

Full Length Research Paper

Anti-nociceptive, anxiolytic and anticonvulsant effects of an aqueous leaf extract of *Leea guineensis* G. Don (Family: Leeaceae)

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Leea guineensis G. Don (Family: Leeaceae) is an evergreen shrub or small tree used in Ghanaian traditional medicine to treat various ailments including epileptic fits and pain. However, little scientific evidence exists to support its use. The present study examined the anti-nociceptive, anti-anxiety and anticonvulsant effects of the aqueous leaf extract of *L. guineensis* (LGE) in murine models of pain (formalin test), anxiety (elevated plus-maze and light/dark box tests) and convulsion (pentylentetrazole-, picrotoxin- and maximal electroshock-induced seizures tests). LGE (30 to 300 mg/kg, *p.o.*) and the positive control morphine (3 to 10 mg/kg, *i.p.*) exerted profound dose-dependent anti-nociceptive activity in both phases of the formalin test. LGE (30 to 300 mg/kg, *p.o.*) also showed anxiolytic effects similar to diazepam (0.1 to 1.0 mg/kg, *i.p.*) but contrary to pentylentetrazole (3 to 30 mg/kg, *i.p.*). LGE increased the frequency and duration of open arm exploration while decreasing the protected forms of stretch-attend postures and head-dips in the elevated plus-maze. It also decreased the emergence latency of mice into the lit compartment of the light/dark box and increased the amount of time spent there. LGE (30 to 300 mg/kg, *p.o.*) also exhibited significant anticonvulsant activity by protecting mice against pentylentetrazole-, picrotoxin- and maximal electroshock-induced seizures. At all the doses used, LGE produced no motor deficits in mice. Together, the results suggest that the extract has anti-nociceptive, anxiolytic and anticonvulsant properties which support its traditional use.

Key words: *Leea guineensis*, seizures, maximal electroshock, formalin test, elevated plus-maze, light/dark box.

INTRODUCTION

Leea guineensis G. Don (Family: Leeaceae) is an evergreen shrub or small tree that grows to about 6 m high and is widespread in moister woodland and forest areas of tropical Africa. It is commonly known as *Agyaben*, *Okatanini* or *Okatakya* by the Akans in Ghana. The plant has been used traditionally for the treatment of various ailments in the West African sub-region. The leaves are used to treat toothache, rheumatism, skin ulcer, vertigo, epileptic fits and paralysis (Burkill, 1985; Irvine, 1961; Mshana et al., 2000). The roots and seeds are also used as a purgative (Irvine, 1961).

Not much pharmacological studies have been done on

the plant. Falodun et al. (2007) have shown that the aqueous leaf extract has anti-inflammatory activity in the carrageenan-induced rat paw oedema test model. Phytochemical evaluation of the woods and leaves revealed volatile constituents including terpenoids (Op de Beck et al., 2000) and hydrophilic flavonoids namely quercetin-3'-sulphate-3-O- α -L-rhamnopyranoside, quercetin-3,3'-disulphate and quercetin-3,3',4'-trisulphate, together with kaempferol, quercetin, quercitrin, mearnsitrin, gallic acid and ethyl gallate (Op de Beck et al., 2003). These flavonoids were found to have free radical scavenging activity when evaluated in the DPPH assay (Op de Beck et al., 2003). Our search has revealed that there are no scientific reports available in established literature to support the effectiveness of the plant in the treatment of pain and epilepsy. In this study, we investigated the anti-nociceptive, anxiolytic and anticon-

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vulsant effects of the aqueous leaf extract of the *L. guineensis* in mice.

MATERIALS AND METHODS

Plant collection and extraction

Fresh leaves of *L. guineensis* were collected from the Botanic Gardens of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, in the month of September, 2007 and authenticated by Dr. Kofi Annan of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, KNUST, Kumasi, Ghana. A voucher specimen of the plant (LEEACEAE/FP/08/26) has been deposited at the Faculty's Herbarium. The fresh leaves of *L. guineensis* were blended and then macerated with distilled water at room temperature for 24 h. The mixture was filtered and the extract obtained by concentrating the filtrate to dryness using a freeze-dryer. The dried aqueous extract, herein referred to as LGE or the extract, was then kept in a refrigerator (4°C). The yield was 12%.

Animals

Cancer Research (ICR) mice (267 males, 84 females; 30±5 g) were used. The animals were purchased from the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon and housed in the animal house of the Department of Pharmacology, KNUST, Kumasi. Animals were housed in groups of five in stainless steel cages (34 · 47 · 18 cm) with soft wood shavings as bedding, fed with normal pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions (temperature 25±2°C, relative humidity 60 to 70%, and 12 h light-dark cycle). All behavioural experiments were carried out under dim light. To acclimatize the animals to the test conditions, they were brought to the laboratory a week before the experiments. All procedures and techniques used in these studies were in accordance with accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC) and were approved by the local Departmental Ethics Committee.

Drugs and chemicals

The following drugs and chemicals were used: Formalin, theophylline (BDH, Poole, England); naloxone, pentylentetrazole (PTZ), picrotoxin (Sigma-Aldrich Inc., St. Louis, MO., USA); diazepam (INTAS, Gujarat, India); carbamazepine (Tegretol®, Novartis, Basel, Switzerland) and morphine (Phyto-Riker, Accra, Ghana). Doses of drugs were selected based on data from literature and preliminary experiments in our laboratory.

Phytochemical analysis

The freshly prepared aqueous extract was subjected to preliminary phytochemical analysis using methods described by Trease and Evans (1989) to determine the presence of alkaloids, saponins, cardiac glycosides, flavonoids and tannins.

The formalin test

The anti-nociceptive effect of the extract was evaluated using the formalin test. The test was carried out as described previously

(Hunskar and Hole, 1987; Malmberg and Yaksh, 1992) with few modifications. Male mice were randomly divided into seven groups (n=5) and each animal acclimatized to the testing environment (Perspex chamber, 15-15-15 cm) for 30 min. Mice were pre-treated with LGE (30, 100 and 300 mg/kg, *p.o.*), morphine (1, 3 and 10 mg/kg, *i.p.*) or vehicle (10 ml/kg, *p.o.*) 30 min (*i.p.*) or 1 h (*p.o.*) before the induction of nociceptive behaviours in the animals by intraplantar injection of 10 μ l of 5% formalin. Animals were immediately returned individually into the testing chamber. A mirror angled at 45° beneath the floor of the chamber allowed an unobstructed view of the paws. The behaviour of the animals was then captured (1 h) for analysis by a camcorder (Everio™ model GZ-MG1300, JVC, Tokyo, Japan) placed directly opposite to the mirror and attached to a computer. Pain responses were scored for 1 h, starting immediately after formalin injection. A nociceptive score was determined for each 5-min time block by measuring the amount of time spent biting/licking of the injected paw (Hayashida et al., 2003). The scoring of pain responses was done with the aid of the public domain software JWatcher™ Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia available at <http://www.jwatcher.ucla.edu/>). Average nociceptive score for each time block was calculated by multiplying the frequency and time spent in biting/licking. Data were expressed as the mean ± S.E.M. scores between 0 to 10 and 10 to 60 min after formalin injection.

The elevated plus-maze test

This test was carried out as described previously (Lister, 1987; Pellow et al., 1985) with modifications. The apparatus was made of Plexiglas and consisted of two open arms (30-5-0.5 cm) and two enclosed arms (30-5-15 cm). The arms extended from a central platform (5-5 cm) forming a plus-sign with like arms opposite each other. The maze was elevated 60 cm from the floor, and placed in a lit room (~750 lux).

