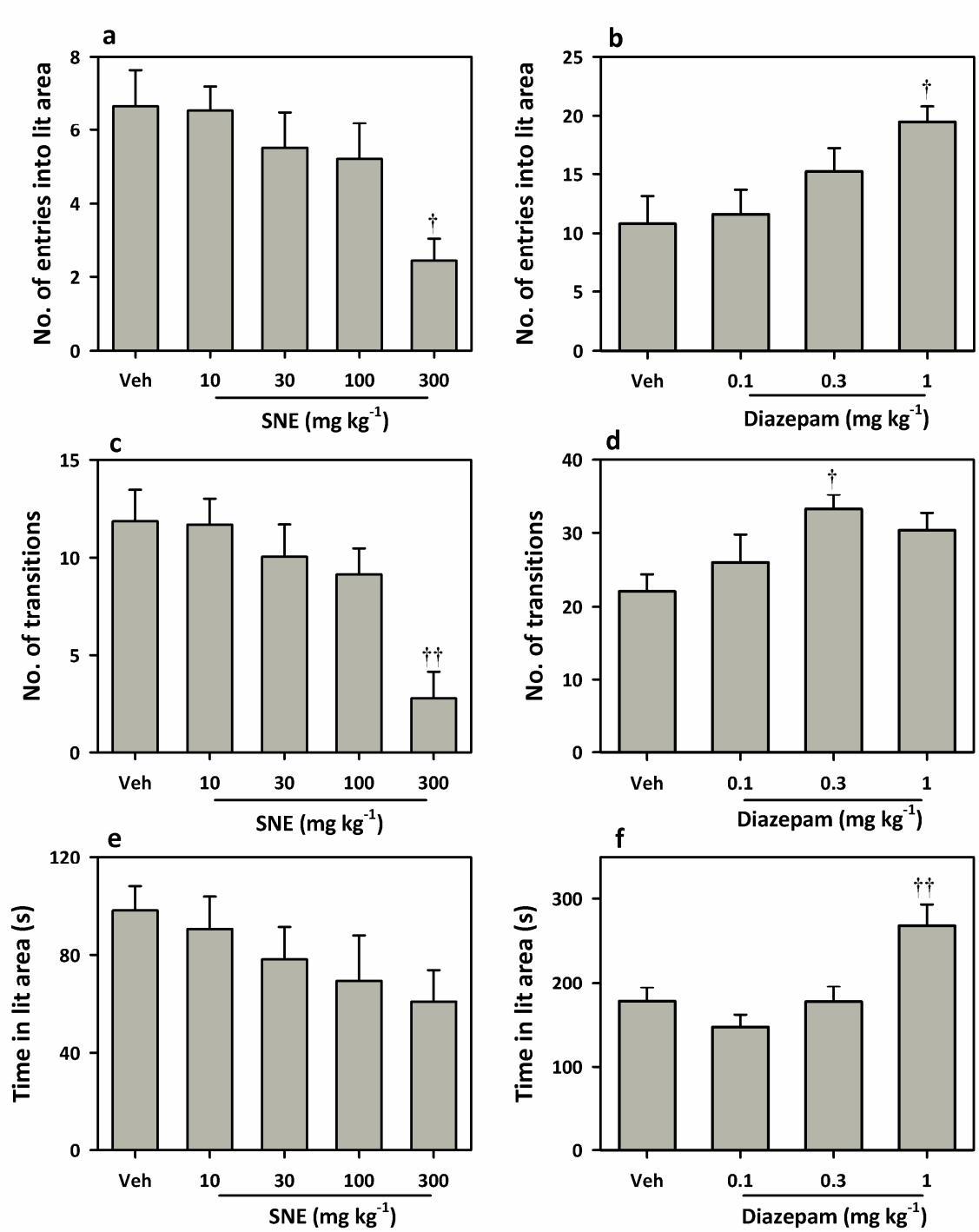

Figure 3 Effects of SNE (10-300 mg kg^{-1}, *p.o.*) and diazepam (0.1-1.0 mg kg^{-1}, i.p.) on the number entries into lit area (a, b), number of transitions (c, d) and time spent in lit area (e, f) by mice in the light/dark box test. Data are presented as mean ± S.E.M. (n=5). **P*<0.05; ***P*<0.01; ****P*<0.001 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls *post hoc* test).

