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Abstract

Background: *Pseudospondias microcarpa* is a plant used for managing various diseases including CNS disorders. Previous studies showed sedative and anticonvulsant effects, suggesting possible anxiolytic activity. This study therefore assessed the anxiolytic effects of *P. microcarpa* hydroethanolic leaf extract (PME) in mice.
Methods: In the present study, anxiolytic-like effect of the extract in behavioural paradigms of anxiety – the elevated plus maze (EPM), light/dark box (LDB), social interaction test and stress-induced hyperthermia (SIH) – was evaluated.
Results: Mice treated with PME (30–300 mg kg^{-1}, p.o.) exhibited anxiolytic-like activity similar to diazepam in all the anxiety models used. The extract increased open arm activity ($p<0.05$) in the EPM as well as increasing the time spent in the lit area in relation to the time spent in the dark area of the LDB. Sociability and preference for social novelty significantly ($p<0.05–0.001$) increased in mice treated with PME. In the SIH paradigm in mice, both PME and the benzodiazepine receptor agonist, diazepam, significantly ($p<0.05$) reduced the stress-induced increase in rectal temperature. The extract did not impair motor coordination and balance in the beam walk test.
Conclusions: Results of the present study indicate that PME possesses anxiolytic-like effects in mice.

Keywords: anxiolytic; elevated plus maze (EPM); *Pseudospondias microcarpa*; stress-induced hyperthermia (SIH).

Introduction

Anxiety disorders are the most prevalent class of psychiatric conditions in the world [1, 2]. Benzodiazepines (BDZs) have been widely used for the treatment of several anxiety disorders, although these compounds have well-known side effects such as sedation, muscle relaxation, amnesia, tolerance and dependence [3–5]. Thus, it is important to explore and develop new therapies for the treatment of anxiety disorders. Various medicinal plants including *Passiflora incarnata* L., *Valeriana officinalis* L. and *Piper methysticum* (*kava-kava*) have been used as anxiolytic agents worldwide [6]. Therefore, the study of medicinal plants could provide new therapeutic options for the management of anxiety disorders [7].

Pseudospondias microcarpa is one of such plants used for managing various diseases including central nervous system (CNS) disorders. In Ghana it is locally known as *katawani* (Twi) literally meaning 'close your eyes' because the tree supposedly has a sedative effect on those who sit or sleep under it. The plant is therefore used in Ghana as a sedative and for treatment of general CNS disorders [8]. Other medicinal uses of the bark and leaves are treatment of arthritis, rheumatism, eye problems, kidney disorders, naso-pharyngeal infections, stomach complaints, malaria and jaundice [8]. Presence of saponins, phenols, terpenoids, flavonoids, cardiac glycosides and coumarines in

***Corresponding author: Donatus Wewura Adongo,** Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, Phone: +233243191409, E-mail: donatusadongo@yahoo.com
Priscilla Kolibea Mante and Eric Woode: Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
Kennedy Kwami Edem Kukuia: Department of Pharmacology and Toxicology, University of Ghana School of Pharmacy, College of Health Sciences, University of Ghana, Accra, Ghana. http://orcid.org/0000-0003-2948-9615
Elvis Ofori Ameyaw: Department of Biomedical and Forensic Sciences, School of Biological Science, University of Cape Coast, Cape Coast, Ghana
Iliya Hosea Azi: Department of Pharmacology, University of Jos, Jos, Nigeria

both the methanol-methylene chloride and aqueous bark extracts of *P. microcarpa* have been reported [9]. Also, the leaves contain alkaloids, tannins, terpenoids and steroids [10]. The plant also possesses antimicrobial properties [11], potent antioxidant effect [9] and anti-plasmodial properties [12].

From our previous study, preliminary phytochemical screening of the hydroethanolic leaf extract of *P. microcarpa* (PME) showed the presence of flavonoids, tannins, saponins, glycosides, terpenoids and alkaloids [13]. In addition, the extract possesses CNS depressant, anticonvulsant and analgesic activity without affecting motor coordination in the animal models used [13].

Drugs that stimulate $GABA_A$ receptors, such as BDZs and barbiturates, have sedative, anxiolytic and antiseizure effects via $GABA_A$-mediated reduction of neuronal excitability [14]. The extract, like the BDZ receptor agonist, diazepam (DZP), has sedative as well as anticonvulsant properties and could therefore possess anxiolytic-like properties. Furthermore, in a previous study, the extract produced an antidepressant-like effect in animal models dependent on the 5-hydroxytryptamine (5-HT) system [15]. Various reports have shown that 5-HT based antidepressants possess anxiolytic effects [16, 17], suggesting possible anxiolytic-like properties for PME. Therefore, this study explored the anxiolytic effect of PME in various classical models of anxiety.

Materials and methods

Collection of plant material

Fresh leaves of *P. microcarpa* were collected from the campus of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, near the Department of Agricultural Engineering (6°40.626′N, 1°34.041′W) during the month of August, 2010, and authenticated at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, KNUST, Kumasi, Ghana. A voucher specimen (KNUST/HM1/2013/L005) was kept at the herbarium of the faculty.

Plant extraction

Leaves of the plant were room-dried for 7 days and pulverised into fine powder. The powder was extracted by cold percolation with 70% (v/v) ethanol in water over a period of 72 h and the resulting extract concentrated into a syrupy mass under reduced pressure at 60 °C in a rotary evaporator. It was further dried in a hot air oven at 50 °C for a week and kept in a refrigerator for use. The yield was 20.5% (w/w). In this study the crude extract is subsequently referred to as PME or extract.

Animals

Male imprinting control region (ICR) mice (20–25 g) were purchased from the Noguchi Memorial Institute for Medical Research, Accra, Ghana, and kept in the animal house of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The animals were housed in groups of five in stainless steel cages (34 cm×47 cm×18 cm) with soft wood shavings as bedding and housing conditions controlled: temperature maintained at 24–25 °C, relative humidity 60–70%, and 12 h light-dark cycle. They had free access to tap water and food (commercial pellet diet, GAFCO, Tema, Ghana). A period of at least 1 week for adaptation to the laboratory facilities was allowed. The studies were conducted in accordance with accepted principles for laboratory animal use and care [18]. Approval for this study was obtained from the Faculty Ethics Committee.

Drugs and chemicals

Pentylenetetrazole (PTZ; Sigma-Aldrich Inc., St. Louis, MO, USA) and DZP (INTAS, Gujarat, India) were used as reference anxiogenic and anxiolytic drugs, respectively.

Elevated plus maze test

This test has been widely validated for measuring anxiolytic and anxiogenic-like activities in rodents [19, 20]. The apparatus was made of Plexiglas and consists of two open (30 cm×5 cm) and two closed (30 cm×5 cm×15 cm) arms, extending from a central platform (5 cm×5 cm) and elevated to a height of 60 cm above the floor in a lit room (~750 lux). A rim of Plexiglas (0.5 cm in height) surrounded the perimeter of the open arms to provide additional grip and thus prevented the mice falling off [21].

Mice were randomly assigned to 10 experimental groups: vehicle-control, PME (30, 100 or 300 mg kg^{-1}, p.o.), DZP (0.1, 0.3 or 1.0 mg kg^{-1}, i.p.) and PTZ (3, 10 or 30 mg kg^{-1}, i.p.). DZP and PTZ served as reference anxiolytic and anxiogenic drugs, respectively. Thirty minutes after intraperitoneal injection and 1 h after oral administration, mice were placed individually in succession in the central platform of the maze for 5 min and their behaviour videotaped. Behavioural parameters were scored from the videos using the public domain software JWatcher™ Version 1.0 (University of California, Los Angeles, CA, USA, and Macquarie University, Sidney, Australia, available at http://www.jwatcher.ucla.edu/) as follows: (i) number of entries and time spent in each arm, i.e. closed and open arms, (ii) number and duration of protected and unprotected stretch-attend postures and (iii) number and duration of protected and unprotected head dipping.