Male mice were randomly assigned to ten groups (n = 6): vehicle-control (distilled water, 10 ml/kg, *p.o.*), LGE (30 to 300 mg/kg, *p.o.*), diazepam (0.1 to 1.0 mg/kg, *i.p.*) and pentylentetrazole (3 to 30 mg/kg, *i.p.*). Diazepam and pentylentetrazole served as reference anxiolytic and anxiogenic drugs, respectively. Thirty minutes after *i.p.* injection with diazepam and pentylentetrazole and 1 h after oral treatment with the extract and vehicle, mice were placed individually in succession in the central platform of the maze for 5 min and their behaviour recorded with a camcorder (Everio™, model GZ-MG1300, JVC, Tokyo) placed 100 cm above the floor. The digitized video of each 5 min trial were later scored using JWatcher™ Version 1.0 for behavioural parameters including: (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 ledge 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.

To compute total distances travelled by the mice, the software Behavior Collect (http://cas.bellarmine.edu/tietjen/Downloads/computer_programs_f_or_data_colle.htm) was used to obtain raw XY data from the videos. These data were then exported into Microsoft® Office Excel 2007 and further analyzed. Distance between two X-Y coordinate pairs was calculated from the formula:

$$\sqrt{[(X_1 - X_2)^2 + (Y_1 - Y_2)^2]}$$

The light/dark box test

The test was carried out as described earlier (Ardayfio and Kim, 2006) but with modifications. The apparatus was a wooden box (45 × 30 × 30 cm) divided into two compartments by a wooden board with a small opening (7.7 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 floor. The smaller compartment was painted black and had a cover that was closed during testing.

Male mice were divided into ten groups (n=6) and received treatments similar to that described previously for the elevated plus-maze test. One hour (*p.o.*) or 30 min (*i.p.*) after drug administration, each mouse was placed in the dark compartment (head facing a corner) and the compartment covered. The sessions were videotaped and later scored to determine the latency to emerge into the lit compartment and the percentage of time spent in the lit compartment.

Pentylentetrazole-induced seizure test

The test was carried out similar to that described by Swinyard and Kupferberg (1985). Female mice were divided into seven groups (n=6) and received the extract (30 to 300 mg/kg, *p.o.*), diazepam (0.1 to 1.0 mg/kg, *i.p.*) or vehicle (distilled water, 10 ml/kg, *i.p.*). Thirty minutes (*i.p.*) or 1 h (*p.o.*) after the treatments, mice were injected with pentylentetrazole (85 mg/kg) subcutaneously. The animals were placed individually in an observation chamber and videotaped for 30 min. The videos were later scored with JWatcher™ Version 1.0 to determine the latency to first myoclonic jerks, the latency to tonic convulsions and the frequency and duration of tonic convulsions for each mouse.

Picrotoxin-induced seizure test

The procedure used was the same as the one described previously for pentylentetrazole-induced seizures except that instead of pentylentetrazole, picrotoxin (10 mg/kg, *i.p.*) was administered to mice (Mackenzie et al., 2002; Ngo Bum et al., 2004; Swinyard, 1969). Latency to myoclonic jerks, latency to tonic convulsions as well as the frequency and duration of tonic convulsions were recorded from the videos as in the pentylentetrazole seizure test.

Maximal electroshock seizure test (MEST)

The test was performed as described by Schmutz et al. (1990) with some modifications. Male mice were divided into seven groups (n=10) and received LGE (30 to 300 mg/kg, *p.o.*), vehicle (10 ml/kg, *p.o.*) or carbamazepine (3 to 30 mg/kg, *p.o.*). One hour after the treatments, tonic convulsions of the hind extremities of mice were induced by passing alternating current (50 Hz, 60 mA, 0.2 s) from an electroconvulsive therapy (ECT) apparatus (Model 7800, Ugo Basile, Camerio, Italy) via ear electrodes. The current used was predetermined before experimentation and was the maximal current that caused hind limb extension in all mice in the trials. Mice were restrained by gripping the loose skin on their back. The number of animals protected from tonic hind limb extension seizure and the time spent in this position were determined in each group.

Effect on motor coordination - rotarod test

The effect on motor co-ordination was assessed using rotarod apparatus (Model 7600, Ugo Basile, Cormerio, Italy) rotating at a speed of 25 rpm. This apparatus consists of a base platform and a rotating rod of 3 cm diameter with a non-skid surface. The rod, 50 cm in length, is divided into five equal sections by six disks. Five mice were tested simultaneously.

Male mice were initially selected for their ability to remain on the rotarod for at least two consecutive 60 s trials before the test day. Mice were randomly divided into seven groups (n=6) and received LGE (30 to 300 mg/kg, *p.o.*), diazepam (0.1 to 1.0 mg/kg, *i.p.*) or vehicle (10 mg/kg, *i.p.*). Thirty minutes (*i.p.*) or 1 h (*p.o.*) after the treatments, the latencies to fall from the rod were measured. Mice that stayed on the rotarod for more than 60 s were given the maximum score, 60 s.

Statistical analysis

Data are presented as mean±SEM. Data were analyzed using one-way analysis of variance (ANOVA) with drug treatment as a between-subjects factor. Whenever ANOVA was significant, further comparisons between vehicle- and drug- treated groups were performed using the Newman-Keuls test. GraphPad Prism for Window 5 (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis and *P*<0.05 was considered statistically significant.

RESULTS

Phytochemical analysis

Preliminary phytochemical analysis of LGE showed the presence of alkaloids, cardiac glycosides and flavonoids (data not shown). Tannins were not detected in the extract.

The formalin test

Intraplantar injection of formalin in mice caused a typical pattern of biting and licking behaviour. The first phase started immediately after injection of formalin and diminished gradually in about 10 min. The second phase started at about 15 min, peaking at about 25 min and lasted until 1 h. Administration of LGE (30 to 300 mg/kg, *p.o.*) produced significant and dose-dependent inhibition of formalin-induced nociception in both first ($F_{3,16}=17.18$, $P<0.0001$; Figures 1a and b) and second ($F_{3,16}=40.17$, $P<0.0001$, Figures 1a and b) phases. Morphine also produced marked inhibition of the nociceptive score in both the first ($F_{3,16}=33.24$, $P<0.0001$, Figures 1c and d) and the second ($F_{3,16}=40.42$, $P<0.0001$, Figures 1c and d) phases of the formalin test. Potency of the extract in both phases was comparable (ED_{50} values: 16.37 ± 4.57 and 14.06 ± 4.39 mg/kg, respectively; Table 1). LGE was however less potent than morphine (ED_{50} : 0.09 ± 0.03 and 0.11 ± 0.04 mg/kg respectively; Table 1) in both phases.