Light-dark box test

The administration of SNE (10-300 mg kg^{-1}) dose-dependently and significantly reduced the number of entries into the light compartment ($F_{4,24}$=3.429, P=0.0237, fig 3a), the number of transition ($F_{4,24}$=5.723, P=0.022, fig 3c) and not significantly, the time spent in the light area ($F_{4,25}$ =0.3722, P=0.8262, fig 3e) of the light-dark box model. Conversely, diazepam (0.1-1.0 mg kg^{-1}), dose-dependently and significantly increased the number of entries into the light

compartment ($F_{4,24}$=4.345, P=0.0181, fig.3b), the number of transitions ($F_{3,18}$=3.771, P=0.0292, fig 3d) and the total time spent in the lighted area ($F_{3,17}$ =7.470, P=0.0021, fig 3f).

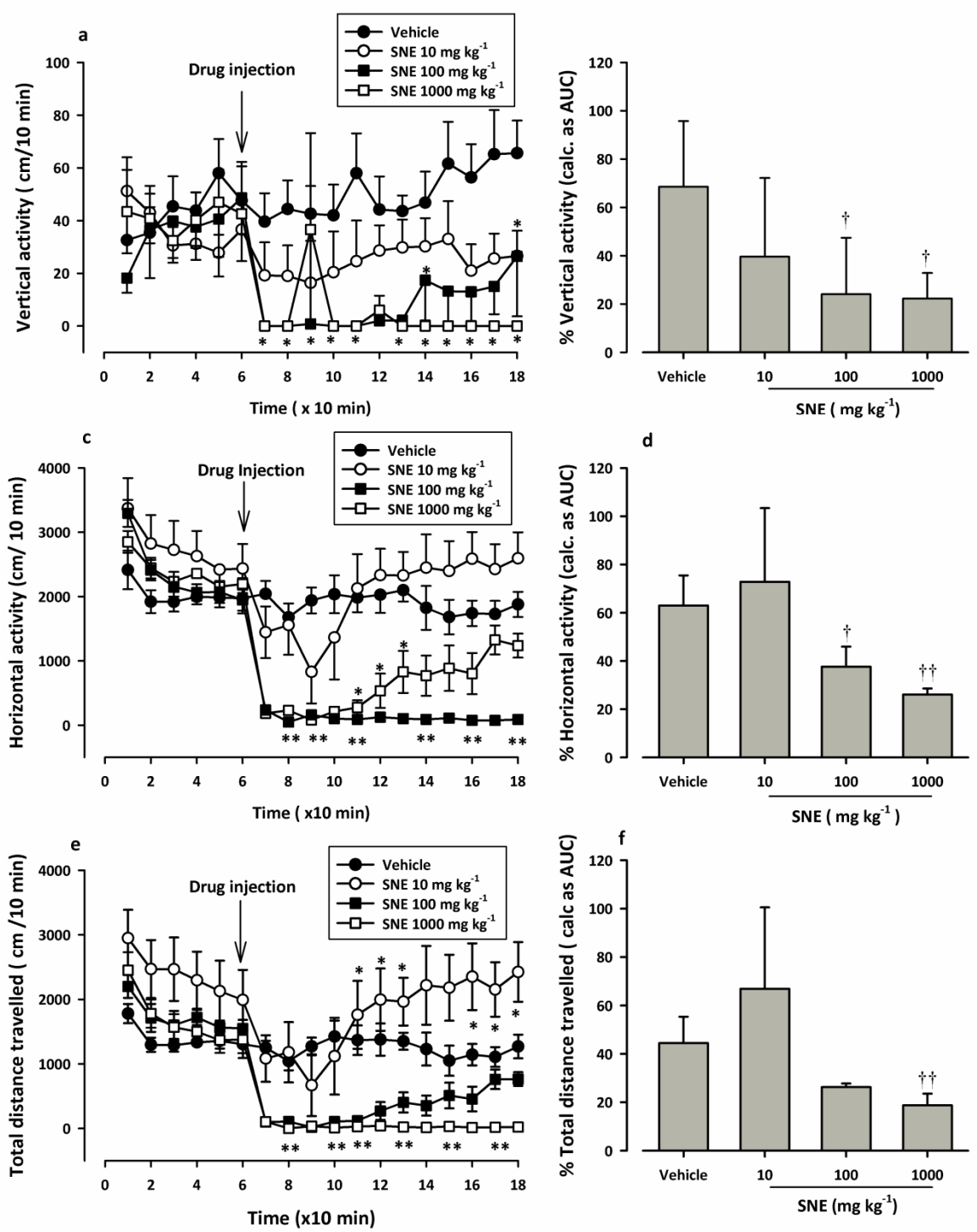

Figure 4 Effect of SNE (10-1000 mg kg^{-1}, *p.o.*) on vertical activity (a, b) and horizontal activity (c, d) and total distance travelled (e, f) by of mice in the Versamax activity monitor. Panels a, c and e shows the time-course curves measured over a three-hour period and panels b, d and f represents the percent effects (calculated as AUC). Each point/column represents mean ± S.E.M (n = 5). **P*<0.05; ** *P*<0.01; ****P*<0.001; compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's *post hoc* test). †*P*<0.05;††*P*<0.01; †††*P*<0.001; compared to vehicle-treated group (One-way ANOVA followed by Newman-Keul's *post hoc* test).