Entry into an arm was defined as the animal placing all four paws into the arm. Protected head dipping was defined as the mouse stretching to dip its head into the open space and observing the environment with the body remaining in a closed arm or the central platform, while in unprotected head dipping, the mouse dips its head into the open space and observes the environment with the body being in an open arm. Protected stretch-attend postures (PSAPs) were defined as the mouse stretching forward and retracting without moving forward its feet whilst in the closed arm or central platform of the maze, whereas unprotected stretch-attend postures (USAPs) were

defined as the mouse stretching forward and retracting without moving forward its feet whilst in the open arm.

To compute total distances travelled by the mice, the software Behaviour Collect (http://cas.bellarmine.edu/tietjen/DownLoads/computer_programs_for_data_colle.htm) was used to obtain raw XY data from the videos. Distance between two X-Y coordinate pairs was calculated from the following formula: $\sqrt{[(X_1-X_2)^2+(Y_1-Y_2)^2]}$.

Light/dark box test

Anxiety-related behaviour was further tested in the light/dark box (LDB) exploration test as described by Crawley and Goodwin [22] with modifications. The apparatus was a wooden box (36 cm long×33 cm wide×30 cm deep) divided into two compartments by a wooden board with a small opening (8 cm×8 cm) connecting the compartments. The larger compartment comprised two thirds of the apparatus, painted white, open and illuminated by a 60-W lamp placed 50 cm above the compartment. The smaller compartment was painted black and had a cover that was closed during testing. Male ICR mice were divided into 10 groups (n=5) and treated with PME, DZP, PTZ and the vehicle as described above for the elevated plus maze (EPM) test. At the beginning of the experiments, mice were placed individually at a far corner of the dark compartment facing the light compartment and videotaped with a digital video camera for a period of 5 min. Behaviours of the animals from the videos were analyzed for the following parameters: (1) the latency to emerge from the dark compartment with all four paws into the light compartment, (2) total time spent in each compartment, and (3) total number of transitions between the compartments.

Social interaction test

Test for sociability and preference for social novelty was conducted as previously described [23, 24]. The apparatus comprised a rectangular, three-chambered box. Each chamber was 20 cm×40 cm×22 cm, and the dividing walls were made from clear Plexiglas, with small square openings (5 cm×3 cm) allowing access into each chamber. An animal was placed in the middle chamber with the dividers closed to allow it to explore the middle chamber for 5 min. After this 5-min habituation period, an unfamiliar male (stranger 1) that had no prior contact with the subject mouse was placed in one of the side chambers. Placement of stranger 1 in the left or right side chambers was systematically alternated between trials. The stranger mouse was enclosed in a small, circular wire cage (11 cm high, with a bottom diameter of 9 cm and bars spaced 0.5 cm apart) that allowed nose contact between the bars but prevented the stranger mouse from initiating any social contact and limited the possibility of aggressive interactions. The subject mouse was first placed in the middle chamber and allowed to explore the entire social test box for 10 min, which was videotaped. Measures were taken of time spent and entries into the chamber containing the unfamiliar mouse in a wire cage (stranger side) and the chamber containing only the empty wire cage on the opposite side of the apparatus (empty side) for 10 min. An entry was defined as all four paws in one chamber. The duration and number of direct (active) contacts between the subject mouse and the containment cup housing or not housing the stranger 1 mouse, for each chamber, was also analyzed. Direct contact between the subject mouse and the containment cup or stretching of the body of the subject mouse in an area 3–5 cm around the cup is counted as an active contact or sniffing.

A 10-min test to quantitate preference for social novelty began immediately after the test for sociability. The original stranger mouse (stranger 1) remained in its wire cage, and a new unfamiliar mouse (stranger 2) was placed in the wire cage which was previously empty during the sociability test. Identical measures as previously described were scored: time spent in each chamber, entries between chambers and time spent sniffing each wire cage. Stranger 1 and stranger 2 animals originated from different home cages and had never been in physical contact with the subject mice or each other. After each test, the wire cages and apparatus were wiped with 70% ethanol and allowed to air-dry. In this test, male ICR mice were divided into 10 groups (n=7) and treated with PME, DZP, PTZ and the vehicle as described above for the EPM test before behavioural was performed.

Stress-induced hyperthermia

This test is based on the principle that mice have a natural hyperthermic response to stress, which reflects the level of anxiety. Test procedure for the modified stress-induced hyperthermia (SIH) was adopted from Van der Heyden et al. [25]. The test involves two measures of rectal temperature repeated in the same animal with a 10-min interval. Mice were singly housed in smaller cages (26 cm×21 cm×14 cm) for 24 h before testing with free access to food and water.

On the morning of the experiment, animals were first divided into seven groups and treated with PME (30, 100 or 300 mg kg^{-1}, p.o.), DZP (0.1, 0.3 or 1.0 mg kg^{-1}, i.p.) or normal saline. Thirty minutes after intraperitoneal injection and 1 h after oral administration, each animal was removed from the holding cage, and rectal temperature was measured to the nearest 0.1 °C by an ELLAB instruments (Copenhagen, Denmark) thermometer via a lubricated thermistor probe (2 mm diameter) inserted 20 mm into the rectum while the mouse was handheld near the base of the tail. The probe was left in place until steady readings were obtained. This temperature was recorded as the baseline rectal temperature (T_1). The animal was immediately placed back to the holding cage, and after a 10-min interval the second rectal temperature (T_2) was taken. SIH was assessed as the difference between the second measurement and the first measurement. The first measurement was used to evaluate whether the test compound by itself would have a potential effect on basal body temperature.

Beam walk test

The test was carried out as described previously [26]. Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by wooden supports to a goal box (enclosed hamster house). Three trials were performed for each mouse and were designed such that the mice tested would be aware that there was a goal box that could be reached. A ruler was used because the mice found this easy to cross and, at the same time, it induced minimum anxiety.

On the day of the experiment, mice were randomly divided into seven groups: saline-treated control group; DZP group (0.1, 0.3, 1 or 3 mg kg^{-1}, i.p.) and PME group (30, 100, or 300 mg kg^{-1}, p.o.). Mice were placed on the beam at one end and allowed to walk to the goal

box. Mice that fell were returned to the position they fell from, with a maximum time of 60 s allowed on the beam. Measurements taken were time on beam and number of foot slips (one or both hind limbs slipped from the beam).

Statistical analysis

A sample size of five to eight animals was utilised. All data are presented as mean±SEM. To compare differences between groups, one-way ANOVA was performed with Newman-Keuls's test as post hoc and two-way ANOVA followed by Bonferroni's test as post hoc. GraphPad Prism for Windows 5 (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis. $p<0.05$ (Newman-Keuls's test or Bonferroni's test) was considered statistically significant.