The elevated plus-maze test

Figures 2 and 3 show the effects of LGE, diazepam and

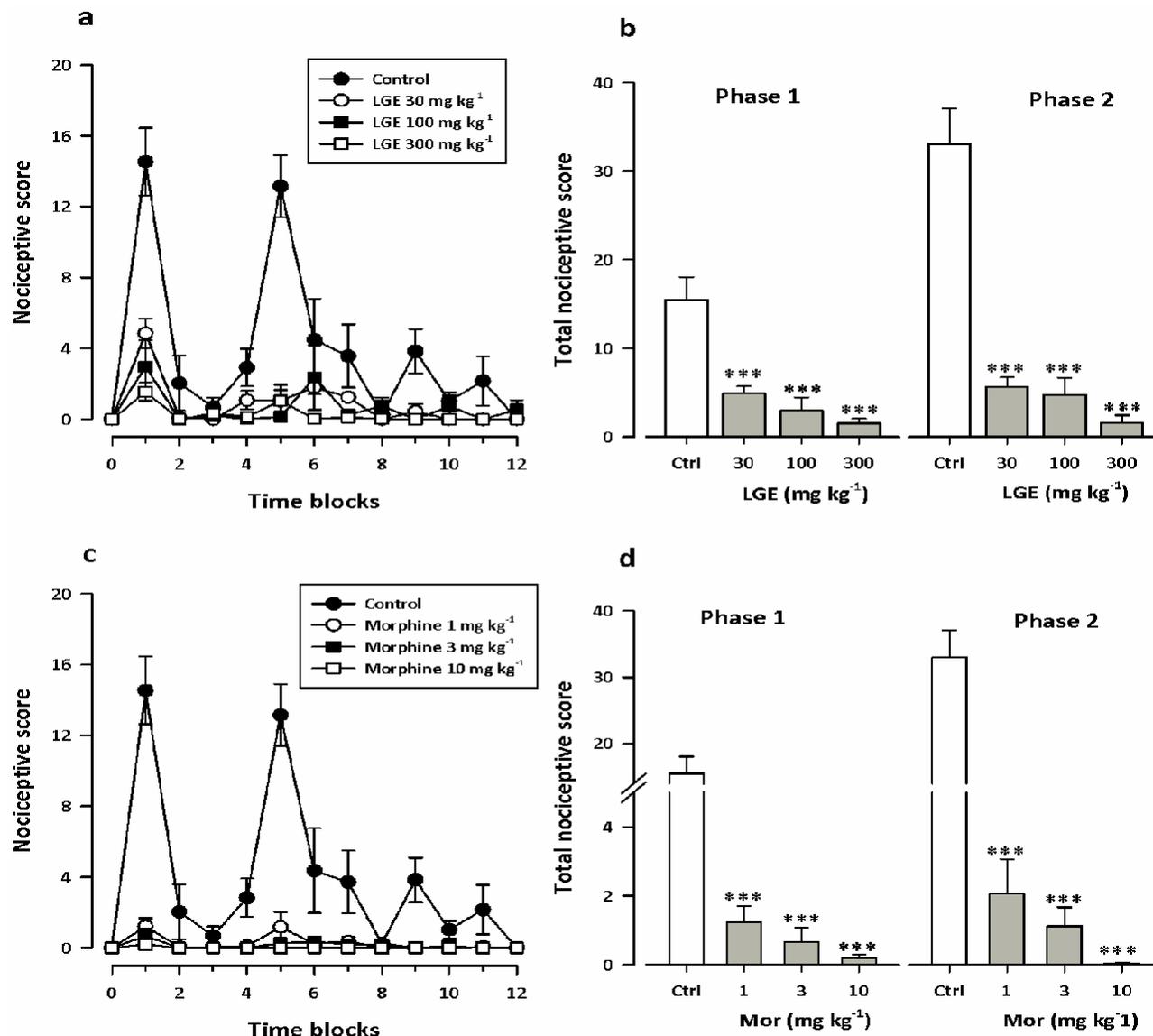


Figure 1. Effect of LGE (30 to 300 mg/kg) and morphine (1 to 10 mg/kg) on the time course curves (a, c) and the total nociceptive score (AUCs) (b, d) of formalin test in mice. Nociceptive scores are shown in 5 min time blocks up to 60 min for the time course curves. Data are presented as mean ± SEM (n=5). ****P*<0.001 compared to vehicle-treated control group (one-way ANOVA followed by Newman-Keuls *post hoc* test).

Table 1. ED₅₀ values of LGE and morphine in the formalin test.

Drug	ED ₅₀ (mg/kg)	
	Phase 1	Phase 2
LGE	16.37±4.57	14.06±4.39
Morphine	0.09±0.03	0.11±0.04

PTZ on conventional elevated plus-maze parameters. Oral administration of LGE (30 to 300 mg/kg) to mice increased the number of open arm entries ($F_{3,16}=5.21$, $P=0.0106$, Figure 2a), % open arm entries ($F_{3,16}=7.65$, $P=0.0022$, Figure 2d) and % time spent in open arms ($F_{3,16}=4.18$,

$P=0.0230$, Figure 2g). The increase was dose-dependent and statistically significant ($P<0.05$) at 100 and 300 mg/kg for the number of open arm entries and at only 300 mg/kg for the open arm time. In comparison, treatment of mice with the anxiolytic drug, diazepam (0.1 to 1.0 mg/kg, i.p.)