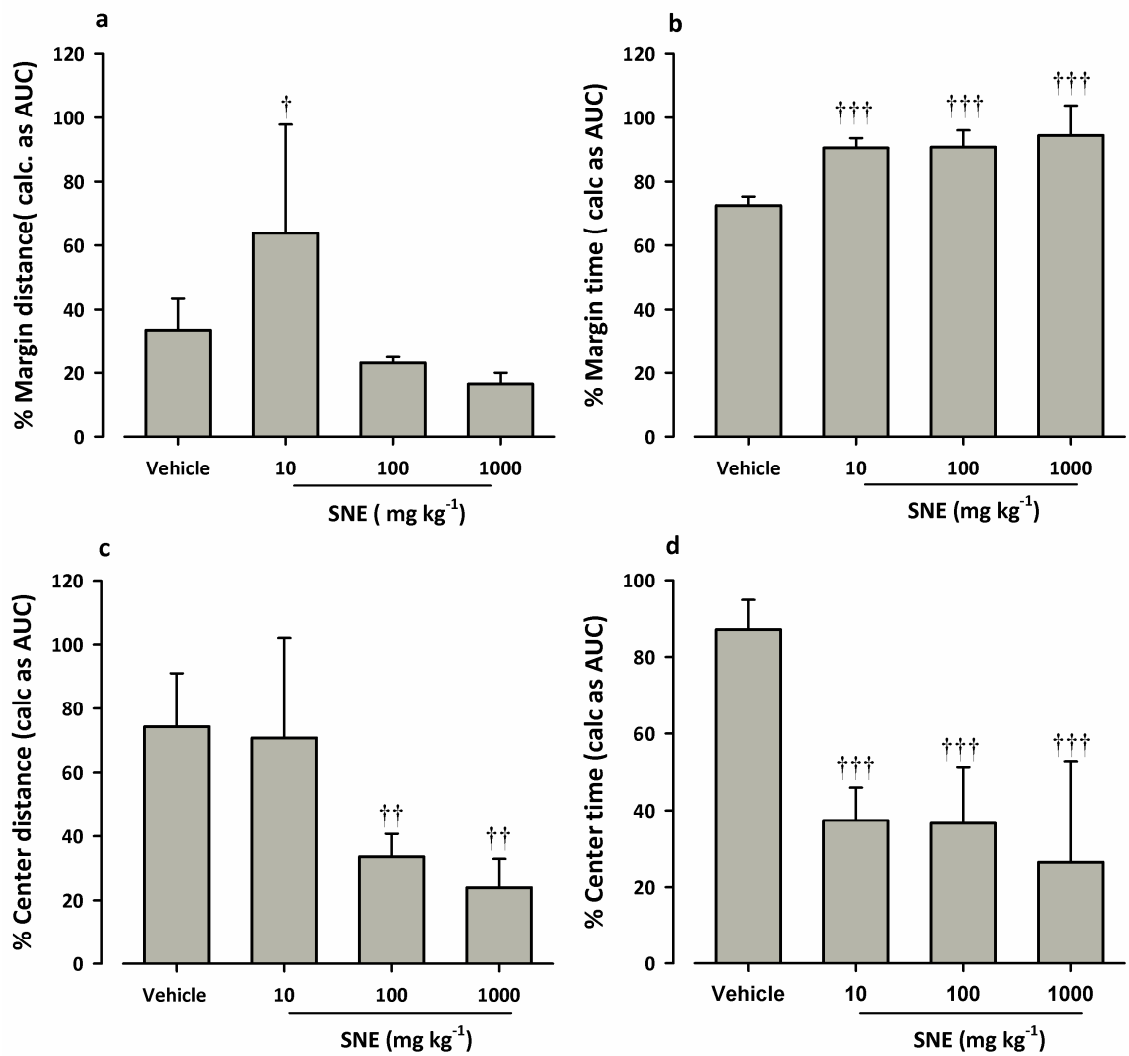

Figure 5 Effect of SNE (10-1000 mg kg^{-1}, *p.o.*) on margin (thigmotactic) distance (a), margin (thigmotactic) time (b), center distance (c) and center time (d) of mice in the Versamax activity monitor. Each column represents mean±S.E.M (n = 5). $^{\dagger}P<0.05$; $^{\dagger\dagger}P<0.01$; $^{\dagger\dagger\dagger}P<0.001$; compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls test).

Effects on anxiety parameters

These as measured by VAMS are as follows: the margin (thigmotactic) distance covered and duration, the distance moved at the centre and the time spent, and the time spent at the corners [the left front (LF), right front (RF), the left rear (LR) and the right rear (RR)].

SNE (10-1000 mg kg^{-1}) significantly decreased the % margin distance ($F_{3,16}$= 6.91, P=0.003, fig. 5a) and increased % time spent at the margin ($F_{3,16}$ =14.31, P<0.001, fig.5b) of the observation cage. The % distance ($F_{3,16}$=9.39, P<0.0001; fig.5c) and % total time spent ($F_{3,16}$ = 14.30, P<0.001, fig.5d) at the centre were also decreased significantly and dose-dependently by SNE. There was no significant difference between the time spent in the various corners of the cage in mice pre-treated with SNE and those pre-treated with the vehicle (fig. 6a-d)