Results

Effects in the elevated plus maze test

Spatiotemporal effects

Oral administration of PME significantly increased number and percentage number of open arm entries as revealed by one-way ANOVA ($F_{3,16}=4.799$, $p=0.0143$; Figure 1A and $F_{3,16}=6.153$, $p=0.0055$; Figure 1D, respectively). Although one-way ANOVA revealed no significance for duration and percentage duration in the open arms, post hoc (Newman-Keuls) analysis showed statistical significance at 300 mg kg^{-1} ($p<0.05$). PME also decreased the time spent in the closed arms with statistical significance occurring at 100 and 300 mg kg^{-1} (both at $p<0.05$). A two-way ANOVA showed significant arm type effect where the number of open arm entries ($F_{1,24}=18.28$, $p=0.0027$; Figure 1A) and the open arm time ($F_{1,24}=22.31$, $p=0.0015$; Figure 2A) increased significantly compared to the closed arm entries and closed arm time. Administration of DZP caused significant and dose-dependent increase in number of open arms entries ($F_{3,16}=7.753$, $p=0.0020$; Figure 1B) and the percentage number of open arm entries ($F_{3,16}=7.132$, $p=0.0029$; Figure 2E). DZP also caused a significant and dose-dependent increase in the amount of time spent in the open arms ($F_{3,16}=4.606$, $p=0.0166$). Two-way ANOVA also showed a significant effect for number of open arm entries ($F_{1,24}=24.86$, $p=0.0011$) and the open arm time ($F_{1,24}=45.41$, $p=0.0001$) in mice treated with DZP. PTZ (3–30 mg kg^{-1}) significantly increased open arm avoidance by decreasing the number of entries ($F_{3,16}=7.197$, $p=0.0028$; Figure 1C), percentage number of entries ($F_{3,16}=8.119$, $p=0.0016$; Figure 1F) and percentage time spent ($F_{3,16}=4.859$, $p=0.0137$; Figure 2F) in the open arms of the EPM. A two-way ANOVA showed a significant effect for PTZ where the number of open arm entries ($F_{1,24}=30.31$, $p=0.0006$; Figure 1C) and the open arm time ($F_{1,24}=16.53$, $p=0.0036$; Figure 2C) decreased compared to the closed arm entries and closed arm time.

Risk assessment behaviour

Effects of PME on risk assessment parameters in the EPM are shown in Figures 3 and 4. Pretreatment of mice with PME significantly increased the number ($F_{3,16}=6.011$, $p=0.0061$) of USAPs and reduced percentage number ($F_{3,16}=6.001$, $p=0.0123$) of PSAPs in mice. A two-way ANOVA showed significant stretch-attend posture type effect where the number ($F_{1,24}=37.67$, $p=0.0004$) of USAPs by mice increased significantly compared to the PSAPs. The extract also significantly reduced the number ($F_{3,16}=9.406$, $p=0.0008$) of protected head dips (PHDs). In addition, two-way ANOVA showed significant head dip type effect where the number ($F_{1,24}=32.46$, $p=0.0005$) of unprotected head dips (UHDs) by mice increased significantly compared to the PHDs. DZP significantly reduced the number ($F_{3,16}=4.706$, $p=0.0154$) and percentage number ($F_{3,16}=10.41$, $p=0.0005$) of PSAPs. Furthermore, it significantly reduced the number ($F_{3,16}=9.958$, $p=0.0006$) and percentage number ($F_{3,16}=6.634$, $p=0.004$) of PHDs. In contrast to PME, PTZ significantly increased the number ($F_{3,16}=6.262$, $p=0.0051$) and percentage number ($F_{3,16}=7.814$, $p=0.0020$) of PSAPs. It also significantly decreased number ($F_{3,16}=3.933$, $p=0.0281$) of USAPs. Moreover, PTZ significantly increased the percentage number ($F_{3,16}=20.20$, $p<0.0001$) of PHDs.

Total distance travelled

Figure 5 shows the effects of PME, DZP and PTZ on total distance travelled in the EPM. Treatment of mice with the extract ($F_{3,16}=0.5352$, $p=0.6645$) and DZP ($F_{3,16}=2.707$, $p=0.0799$) did not have any significant effect on the total distance travelled in the EPM compared to the vehicle treated animals. However, PTZ caused a significant decrease ($F_{3,16}=19.60$, $p<0.0001$) in the total distance travelled. Comparing the 3D line plots, PME and DZP treated animals seemed to have made a greater number of visits into the open arms than the closed arms of the EPM. In contrast, PTZ treated animals made more closed arm entries than open arm entries.

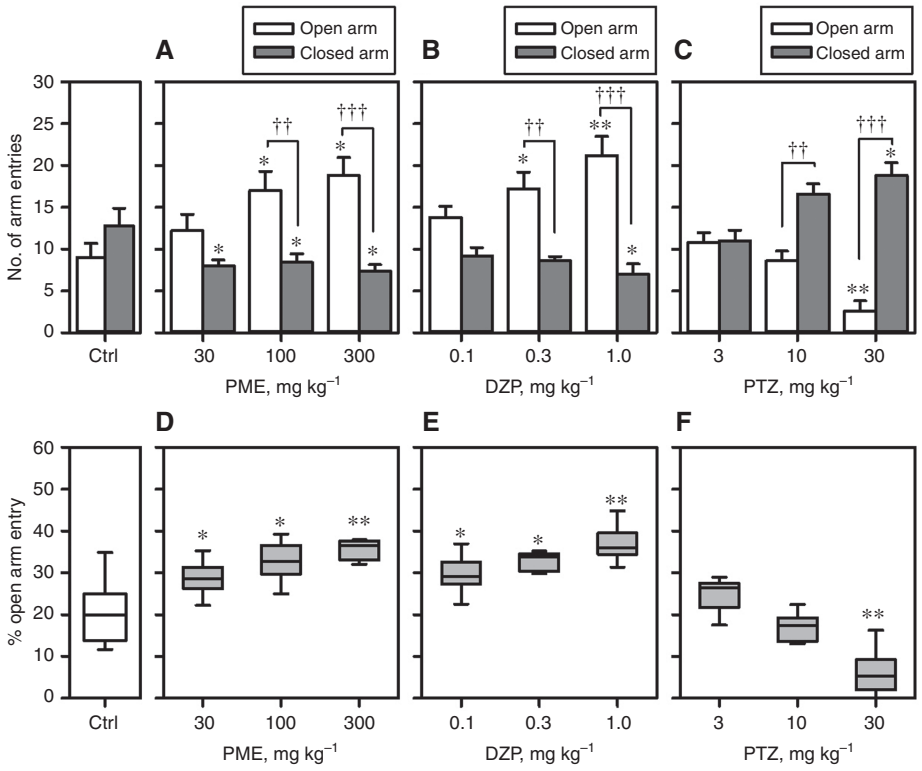

Figure 1: Effects of PME (30–300 mg kg^{-1}), DZP (0.1–1.0 mg kg^{-1}) and PTZ (3–30 mg kg^{-1}) on mice behaviour on the EPM over a 5-min test period.

Data are presented as group mean±SEM. The lower and upper margins of the boxes (D, E and F) represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. Significantly different from control: *p<0.05, **p<0.01 (one-way ANOVA followed by Newman-Keuls's post hoc test) and significant difference when open arm and closed arm were compared: ††p<0.01, †††p<0.001 (two-way repeated measures ANOVA followed by Bonferroni's post hoc).