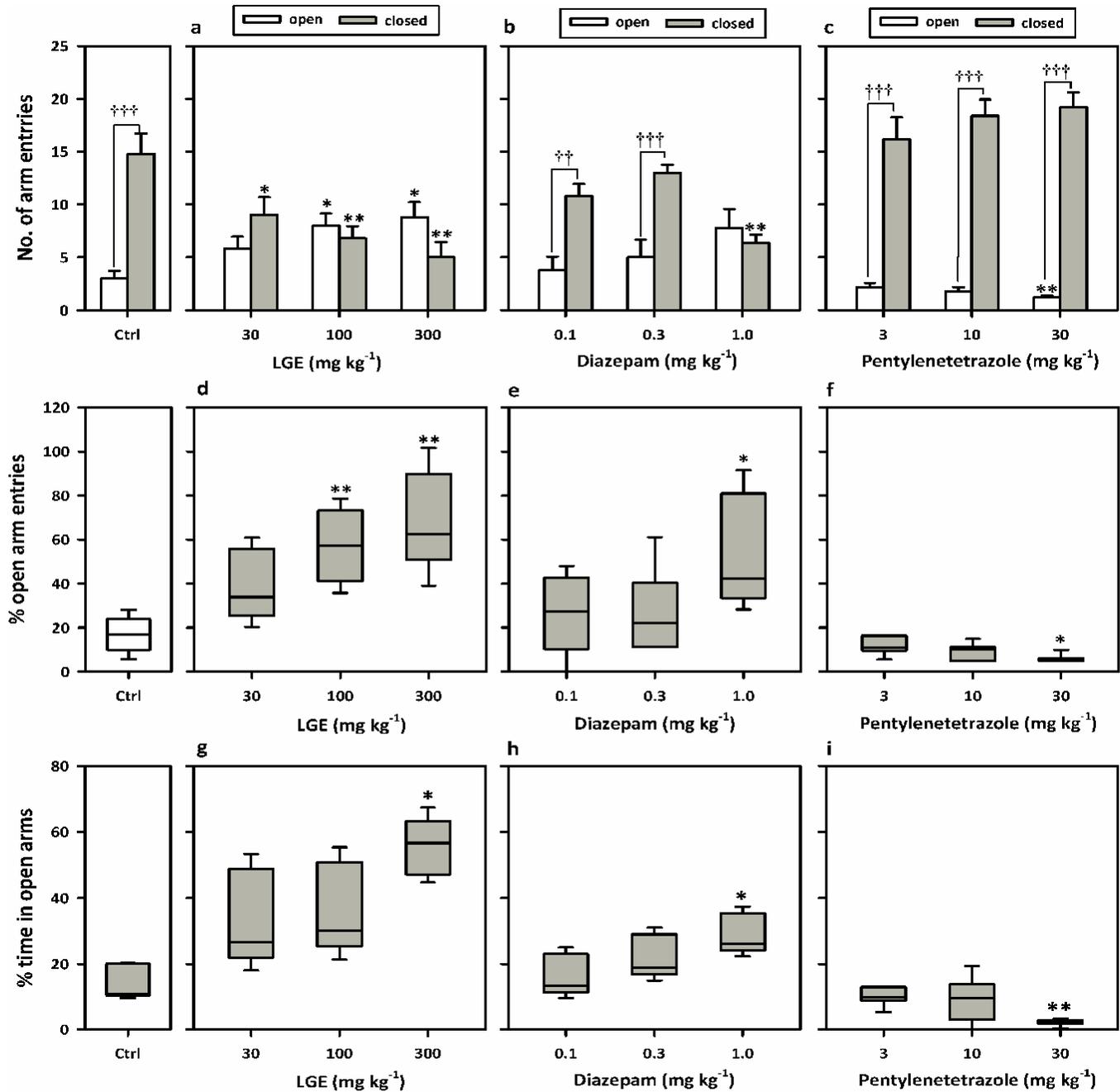


Figure 2. Effect of LGE (30 to 300 mg/kg, *p.o.*), diazepam (0.1 to 1.0 mg/kg, *i.p.*) and pentylenetetrazole (3 to 30 mg/kg, *i.p.*) on open arm entries (a, b, c), percentage open arm entries (d, e, f) and percentage time spent in open arms (g, h, i) of the EPM over a 5 min test period in mice. Data are presented as mean±SEM ($n=6$). The lower and upper margins of the boxes (d-i) 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. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (one-way ANOVA followed by Newman-Keuls test). † $P<0.05$, †† $P<0.01$, ††† $P<0.001$ (two-way ANOVA followed by Bonferroni's *post hoc*). Ctrl, Control.

caused a dose-dependent increase in percentage open arm entries ($F_{3,16}=3.21$, $P<0.05$, Figure 2e) and the percentage of open arm time ($F_{3,16}=5.13$, $P=0.0113$, Figure 2h). The drug also increased the number of open arm entries but not statistically significant on one-way ANOVA ($F_{3,16}=2.19$, $P=0.1296$, Figure 2b). The effect of

pentylenetetrazole (3 to 30 mg/kg, *i.p.*) was contrary to that of LGE and diazepam. Pentylenetetrazole caused significant decrease in the percentage of open arm entries ($F_{3,16}=3.60$, $P=0.0367$, Figure 2c) and the percentage of time spent in the open arms ($F_{3,16}=5.34$, $P=0.0097$, Figure 2f) with statistical significance at the dose of 30 mg/kg

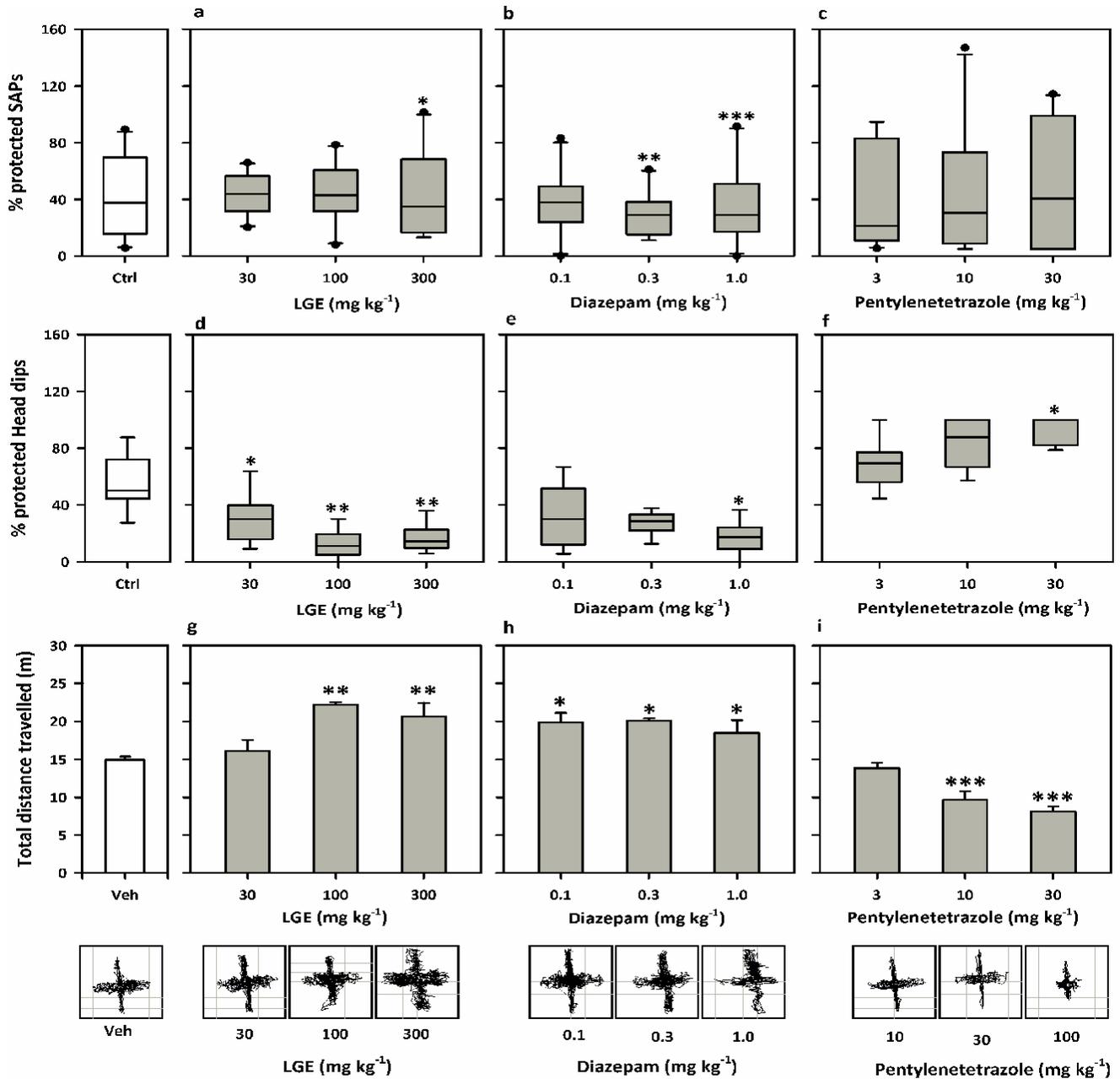


Figure 3. Effect of LGE (30 to 300 mg/kg, *p.o.*), diazepam (0.1 to 1.0 mg/kg, *i.p.*) and pentylenetetrazole (3 to 30 mg/kg, *i.p.*) on percentage protected stretch-attend postures (a-c), percentage protected head-dips (d-f) and total distance travelled (g-i) in the EPM over a 5 min test period in mice. Data are presented as mean±SEM (n=6). The lower and upper margins of the boxes (a-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. **P*<0.05, ***P*<0.01, ****P*<0.001 compared to vehicle-treated control group (one-way ANOVA followed by Newman-Keuls test).

(*P*<0.05). Pentylenetetrazole also decreased the number of open arm entries but was not statistically significant at doses used.