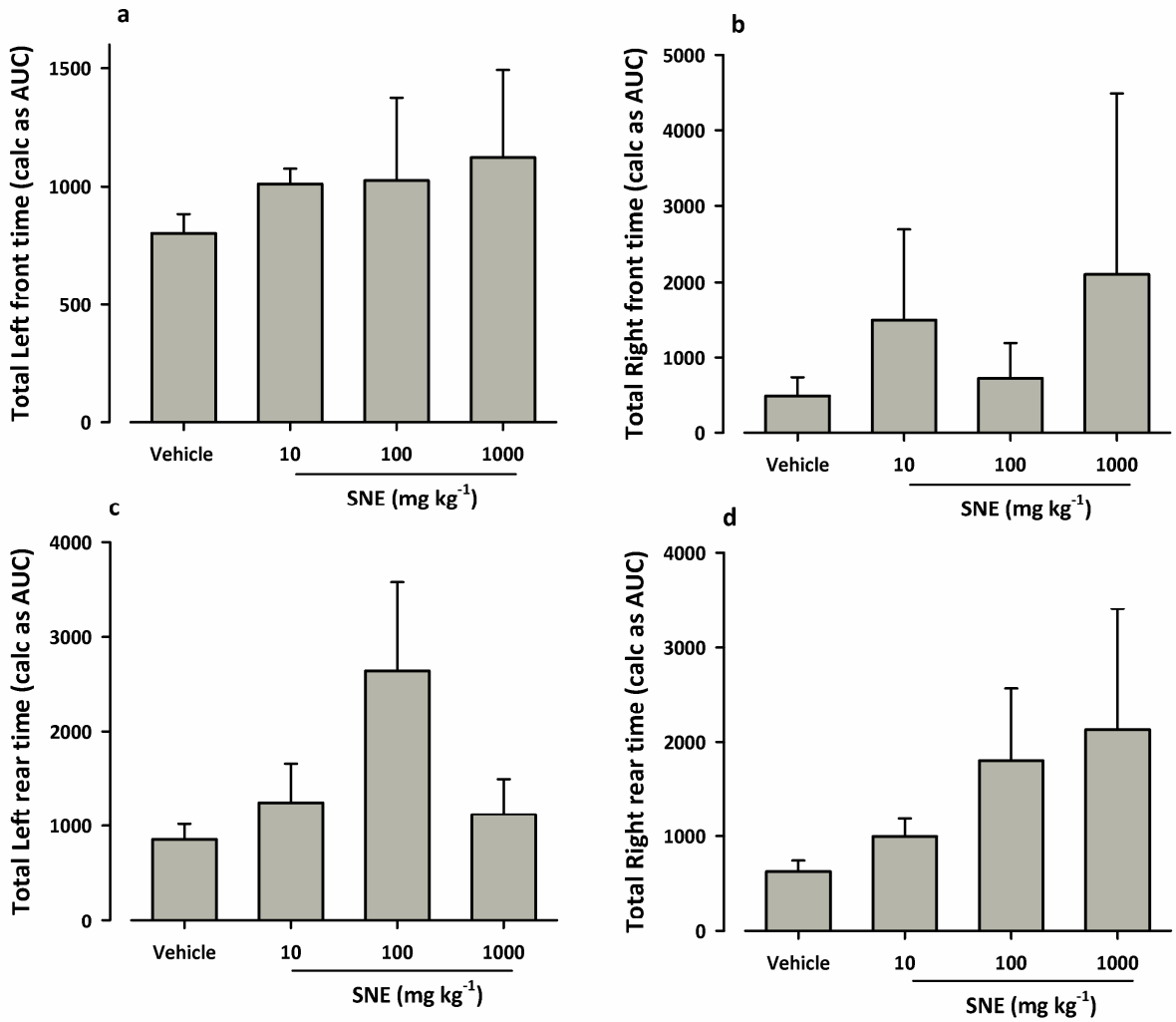

Figure 6 Effect of SNE (10-1000 mg kg^{-1}, *p.o.*) on the time spent by mice at the left front (a), right front (b), left rear (c), right rear (d) areas of the observation chamber of the Versamax activity monitor over 180 min. Values are mean±S.E.M. (n=5). $^{\dagger}P<0.05$; $^{\dagger\dagger}P<0.01$; $^{\dagger\dagger\dagger}P<0.001$; compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls test).

Pentobarbitone-induced sleeping time

Pentobarbitone induced sleep in all the mice used. Sleep duration was significantly and dose-dependently increased ($F_{3,20}$=11.11, P=0.0002, fig. 7) by the extract (30-300 mg kg^{-1}) in the mice. Diazepam, the reference drug, also increased significantly the sleeping time of the mice ($F_{4,25}$=4.050, P=0.0115, fig.7).