Effects in the LDB box test

In the light-dark box test (Figure 6), one-way ANOVA shows that oral administration of PME (30–300 mg kg^{-1}) significantly decreased the latency ($F_{3,16}$=3.977, p=0.0271) into the lit compartment and also increased time spent ($F_{3,16}$=3.458, p=0.0415) in the lit compartment without affecting inter-compartmental transitions. Two-way ANOVA (treatment×box type, i.e. lit or dark) revealed no significant box type effect ($F_{1,24}$=2.667, p=0.1411) when duration in lit box was compared to the dark box. However, Bonferroni's post hoc analysis showed a significance at the dose of 300 mg kg^{-1} (p<0.001). DZP produced effects that were similar to those produced by PME. It decreased the latency into the lit compartment and increased time spent in the lit compartment ($F_{3,16}$=5.884, p=0.0066 and $F_{3,16}$=4.894, p=0.0134, respectively). A two-way ANOVA showed a significant effect ($F_{1,24}$=12.74, p=0.0073) where mice spent more time in the lit box compared to the dark box. In contrast to PME and DZP, PTZ significantly decreased the time spent by mice in the lit box ($F_{3,16}$=8.715, p=0.0012) and increased significantly the time spent in the dark box ($F_{3,16}$=8.711, p=0.0012). PTZ also significantly decreased the number of inter-compartment transitions ($F_{3,16}$=7.163, p=0.0029) and increased significantly the latency to enter lit compartment ($F_{3,16}$=4.832, p=0.014). In addition, two-way analysis of the effects of PTZ revealed a significant effect ($F_{1,24}$=1011, p<0.0001) where mice spent less time in the lit compartment compared to the time spent in the dark compartment.

Effects on sociability and preference for social novelty

In the sociability test, mice treated with PME and DZP demonstrated a significant preference for spending time in the chamber containing stranger 1 than the empty chamber [Figure 7; overall repeated measures ANOVA — following significant main effect of side on duration: PME

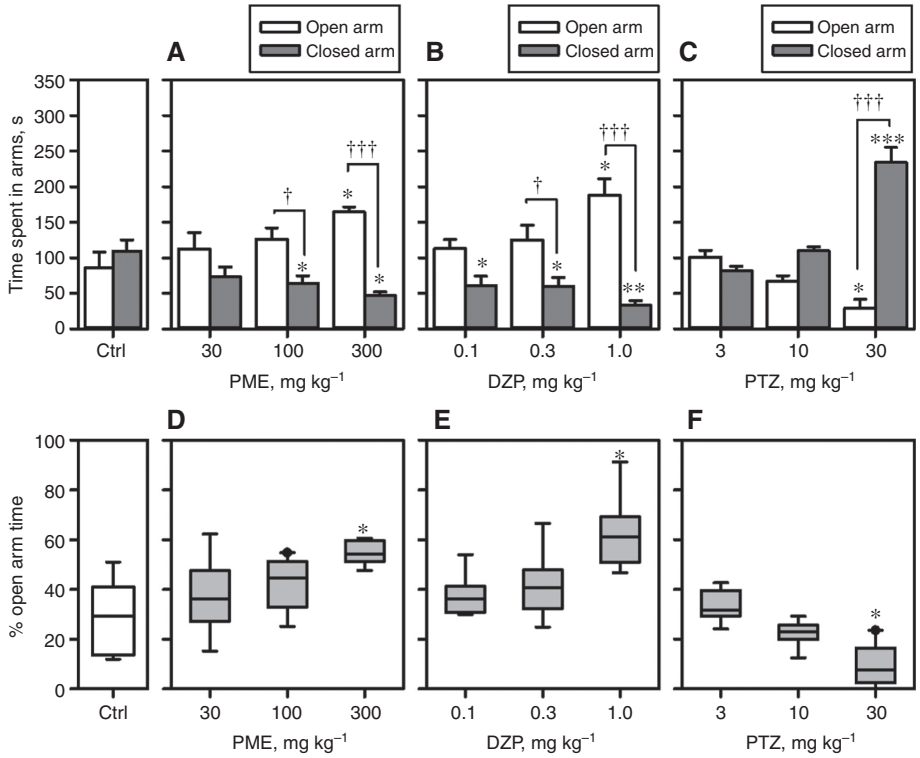

Figure 2: Effects of PME (30–300 mg kg^{-1}), DZP (0.1–1.0 mg kg^{-1}) and PTZ (3–30 mg kg^{-1}) on mice behaviour on the EPM over a 5-min test period.

Data are presented as group mean±SEM. The lower and upper margins of the boxes (D, E and F) represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. Outlying points are shown individually. Significantly different from control: *p<0.05, **p<0.01, ***p<0.01 (one-way ANOVA followed by Newman-Keuls's test) and significant difference when open arm and closed arm were compared: †p<0.05, †††p<0.001 (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

($F_{2,72}$=136.2, p<0.0001) and DZP ($F_{2,72}$=132.9, p<0.0001)]. Subjects generally spent more time in either side of the apparatus than in the middle chamber. PME treated mice spent more time sniffing the wire cage containing the unfamiliar mouse than the empty wire cage ($F_{1,48}$=140.3, p<0.0001) with Bonferroni's post hoc analysis revealing significance at all the doses used (all p<0.001). A similar effect was observed for DZP ($F_{1,48}$=90.06, p<0.0001). PME and DZP treated mice exhibited a significant preference to spend time in the chamber containing the novel stranger 2, as compared to time spent in the chamber containing the now-familiar stranger 1 [Figure 8; two-way ANOVA — following significant main effect of side on duration: PME ($F_{2,72}$=185.0, p<0.0001) and DZP ($F_{2,72}$=80.02, p<0.0001)]. A significant preference for social novelty (sniffing duration) was also observed for PME ($F_{1,48}$=64.51, p<0.0001) and DZP ($F_{1,48}$=47.10, p<0.0001), where mice spent more time sniffing the novel stranger than stranger 1. In contrast to PME and DZP, PTZ showed a decrease in sociability by significantly decreasing time spent in the chamber containing stranger 1 compared to the empty chamber ($F_{2,72}$=19.0, p<0.0001) with post hoc analysis showing significance at 30 mg kg^{-1} (p<0.001). Mice also spent more time sniffing the empty wire cage than stranger 1 ($F_{1,48}$=5.059, p=0.0291). A significant preference to spend time in the chamber containing the now-familiar stranger 1, as compared to time spent in the chamber containing the novel stranger 2 was observed for PTZ ($F_{2,72}$=52.25, p<0.0001). Mice administered with PTZ also showed an increased preference for sniffing stranger 1 compared to stranger 2 ($F_{1,48}$=9.578, p=0.0033). Differences in number of entries into the two sides were not observed during the test for sociability and social preference for all treated groups (p>0.05).