As regards the ethological measures of anxiety, LGE caused significant decrease in the percentage protected stretch-attend postures ($F_{3,16}=5.78$, $P=0.0071$, Figure 3a) as well the percentage protected head-dips ($F_{3,16}=6.501$,

$P=0.0044$, Figure 3d). Diazepam showed anxiolytic effects similar to the extract. It decreased the percentage protected stretch-attend postures ($F_{3,16}=10.63$, $P=0.0004$, Figure 3b) as well the percentage protected head-dips ($F_{3,16}=4.046$, $P=0.0256$, Figure 3e). On the other hand, pentylenetetrazole increased percentage protected stretch-attend postures ($F_{3,16}=0.7582$, $P=0.5338$, Figure 3c) and

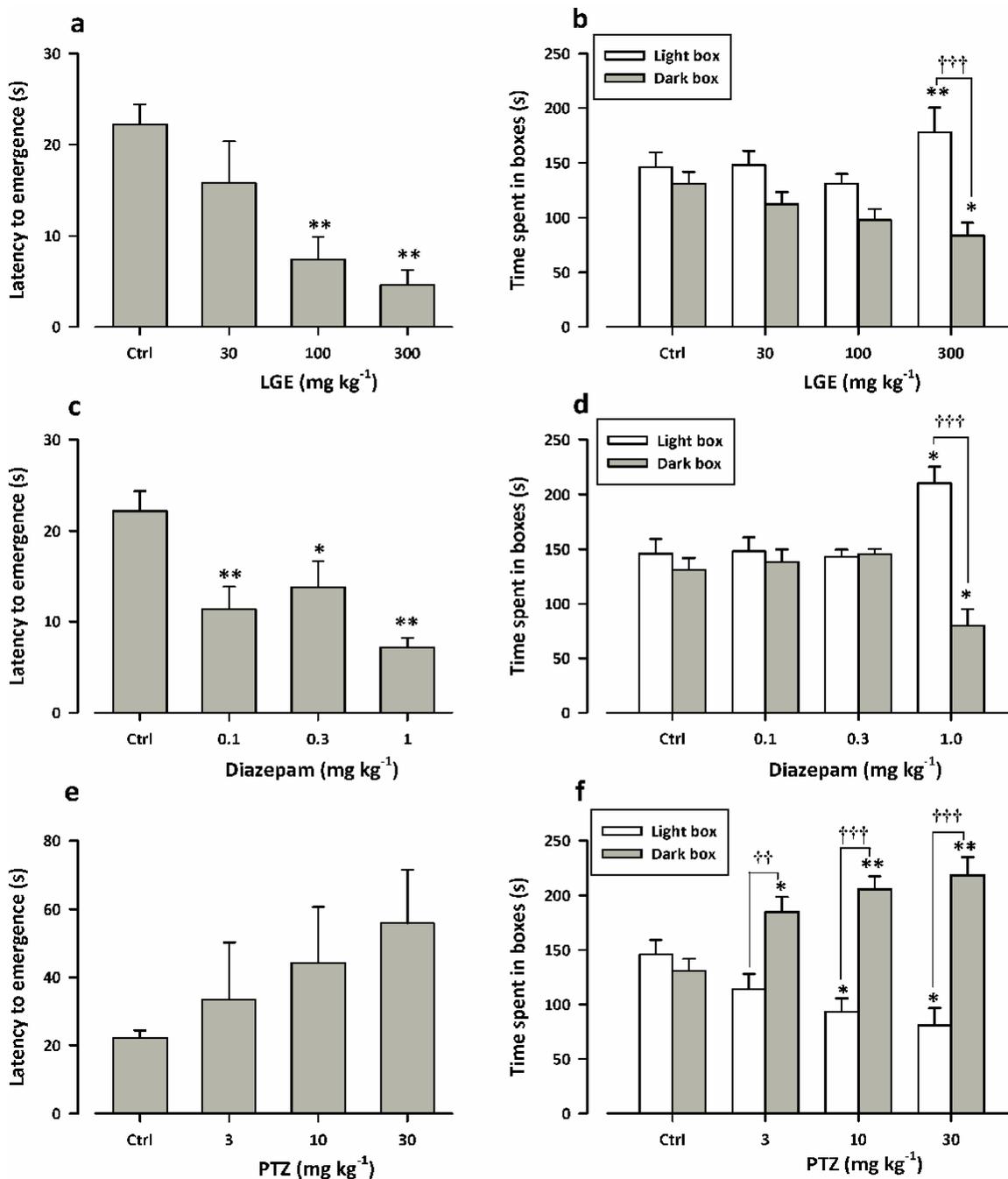


Figure 4. Effect of LGE (30 to 300 mg/kg, *p.o.*), diazepam (0.1 to 1.0 mg/kg, *i.p.*) and pentylenetetrazole (PTZ; 3 to 30 mg/kg, *i.p.*) on the latency to emerge into lit compartment (a, c, e) and time spent in compartments (b, d, f) of the light-dark box over a 5 min test period in the mice. Each bar represents mean \pm S.E.M. ($n=6$). * $P<0.05$, ** $P<0.01$ compared to control group (one-way ANOVA followed by Newman-Keuls test). †† $P<0.01$, ††† $P<0.001$ (two-way ANOVA followed by Bonferroni's *post hoc* test).

percentage protected head dips ($F_{3,16}=3.643$, $P=0.0356$, Figure 3f).

LGE and diazepam significantly increased ($F_{3,16}=8.883$, $P=0.0011$, Figure 3g and $F_{3,16}=4.828$, $P=0.0140$, Figure 3h) the total distance travelled by mice in the EPM while PTZ decreased ($F_{3,16}=17.66$, $P<0.0001$; Figure 3i) it.

The light/dark box test

The extract significantly and dose-dependently decreased the latency to emerge into lit compartment of the light/dark box ($F_{3,16}=7.50$, $P=0.0024$, Figure 4a). Newman-Keuls test showed that only 100 - 300 mg/kg caused significant effect