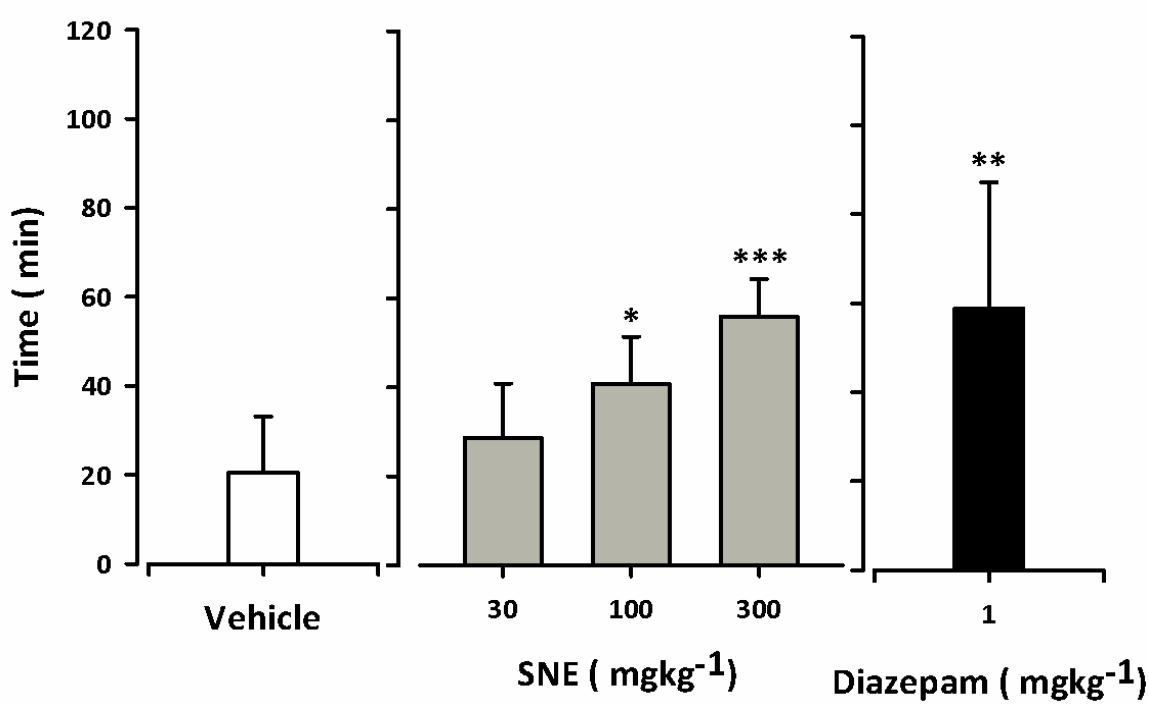

Figure 7 Effect of SNE (30-300 mg kg^{-1}, *p.o.*) and diazepam (1 mg kg^{-1}, i.p.) on pentobarbitone-induced sleeping time in mice. Each column represents mean±S.E.M. (n=10). **P*<0.05; ***P*<0.01; ****P*<0.001 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls test).

DISCUSSION

The elevated plus-maze (EPM) is considered as one of the valid ethological animal models of anxiety since it employs natural stimuli (fear of a novel, brightly lit open space and fear of balance of a relatively narrow and raised platform) capable of inducing anxiety in humans [22-24]. This test has been described as bi-directionally sensitive to both anxiolytic drugs, particularly benzodiazepines and anxiogenic agents used in humans. Generally, anxiolytics are known to increase the percentage number of entry into and duration spent in the open arm of the EPM. Acute oral administration of SNE (10-300 mg kg^{-1}) produced an anxiogenic-like effect in mice as it decreased the number of entries and percentage time spent in the open arm of the maze. Diazepam, in agreement to previously reported studies [25-26], produced an opposite effect to that induced by SNE and this effect has been shown to be mediated via the GABAergic system [27].

Ethological parameters indicative of risk assessment, such as stretch-attend posture and exploratory head dipping, which have been validated to be predictive of anxiety [28-30], were also measured in this study. These parameters have also been shown in the elevated zero-maze to distinguish between anxiogenesis or sedation [31]. SNE induced anxiogenic-like