Stress-induced hyperthermia

In Figure 9, ANOVA revealed a significant effect of PME treatment on the magnitude of the SIH response ($F_{3,36}$=2.883, p=0.0491) with Newman-Keuls's post hoc analysis showing a statistical significance at 300 mg kg^{-1}

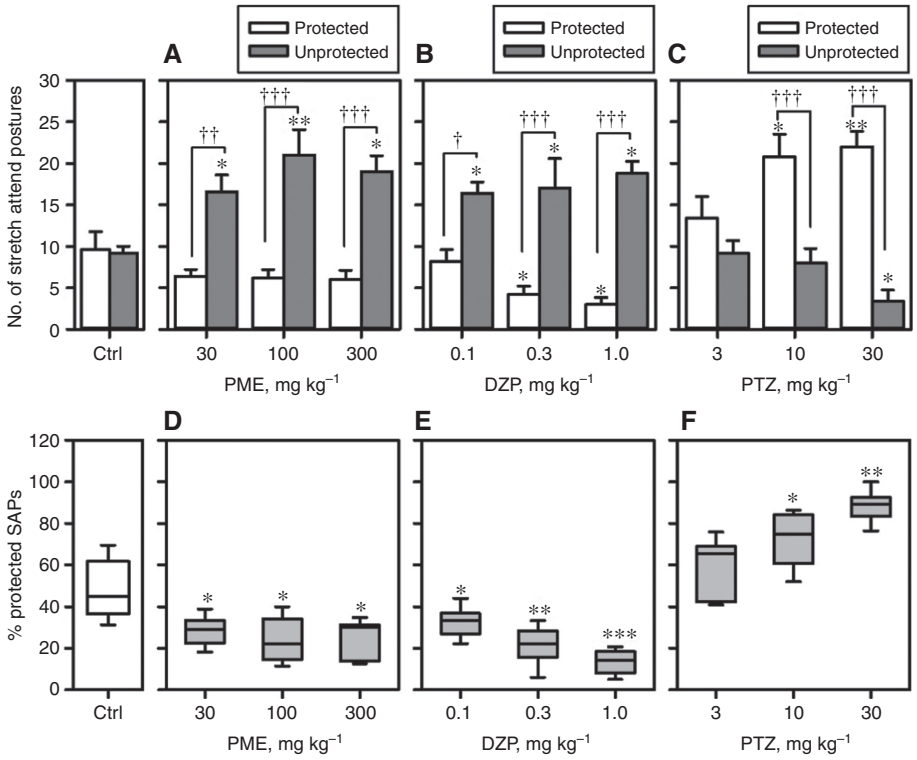

Figure 3: Effects of PME (30–300 mg kg^{-1}), DZP (0.1–1.0 mg kg^{-1}) and PTZ (3–30 mg kg^{-1}) on risk assessment behaviours (protected and unprotected stretch-attend postures) over a 5-min test period in mice on the EPM.
Data are expressed as group mean±SEM. The lower and upper margins of the boxes (D, E and F) represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. Significant difference: *p<0.05, **p<0.01, ***p<0.001 compared to control group (one-way ANOVA followed by Newman-Keuls's post hoc test) and †p<0.05, ††p<0.01, †††p<0.001 when protected and unprotected stretch-attend postures are compared (two-way ANOVA followed by Bonferroni's post hoc test).

(p<0.05). Moreover, the stronger reduction in T_2 than in T_1 following treatment with PME is reflected in a significant stress×treatment interaction ($F_{3,72}$=3.561, p=0.0183). Similar to PME, DZP significantly decreased SIH ($F_{3,36}$=4.003, p=0.0149) and a two-way ANOVA showing a significant stress×treatment interaction ($F_{3,72}$=13.18, p<0.0001).

Effects on motor co-ordination

Figure 10 shows the results of the effect of PME and DZP on motor co-ordination in the mouse beam walk test. One-way ANOVA revealed that pre-treatment of mice with PME (30–300 mg kg^{-1}, p.o.) did not significantly affect the time taken by mice to reach the goal box ($F_{3,16}$ =0.2882, p>0.05) and number of foot slips. DZP did not also have significant effect on the time taken to cross the beam at 0.1–1.0 mg kg^{-1} (p>0.05) except 3.0 mg kg^{-1} which caused significant delay in the time to traverse the beam as well as increasing number of foot slips compared to vehicle-treated group (p<0.01).

Discussion

In this study, administration of the hydroethanolic extract of the leaves of *P. microcarpa* possesses anxiolytic-like effect comparable with that of DZP in pharmacologically validated models of anxiety.

The present study showed that the administration of different doses of PME in mice was able to induce anxiolytic-like effects in the EPM. The EPM has become the most widely used animal model in contemporary preclinical research on anxiety. In addition, the EPM affords a good example of a model based on the study of unconditioned responses to less intense threatening situations [27]. This test is based on the observation that the natural behaviour of rats or mice is to display an aversion to open spaces; therefore, avoidance of the open arms is interpreted as

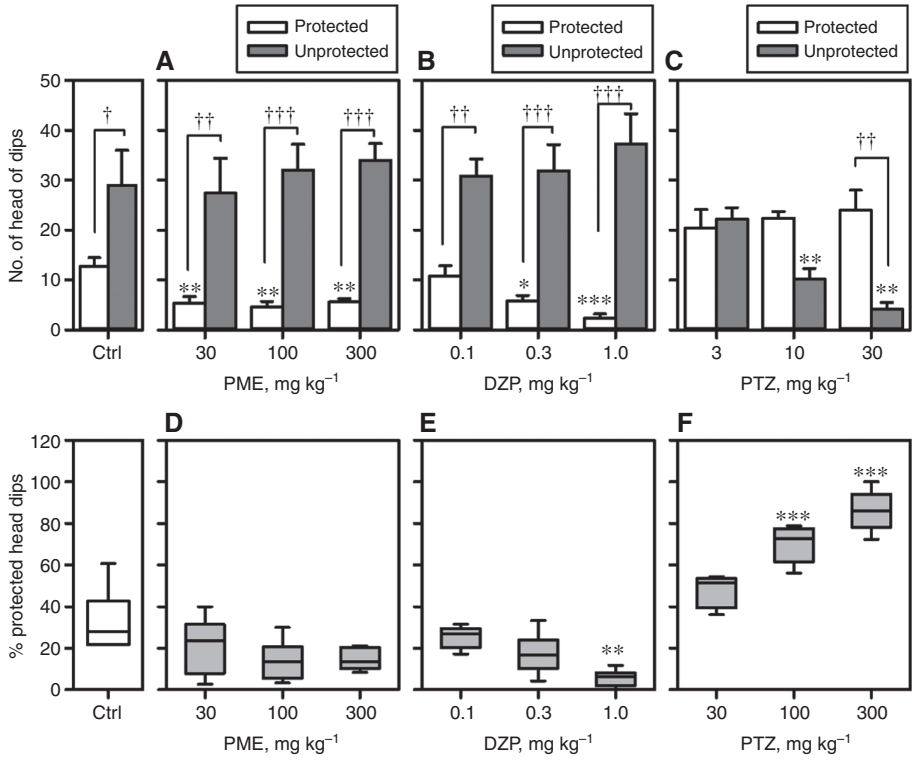

Figure 4: Effects of PME (30–300 mg kg^{-1}), DZP (0.1–1.0 mg kg^{-1}) and PTZ (3–30 mg kg^{-1}) on risk assessment behaviours (PHDs and UHDs) over a 5-min test period in mice on the EPM.
Data are expressed as group mean±SEM. The lower and upper margins of the boxes (D, E and F) represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. Significant difference: *p<0.05, **p<0.01, ***p<0.001 compared to control group (one-way ANOVA followed by Newman-Keuls's test) and †p<0.05, ††p<0.01, †††p<0.001 when PHDs and UHDs are compared (two-way ANOVA followed by Bonferroni's post hoc test).