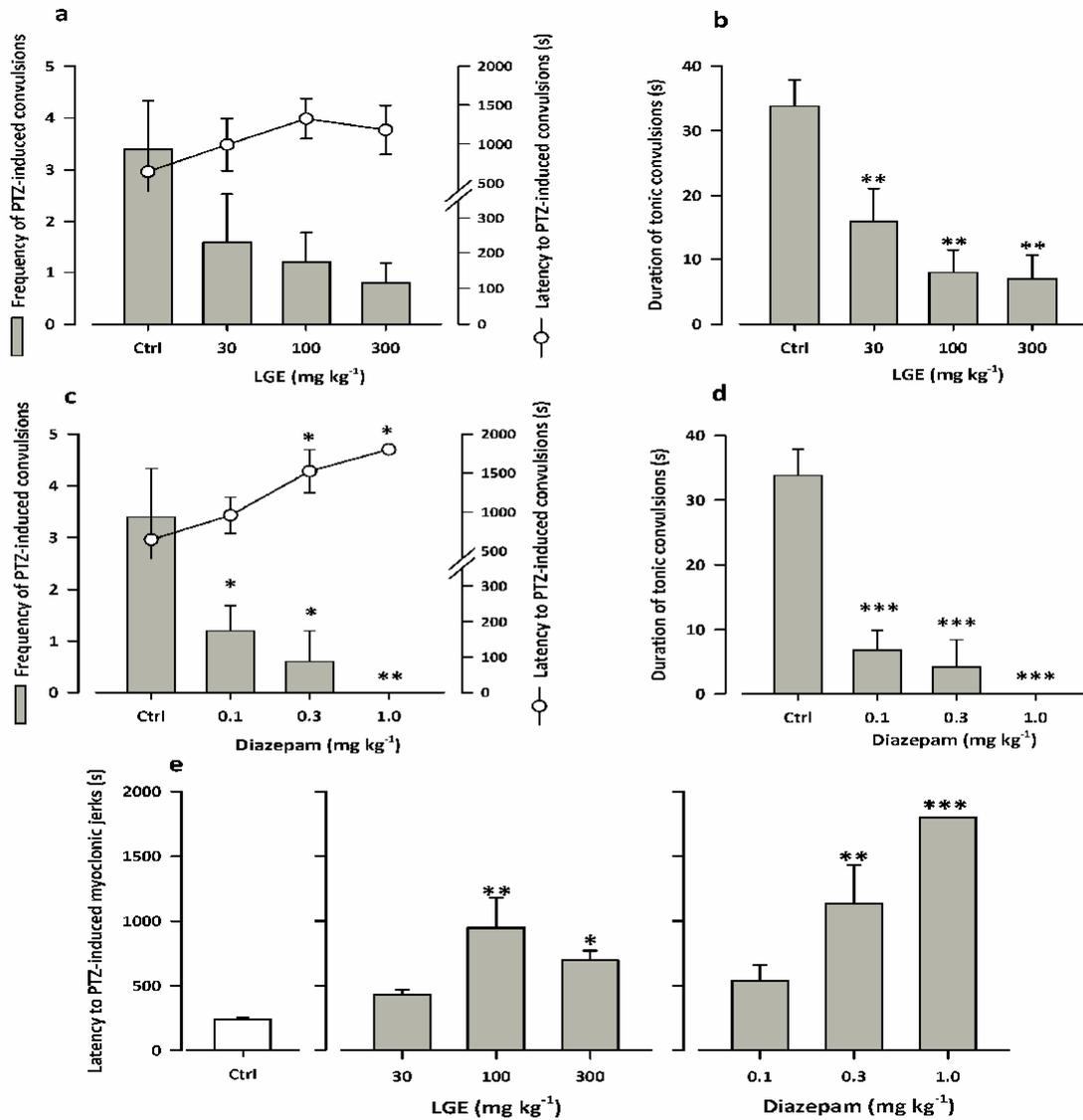


Figure 5. Effect of LGE (30 to 300 mg/kg, *p.o.*) and diazepam (0.1 to 1.0 mg/kg, *i.p.*) on frequency (a, c), latency to (a, c) and duration (b, d) of PTZ-induced tonic convulsions as well as latency to PTZ-induced myoclonic jerks (e) in mice. Each point or column represents the mean ± S.E.M. (n=6). **P*<0.05, ***P*<0.01, ****P*<0.001 compared to control group (one-way ANOVA followed by Newman-Keuls test).

(both *P*<0.01). Diazepam caused similar effect by significantly decreasing the emergence latency of mice ($F_{3,16}=7.95$, *P*=0.0018, Figure 4c). On the contrary, pentylenetetrazole increased the emergence latency although it was not statistically significant ($F_{3,16}=1.03$, *P*=0.4059, Figure 4e). LGE profoundly increased the amount of time spent in the light box ($F_{3,16}=4.99$, *P*=0.0124, Figure 4b) while it decreased the time spent in the dark box ($F_{3,16}=3.49$, *P*=0.0403, Figure 4b). The effect was dose-dependent and reached statistical significance at only 300 mg/kg. Like LGE, diazepam increased the proportion of time spent in the light box ($F_{3,16}=4.825$, *P*=0.0199, Figure 4d) and decreased the time spent in the dark box ($F_{3,16}=5.291$, *P*=0.0148, Figure 4d) both reaching

statistical significant at the dose of 1 mg/kg (*P*<0.05). On the other hand, pentylenetetrazole caused significant decrease in the amount of time spent in the light box ($F_{3,16}=4.12$, *P*=0.0241, Figure 4f) while increasing the time spent in the dark box ($F_{3,16}=8.38$, *P*=0.0014, Figure 4f).

Effect of extract on pentylenetetrazole-induced seizures

The extract showed significant anticonvulsant activity against PTZ-induced seizures. It significantly and dose-dependently delayed the onset to myoclonic jerks ($F_{3,16}=6.29$, *P*=0.0051, Figure 5e) and caused significant