effects by decreasing the number of head dipping whereas in the diazepam treated group, as expected, the number of head dipping increased [29]. However, both SNE and diazepam decreased the number of stretch-attend postures thus suggesting anxiolysis [29]. This indicates that the anxiogenic-like effect observed may not really be so but rather due to sedation. The total number of entries into the arms has been used as an indicator of locomotor activity of rodents in the EPM; however it is an insensitive measure since it has previously been shown that in some circumstances fear/anxiety will inhibit ongoing behavior, including exploration [32-33]. A decrease in total number of entries has therefore been attributed to sedation, locomotor impairment and anxiogenesis [33]. In these instances, measures of the number of stretch-attend postures have

been used to dissociate anxiogenesis from sedation or locomotor impairment. SNE reduced both total arm entries and the number stretch-attend postures thus indicating that the effects observed could be due to sedation and/or locomotor impairment rather than anxiogenesis [34]. A classical example is that the anxiogenic drug m-chlorophenylpiperazine (mCPP), which reduces total entries in the EPM, was shown in the zero-maze to reduce the time spent on the open areas but to increase the number of stretched–attend postures, supporting the conclusion that the effects of mCPP in this test are due to anxiogenesis rather than sedation or locomotor impairment [31]. In addition, SNE has also been shown to produce sedation and motor incoordination in similar doses as used in this study.