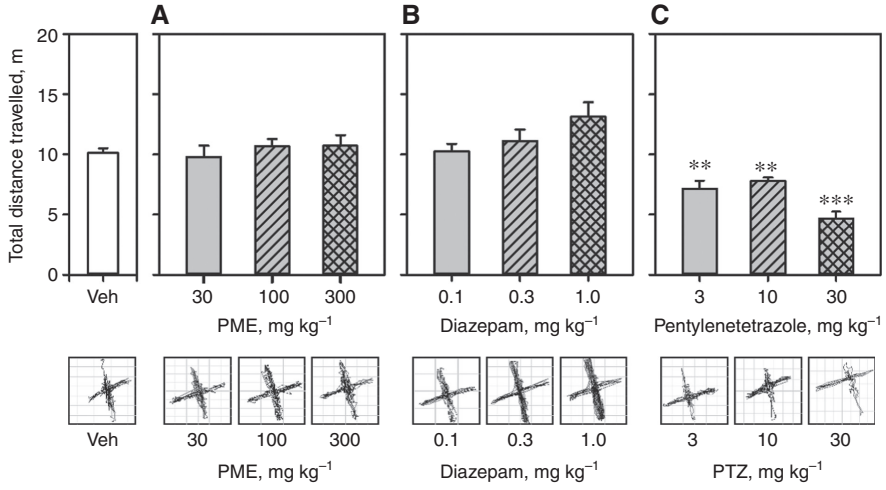

Figure 5: Effects of PME (30–300 mg kg^{-1}), DZP (0.1–1.0 mg kg^{-1}) and PTZ (3–30 mg kg^{-1}) on total distance travelled on the EPM.
Data are presented as group mean±SEM. **p<0.01, ***p<0.001 compared to control group (one-way ANOVA followed by Newman-Keuls's test). Line plots (lower panels) 3D plots were generated from the time and XY data obtained using Sigma Plot Version (Systat Software Inc., Point Richmond, CA, USA).

anxiogenic behaviour [7, 28, 29]. Moreover, the anxiolytic-like effectiveness of a drug can be demonstrated by an increase in exploration of the open arms (time and entries into open arms), while the opposite holds true for drugs with anxiogenic-like effects [19, 20, 29, 30]. The number of closed arm entries also provides a control measure of

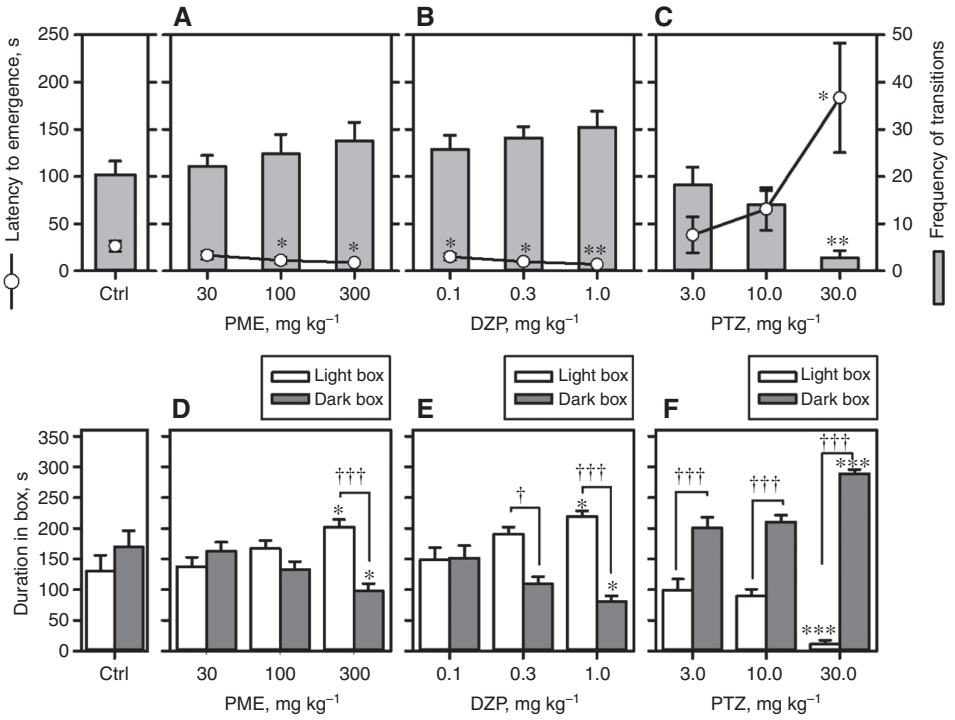

Figure 6: Effects of PME, DZP and PTZ on mice behaviour in the LDB over a 5-min period.
Data are expressed as group mean±SEM. *p<0.05, **p<0.01, ***p<0.001 compared to control group (one-way ANOVA followed by Newman-Keuls's post hoc test). †p<0.05, †††p<0.001 when light compartment was compared with dark compartment (two-way ANOVA followed by Bonferroni's post hoc test).

motor activity [31]. In the present study, PME decreased the avoidance to open arms, increasing the percentage of entries and time in the open arms as well as decreasing number and time in the closed arms indicating an anxiolytic-like effect. These results were similar to the effects observed after administration of the reference anxiolytic drug DZP while showing effects opposite to that of PTZ. These data are in agreement with the results of other studies, where DZP (an anxiolytic) increased the percentage time spent in open arms and open arm entries, while PTZ (an anxiogenic) showed opposite effects in the EPM [32, 33].

Ethological approaches to the EPM have evaluated defensive behaviours, especially those associated with risk assessment, for example, stretch-attend posture and head dipping [29, 33]. These measures of defence have improved the analysis of the effects observed in this paradigm [34–36]. Anxiolytics reduce these risk assessment behaviours (decrease in number of PSAPs or protected head dips), while anxiogenics increase these parameters [21, 33, 37]. Both PME and DZP decreased the number and percentage number of the protected forms of both stretch-attend postures and head-dipping, indicating reduced state of anxiety or fear. However, PTZ increased the protected forms of the risk assessment behaviours which is consistent with its anxiogenic activity. Locomotor effects can confound interpretation of behavioural changes that are used as indices of anxiety reduction [38]. The anxiolytic-like effect of PME seems to be specific, as it did not significantly affect the exploratory activity of the animals in the EPM test (total distance).

As an anxiety model, the light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, novel environment and light [22, 39]. In the light/dark test, the primary indices of anxiety are spatiotemporal, i.e. the time spent in the bright side and the number of transitions made by animals between the two compartments. Anxiolytics have been shown to significantly increase the number of transits between compartments without an increase in spontaneous locomotion [39, 40]. The extract and DZP showed anxiolytic-like effects by decreasing the latency to enter the light compartment as well as increasing time spent in the light. PTZ also showed results consistent with its anxiogenic activity, increasing the latency to enter the light compartment and decreasing both inter-compartment transitions and the time spent in the light.