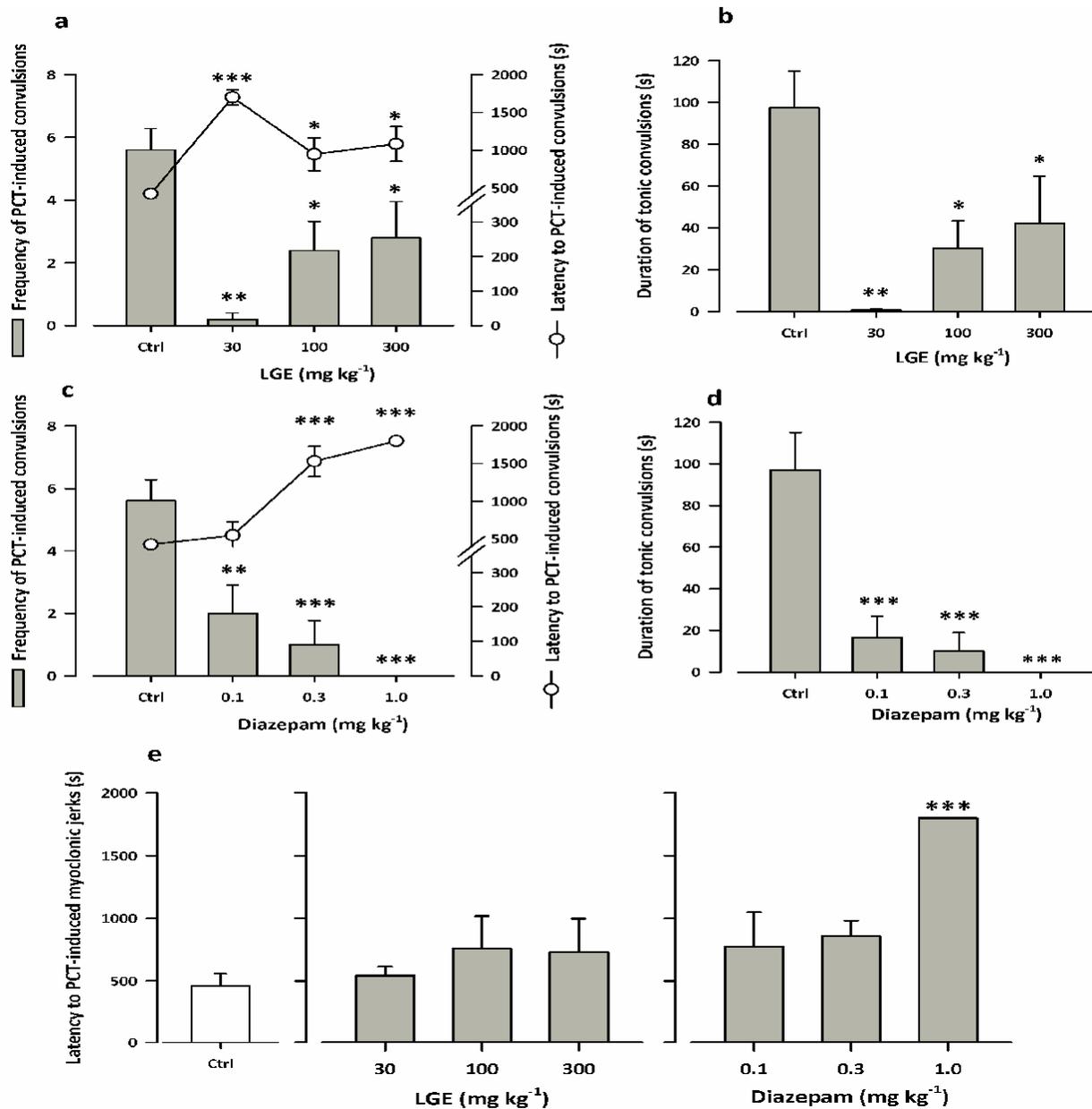


Figure 6. Effect of LGE (30 to 300 mg/kg, *p.o.*) and diazepam (0.1 to 1.0 mg/kg, *i.p.*) on frequency (a, c), latency to (a, c) and duration (b, d) of picrotoxin-induced tonic convulsions as well as latency to picrotoxin-induced myoclonic jerks (e) in mice. Each point or column represents the mean \pm S.E.M. ($n=6$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to control group (one-way ANOVA followed by Newman-Keuls test).

reduction of the duration of tonic convulsions ($F_{3,16}=9.20$, $P=0.0009$, Figure 5b). The extract also delayed the onset of tonic convulsions and decreased the frequency of tonic convulsions but these were not statistically significant ($F_{3,16}=0.95$, $P=0.4385$ and $F_{3,16}=2.39$, $P=0.1065$, respectively, Figure 5a). Diazepam which was used as a reference anticonvulsant caused similar effects. The drug significantly delayed the onset of myoclonic jerks and tonic convulsions ($F_{3,16}=18.18$, $P<0.0001$, Figure 5e and $F_{3,16}=5.10$, $P=0.0115$, Figure 5c, respectively) and significantly

reduced the frequency and duration of tonic convulsions ($F_{3,16}=6.03$, $P=0.006$ and $F_{3,16}=21.34$, $P<0.0001$, respectively; Figures 5c and d).

Effect of extract on picrotoxin-induced seizures

The extract showed profound anticonvulsant effect against picrotoxin-induced seizures by significantly delaying the onset of tonic convulsions ($F_{3,16}=9.72$, $P=0.0007$, Figure 6a) and significantly reducing the frequency and duration

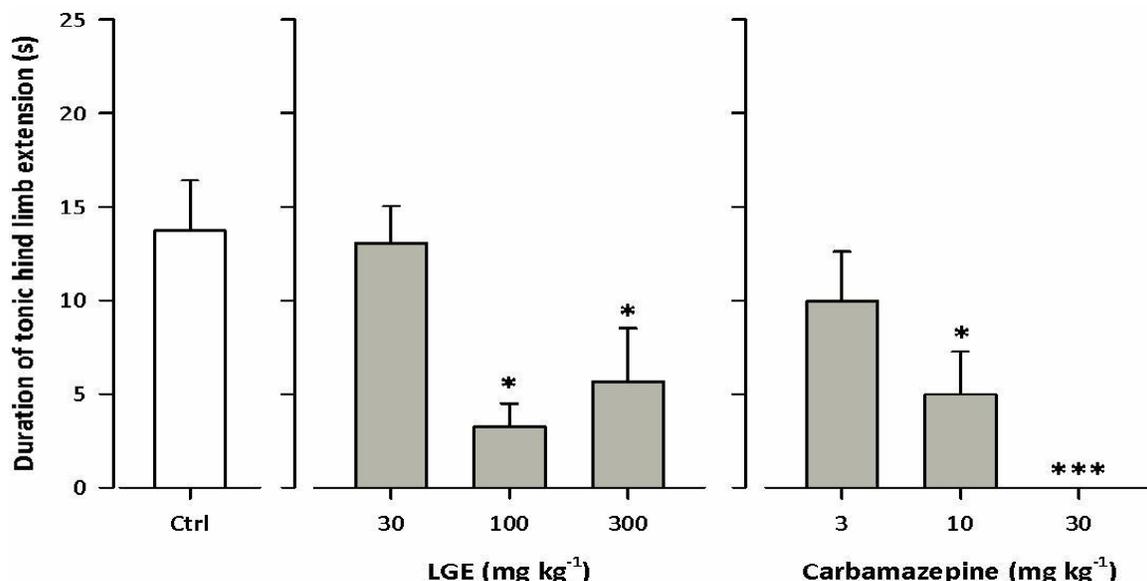


Figure 7. Effect of LGE (30 to 300 mg/kg, *p.o.*) and carbamazepine (3 to 30 mg/kg, *p.o.*) on the duration of MES-induced tonic hind limb extension. Each column represents Mean ± S.E.M. (n=10). **P*<0.05, ***P*<0.01, ****P*<0.001 (one-way ANOVA followed by Newman-Keuls test).

Table 2. Effect of LGE and diazepam on mice on the rotarod.

Drug	Dose (mg/kg)	Time spent on rod (s)
Vehicle	-	47.60±7.99
	30	54.84±3.39
	100	48.47±8.50
LGE	300	39.84±7.77
	0.1	46.93±8.34
	0.3	46.90±8.33
Diazepam	1.0	15.38±7.81*

Data are expressed as mean ± SEM (n=6). **P* < 0.05 compared to vehicle-treated control group (one-way ANOVA followed by Newman-Keuls test).

of tonic convulsions ($F_{3,16} = 7.28$, $P=0.0027$ and $F_{3,16}=6.60$, $P=0.0041$, respectively; Figures 6a and b). Onset of myoclonic jerks were delayed by the extract but this was not statistically significant ($F_{3,16} = 0.53$, $P=0.6682$, Figure 6e). The effects of diazepam on picrotoxin-induced seizures were similar to that of the extract. It significantly delayed the onset of both myoclonic jerks and tonic convulsions ($F_{3,16} = 13.45$, $P=0.0001$, Figure 6e and $F_{3,16} = 25.02$, $P<0.0001$, Figure 6c, respectively) and reduced the frequency and duration of convulsions ($F_{3,16} = 12.81$, $P=0.001$ and $F_{3,16} = 15.96$, $P<0.0001$ respectively, Figures 6c and d).

Effect of extract on maximal electroshock seizures

The extract caused significant reduction of the duration of

maximal electroshock-induced tonic hind limb extension ($F_{3,35}=5.08$, $P=0.0050$, Figure 7) but did not prevent the occurrence of this event at the doses used. Carbamazepine which was used as a reference drug also produced significant reduction of the maximal electroshock-induced tonic hind limb extension ($F_{3,36}=7.35$, $P=0.0006$, Figure 7) and completely prevented its occurrence at 30 mg/kg.

Effect of extract on motor coordination - the rotarod test

At all the doses tested, the extract had no significant effect on the time taken by mice to fall off the rotarod ($F_{3,20}=0.7262$, $P=0.5483$, Table 2). Similar results were observed with diazepam except 1.0 mg/kg which caused

significant decrease in the latency to fall off the rotating rod ($P < 0.05$; Table 2).