The light/dark box paradigm is an ethologically-based approach-avoidance conflict test and is widely used to investigate drugs that affect anxiety [35-37]. Anxiolytic drugs have been known to increase the number of entries and duration spent in the light compartments. Analysis of the results obtained from this test indicates that SNE possess an anxiogenic-like effect since it decreased the number of entries into the lit compartment and also reduced the time spent in this compartment. The number of transitions has been used as an index of activity-exploration and is more sensitive to sedation or psychostimulant effects of drug treatment [38]. Even though anxiolytics are known to generally increase this parameter, sedation or changes in locomotor activity induced by anxiolytics have been known to cause these parameters to be decreased [39]. For instance, diazepam and alprazolam at sedative doses have been reported to cause decreased number of transitions whereas increased transitions were seen with caffeine [38]. Thus the gradual decrease in the transitions observed by SNE could be due to sedation rather than anxiogenesis.

The effects of oral administration of SNE to mice in the observation chamber of the versamax animal activity monitoring system (VAMS) indicate that SNE decreased locomotor activity and produced anxiogenic-like effects dose-dependently. VAMS produces a set of responses, demonstrated by test animals, which can be characterized as locomotor, anxiety or physiological arousal. VAMS is basically a modified open field paradigm test and parameters scored in this test are the same as that of a typical open field. The advantage of this system over the conventional open field paradigm is that scoring of parameters are done by the computer thus excluding human errors of omission which can happen with conventional open field test. Also, the test animals used were made to acclimatize in the VAMS for three hours each day for three days before beginning the actual test, and on the test day too, an hour of primary, drug-free period was allowed before the two hour post treatment assessment was done. This ensures that contrasting behavioral effects of drug treatment are vividly observed and free of any bias due to the animal tested.

Locomotor activity is measured as horizontal and vertical activities, and the total distance travelled by the animal in the observation chamber/cage over a two-hour post treatment. SNE significantly and dose-dependently decreased horizontal and vertical motor activity and the total distance travelled. A drug-induced decrease in spontaneous horizontal motor activity is regarded as an indication of sedation [40]. And since SNE has shown sedative effects in the pentobarbitone-induced sleeping time assay, it confirms sedation as the cause of the reduced locomotor activity observed.

Typical anxiety parameters that can be obtained from VAMS include the margin distance and time at the margins, centre distance travelled and time spent at this area and the total time spent at the left and right front or rear corners of the observation field. This modified open field measures anxiety-related behavior characterized by a normal aversion of experimental animals to

brightly lit environment [41-42]. Thus, when test animals are removed from their acclimatized cages and placed in novel environments, they express anxiety and fear by causing alterations in all or some of the parameters, such as decrease in ambulatory and exploration in the centre of the field with increased peripheral movements or thigmotaxis [43]. These parameters are attenuated by classical anxiolytics but potentiated by anxiogenics. The results obtained suggest the SNE is anxiogenic-like since it reduced entry into the central area and increased the time spent at the margins. Since the general activity of the mice pre-treated with SNE was reduced, it is quite likely that the observed effect were due to sedation but not anxiogenesis.

In summary it is difficult to fully determine the exact effect of SNE on anxiety because of confounding effects of SNE-induced changes in locomotor activity. However, it seems that the anxiogenic-like effects displayed by SNE could be attributed to sedation and/or reduction in locomotor activity.

CONCLUSION

In conclusion, the hydro-ethanolic extract of *Synedrella nodiflora* exhibits anxiogenic-like effects which could be attributed to sedation and reduction in locomotor activity.

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