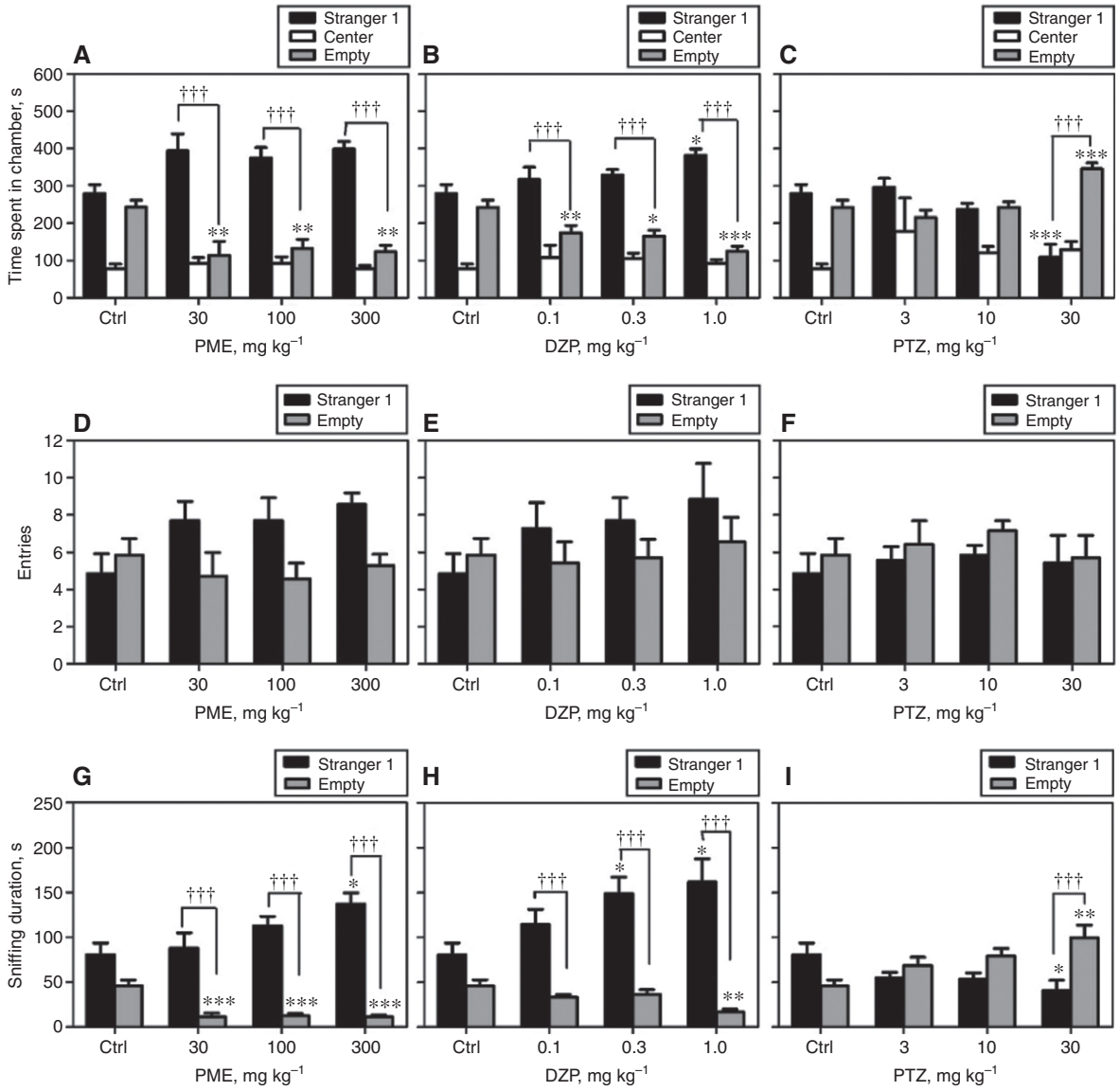

Figure 7: Effects of acute PME (30–300 mg kg^{-1}), DZP (0.1–1.0 mg kg^{-1}) and PTZ (3–30 mg kg^{-1}) treatment on the total time spent in the chambers for PME (A), DZP (B) and PTZ (C); entries into compartments for PME (D), DZP (E) and PTZ (F) and sniffing duration for PME (G), DZP (H) and PTZ (I) in the sociability test.

Data are presented as group mean±SEM. Significantly different from control: *$p<0.05$, **$p<0.01$, ***$p<0.001$ by Newman-Keuls's test and significant difference when stranger 1 was compared to empty cage: †††$p<0.001$ (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

Abnormal social behaviours or decreased levels of social interaction are symptoms of several psychiatric disorders [41]. Crawley's three-chamber social approach test consists of sociability test – tendency to initiate social contact and a social novelty preference test – tendency to initiate social contacts with a new individual as compared to someone familiar from past experience [23]. This test has been successfully employed to study sociability and preference for social novelty as reported by various studies [42–45]. Sociability is defined as the subject mice spending more time in the chamber containing the novel target mouse than in the chamber containing the empty cage whereas preference for social novelty is the ability to spend time with a previously unencountered mouse rather than with a familiar mouse [23, 46].

Pseudospondias microcarpa extract and DZP treated mice spent more time in the side of the social test box containing the unfamiliar stranger than in the empty side. Mice treated with PME and DZP spent more time sniffing

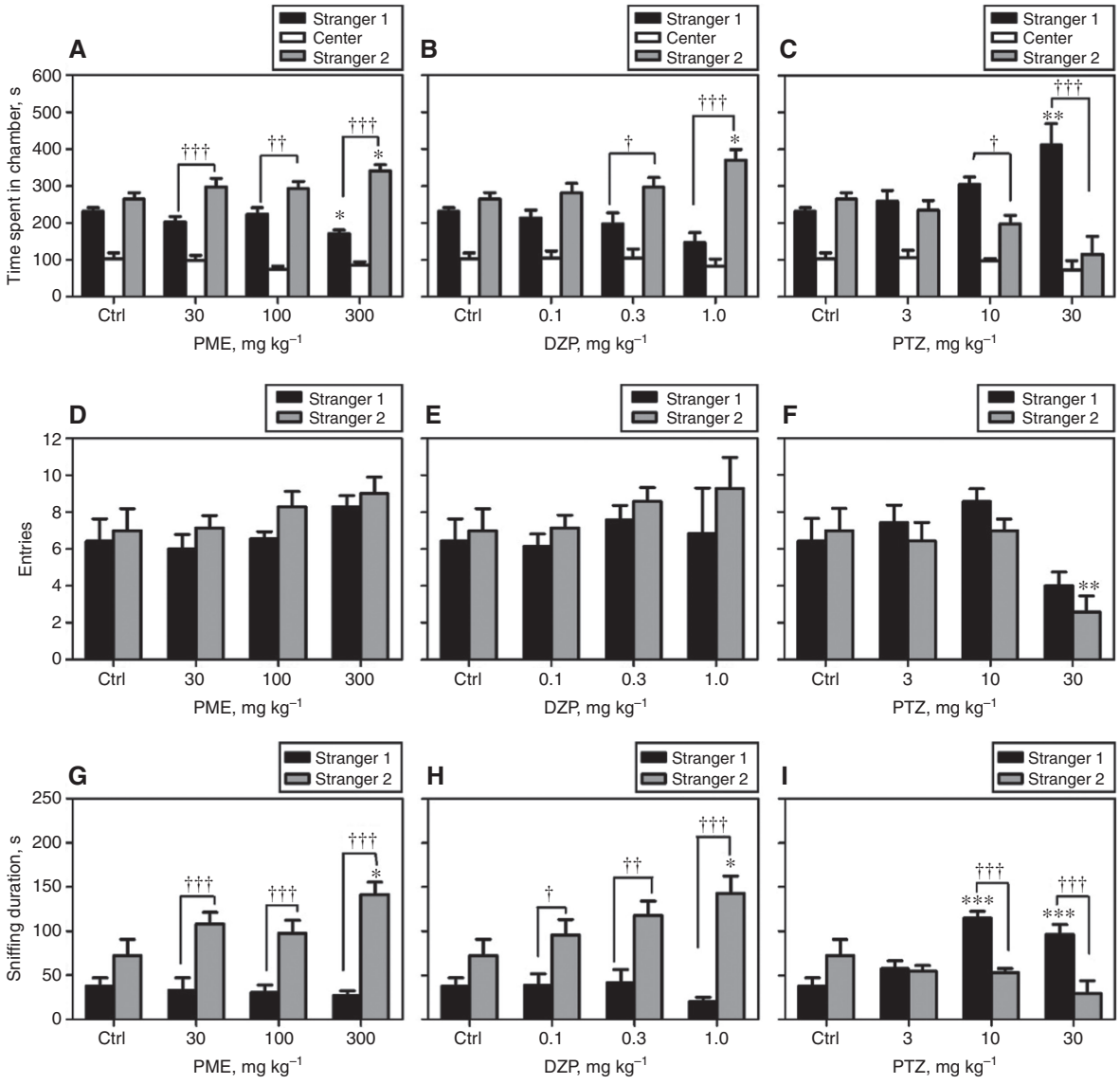

Figure 8: Effects of acute PME (30–300 mg kg^{-1}), DZP (0.1–1.0 mg kg^{-1}) and PTZ (3–30 mg kg^{-1}) treatment on the total time spent in the chambers for PME (A), DZP (B) and PTZ (C); entries into compartments for PME (D), DZP (E) and PTZ (F) and sniffing duration for PME (G), DZP (H) and PTZ (I) in the preference for social novelty test.