DISCUSSION

The present study demonstrated that oral administration of an aqueous leaf extract of *L. guineensis* exerts anti-nociceptive, anxiolytic and anticonvulsant effects in mice.

The anti-nociceptive effect of LGE was evaluated in the formalin test which is a reliable and valid animal model for nociception. The formalin test produces a distinct biphasic nociceptive response. An early phase (neurogenic pain), occurring within seconds of formalin injection, is elicited by direct chemical activation of nociceptive primary afferent fibres. A second, later phase (inflammatory pain), occurs as a result of ongoing activity in primary afferents and increased sensitivity of dorsal horn neurons (Hunskar and Hole, 1987; Munro, 2009; Tjolsen et al., 1992). Since different analgesics act differently in the early and late phases, the formalin test can be used to clarify the possible mechanism of an anti-nociceptive effect of a proposed analgesic (Tjolsen et al., 1992). Centrally acting drugs, such as opioids, inhibit both phases equally (Shibata et al., 1989); however, many NSAIDs and corticosteroids inhibit only the late phase (Hunskar and Hole, 1987). In this study, LGE inhibited both phases of the formalin test. This implies that LGE is effective against both neurogenic and inflammatory pain. It also suggests that LGE may be centrally acting. The inhibitory effect in the second phase also confirms the anti-inflammatory activity of LGE, which has already been demonstrated (Falodun et al., 2007).

Another interesting finding in this study is that LGE showed anxiolytic-like effects in two predictive murine models of anxiety, the EPM and the light/dark box tests. The elevated plus-maze test is probably the most popular of all currently available animal models of anxiety, and affords an excellent example of a model based on the study of unconditioned or spontaneous behaviour (Carobrez and Bertoglio, 2005; Dawson and Tricklebank, 1995; Rodgers and Dalvi, 1997). The test relies on two conflicting innate tendencies in rodents; the rodents' drive to explore a novel environment and their aversion to heights and brightly-lit open spaces (Borsini et al., 2002; Lister, 1987). The EPM test has been demonstrated to be bi-directionally sensitive to both anxiolytic drugs; in particular benzodiazepines (Handley and Mithani, 1984; Lister, 1987; Pellow et al., 1985), as well as compounds which induce anxiety in man (Lister, 1987; Pellow et al., 1985; Pellow and File, 1986). The primary indices of EPM anxiety comprise spatiotemporal measures of open arm avoidance (percentage of entries and time spent in open arms), with anxiolytics generally increasing and anxiogenics decreasing these measures (Carobrez and Bertoglio, 2005; Chen et al., 2006). In this study, LGE increased both the frequency and duration of open arm

exploration in the EPM indicative of anxiolytic activity. Similar to LGE and in agreement with previously published reports (Dalvi and Rodgers, 1996; Rodgers et al., 1997), diazepam induced behaviours typical of an anxiolytic drug, increasing the frequency and duration of open arm exploration by mice. In contrast, PTZ induced anxiety-like behaviours in mice by decreasing entries and time spent in the open arms.

In addition to using spatiotemporal indicators of anxiety in the EPM, ethological measures of "risk assessment", such as stretch-attend postures and head-dipping, have been validated and shown by factor analysis to be a more predictive determinant of anxiety (Griebel et al., 1997; Rodgers et al., 1997; Setem et al., 1999). In agreement with its anxiolytic activity, LGE like diazepam decreased the percentage protected forms of both stretch-attend postures and head-dips whereas pentylenetetrazole increased the performance of these risk assessment behaviours.

LGE also showed anxiolytic-like activity in the light/dark box test. This test exploits rodents' natural aversion to bright areas compared to dark areas and their innate exploratory behaviour (File et al., 2004). When initially placed in the dark compartment, clinically effective anxiolytics have been shown to cause reduction of latency of mice to emerge from the small, dark compartment into the large, brightly lit and open areas (Ardayfio and Kim, 2006) whereas stress and anxiogenic treatments increase emergence latency and the time spent in the dark (Crawley, 1985; Shimada et al., 1995). However, the measurement found to be most consistent and useful for assessing anxiolytic-like action is the time mice spend in the lit area, because this parameter provides the most consistent dose-effect results with drugs (Hascoet and Bourin, 1998). LGE and diazepam significantly and dose-dependently decreased the emergence latency of mice into the lit compartment and increased the amount of time spent in the lit compartment. PTZ also showed results consistent with its anxiogenic activity, increasing the latency to enter the lit compartment and decreasing the time spent in the lit compartment.

Changes in locomotor activity can confound the interpretation of results obtained from the EPM or light/dark box tests (Weiss et al., 1998). Increased locomotor activity in the EPM may be as a result of a nonspecific motor stimulant effect or the consequence of increased exploratory behaviour due to selective anxiolytic effects. Contrarily, decrease in locomotor activity may be due to nonspecific effects (motor impairment, sedation or muscle relaxation) or the result of inhibition of exploratory behaviour due to selective anxiogenic effects in the EPM. This creates a challenge when using only conventional spatiotemporal indicators of anxiety which fail to dissociate false positives and false negatives from real, anxiolytic effects. Fortunately, measures of risk assessment such as head-dipping and stretched, attend posture, considered selective indices of

anxiety rather than locomotor behaviour (Dawson et al., 1995; Weiss et al., 1998), usually help to resolve these conflicts. In this study, LGE and diazepam increased total distance travelled in the EPM while PTZ decreased it. However, the effect of LGE, diazepam and PTZ on risk assessment behaviours in the EPM suggests that the activities exhibited by these agents on anxiety were selective and not due to non-specific changes in locomotor activity. The results of the rotarod test also show that LGE did not induce any significant motor deficits in mice at the doses tested.

Inhibition of seizures induced by pentylenetetrazole and maximal electroshock in laboratory animals is the most common predictive screening test used for characterizing potential anticonvulsant drugs (Krall et al., 1978; Loscher and Schmidt, 1988; Sayyah et al., 2004). The maximal electroshock-induced seizure test (MEST) is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures. Anticonvulsant effect in MEST further indicates the ability of the testing material to prevent seizure spread through neural tissue (Castel-Branco et al., 2009; Piredda et al., 1985). By contrast, the PTZ-induced seizure test represents a valid model for human generalized myoclonic and absence seizures (Loscher and Schmidt, 1988). Anticonvulsant activity against PTZ seizures also identifies compounds that can raise seizure threshold in the brain (Goodman et al., 1953; Mandhane et al., 2007; Piredda et al., 1985). In this study LGE inhibited MES- and PTZ-induced seizures suggesting that *L. guineensis* may contain compounds that have activity against generalized tonic-clonic as well as generalized myoclonic and absence seizures. PTZ is believed to exert its convulsant effect by inhibiting the action of GABA at GABA_A receptors (De Sarro et al., 1999). Picrotoxin, a GABA_A receptor antagonist, has also been shown to elicit seizures by blocking chloride channels linked to GABA_A receptors (Meldrum and Rogawski, 2007). Since LGE showed anticonvulsant activity against PTZ- and picrotoxin-induced seizures, it is highly probable that GABAergic mechanisms are involved in the extract's anticonvulsant action. Further studies are obviously necessary to establish the exact mechanism of action of the extract.

Conclusion

The results from the present study have shown that the aqueous leaf extract of *L. guineensis* possess anti-nociceptive, anxiolytic and anticonvulsant properties. However, further research is necessary to determine the components involved and their mechanism of action in bringing about the observed pharmacological effects.

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