Data are presented as group mean±SEM. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001 by Newman-Keuls's post test and significant difference when stranger 1 was compared to stranger 2: †p<0.05, ††p<0.01, †††p<0.001 (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

the wire cage containing the stranger than the empty wire cage reflecting social approach behaviour. In the social preference test, mice treated with PME and DZP increased preference (increased time spent in chamber and sniffing) for the novel unfamiliar mouse placed in the formerly empty cage (stranger 2). This was interpreted as the ability of mice to discriminate between the two strangers and to recognise the one that had not been encountered before [23]. The increased sociability and preference for social novelty in PME and DZP treated mice is therefore indicative of an increased social interaction. Unlike PME and DZP, PTZ decreased sociability and social novelty preference in mice.

The number of entries between compartments provides an independent measure of general exploratory locomotion [46]. Mice treated with test compounds had no effect on number of entries; showing general exploratory activity did not appear to affect sociability and social novelty preference. An increase in social interaction, without a concomitant increase in motor activity, is indicative of an anxiolytic effect, whereas a specific decrease in social interaction indicates an anxiogenic effect [47].

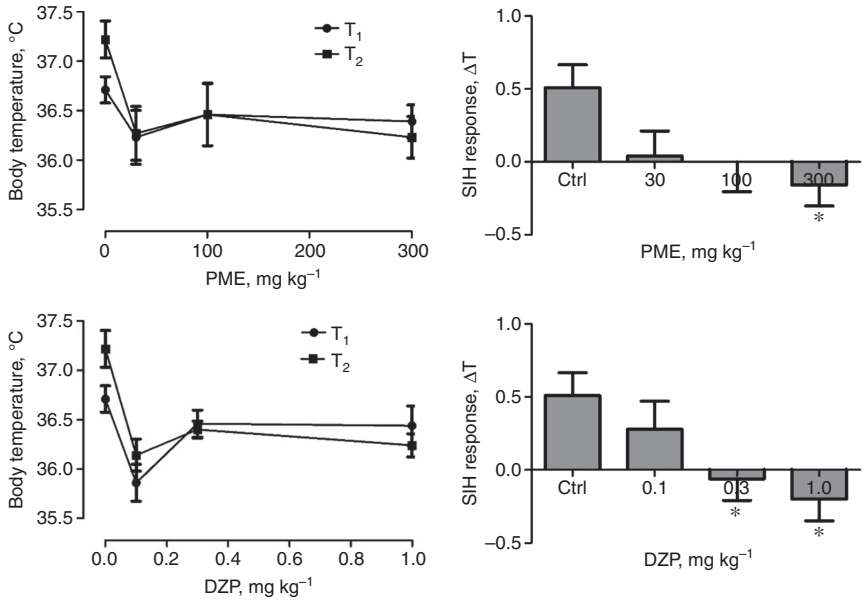

Figure 9: Effects of vehicle or various doses of PME and DZP (a standard anxiolytic drug) on SIH (ΔT), basal temperature (T_1) and T_2. Drugs or vehicle were given orally 60 min before the first rectal temperature measurement (T_1). T_2 was measured 10 min later. The difference between T_2 and T_1, ΔT, is indicated at each dose. Data are presented as group mean±SEM. Significantly different from control: *p<0.05 by Newman-Keuls's post hoc test.

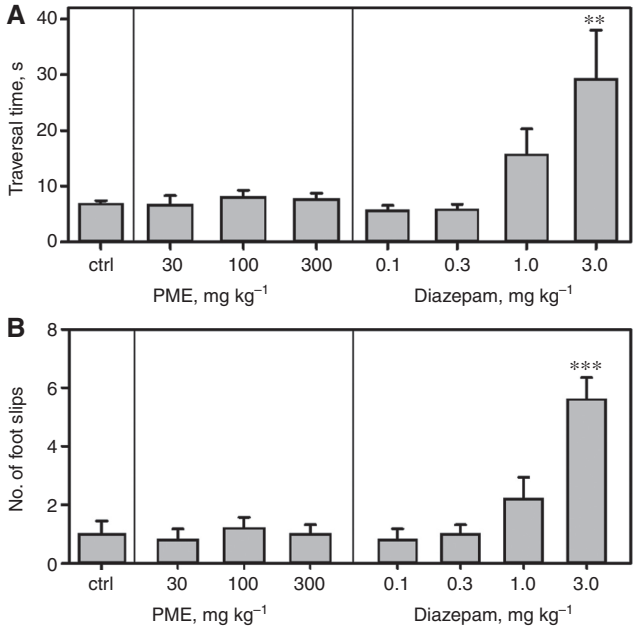

Figure 10: Effect of PME and DZP on motor co-ordination in the mouse beam walk test.
Data are expressed as group mean±SEM. **p<0.01, ***p<0.001 compared to control group (one-way ANOVA followed by Newman-Keuls's post hoc test).

Therefore, from the present study, like DZP [48], PME exhibited an anxiolytic-like effect, while PTZ showed an anxiogenic action [49] in the social interaction test.

SIH in singly housed male mice appears to be a robust, reproducible and easy paradigm to study putative anxiolytic-like effects of drugs [25, 50]. Reduction of SIH (ΔT) by a drug is interpreted as anti-stress or anxiolytic-like effect [25, 50–52]. An unchanged stress-induced hyperthermic response indicates absence of any effect on anxiety or stress, whereas an increased SIH could indicate an anxiogenic-like effect [51, 53]. Clinically effective anxiolytic compounds such as BDZs (including DZP, alprazolam, oxazepam and chlordiazepoxide) and 5-HT_{1A} receptor agonists (such as buspirone and flesinoxan) decrease the SIH, suggesting that GABAergic and serotoninergic mechanisms underlie SIH [50, 52, 53]. However, non-anxiolytic drugs including dopaminergic and noradrenergic compounds do not influence the SIH response [54]. From the present study, PME and DZP significantly antagonised stress-induced hyperthermic response indicative of an anti-stress or anxiolytic-like effect. This further confirms the fact that PME has anxiolytic-like effect and could be acting via GABAergic and serotoninergic mechanisms.

A deficit in motor coordination could affect performance in behavioural tests. Therefore, motor coordination and balance of mice was assessed in the beam walk test by measuring the ability of the mice to traverse a narrow beam to reach an enclosed safety platform or goal box [55, 56]. In this study, PME at the doses used did not impair motor coordination in the beam walk test

indicating an anxiolytic-like effect without motor impairment. However, DZP caused motor deficits in mice as it significantly increased the time to traverse the beam as well as number of foot slips.

The results of the present study indicate that PME possesses anxiolytic-like effects in mice.

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