

Synergistic depressant activity of *Amorphophallus paeoniifolius* in Swiss albino mice

Sir,

Pharmacodynamic synergism results from two drugs directed at a similar receptor target or physiological system.^[1] Synergistic activity of tea samples with antibiotics showed best response against the bacteria.^[2] The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.^[3] Generally neurotransmitters are involved in the work of regulation of central nervous system (CNS) activity. GABA_A receptors are located postsynaptically and they mediate postsynaptic inhibition by increasing the Cl⁻ permeability and hyperpolarizing the cell. GABA_A receptors are target for the several important centrally acting drugs like benzodiazepines, barbiturates, etc.^[4] Similarly the tuber of the Ayurvedic plant *A. paeoniifolius*^[5] were found to have CNS depressant activity in mice.^[6] So the present study was planned to find out the interaction of the extracts with the benzodiazepines and barbiturates receptor agonist.

Amorphophallus paeoniifolius (Araceae) tuber was brought from Asansol market, West Bengal, India in the month of September 2007. The tuber was identified by the Botanical Survey of India, Howrah with ref no. CNH/I-I/ (272)/ 2008/ Tech. II/ 314. The tuber of the plant was dried under shade and made to a fine powder using a laboratory mill, and was extracted with petroleum ether (40-60) (Merck, India) using soxhlet extractor. The percentage yield of the extract was found to be 6.23%. The preliminary phytochemical screening of the petroleum ether extract confirmed the presence of steroids, fats, and fixed oil. The petroleum ether extract of *Amorphophallus paeoniifolius* (PEAP) was used for pharmacological screening.

Male Swiss albino mice (20–25 g) were obtained from animal house of Gupta College of Technological Sciences. The animals were housed under CPCSEA specified environmental condition and fed with standard diet (Tetragon chemie private limited, Bangalore, India), water *ad libitum*. The study received approval from the Institutional Animal Ethics Committee.

All the experiments were conducted on an isolated and noiseless condition. The CNS activity was evaluated using Actophotometer (Technoworld, India) and rota-rod apparatus (Biological Museum, India). The protocol is based as carried

out by Turner.^[7] The petroleum ether extract of *A. paeoniifolius* was administered in the form of suspension in 5% v/v Tween 80 (Burgoyne Burbidges and company, India) as vehicle. The standard drug diazepam (Ranbaxy, India) and phenobarbitone (Nicholas, India) was administered in the form of suspension in 5% v/v Tween 80 as vehicle. The male animal were divided into twelve groups each composed of six animals as follows.

- Group I: Control (received 5% Tween 80 at the dose of 10 ml/kg, i.p.)
Group II: PEAP (100 mg/kg, i.p.)
Group III: PEAP (300 mg/kg, i.p.)
Group IV: PEAP (1000 mg/kg, i.p.)
Group V: Diazepam (0.1 mg/kg, i.p.)
Group VI: Diazepam (0.5 mg/kg, i.p.)
Group VII: Diazepam (1 mg/kg, i.p.)
Group VIII: Phenobarbitone (1 mg/kg, i.p.)
Group IX: Phenobarbitone (5 mg/kg, i.p.)
Group X: Phenobarbitone (20 mg/kg, i.p.)
Group XI: PEAP (250 mg/kg) and diazepam (0.5 mg/kg, i.p.)
Group XII: PEAP (250 mg/kg) and phenobarbitone (12 mg/kg, i.p.)

After 1 h of the administration of the drug, the animals were placed in an actophotometer for 10 min and the locomotor activity was observed. From the dose response curve of each drug, the effective doses were calculated. The synergistic activity of petroleum ether extract in combination with either diazepam (Group XI) or phenobarbitone ((Group XII) was evaluated.

Data obtained from pharmacological experiments was expressed as mean $\pm$ SD. Difference between the control and the treatments in these experiments were tested for significance using ANOVA followed by Dunnett's test. Values of $P < 0.05$ were considered statistically significant.

A spontaneous dose dependent increase in CNS depressant activity was observed with petroleum ether extract, diazepam, and phenobarbitone in Swiss albino mice. The petroleum ether extract of *A. paeoniifolius* at the dose level of 100, 300, and 1000 mg/kg body weight administered i.p. 60 min, the percentage inhibition of the CNS activity by pet-ether extract was found to be 16.53%, 56.77%, and 73.36% respectively. From the dose response curve, the effective dose (ED₅₀) for the CNS depressant activity was calculated to be 250 mg/kg. Diazepam at the dose level of 0.1, 0.5, and 1.0 mg/kg bodyweight administered i.p. 60 min, the percentage inhibition of the CNS activity was found to be 28.23%, 46.72%, and 91.6%, respectively. Further, from the dose response curve the effective dose (ED₅₀) for the CNS depressant activity was calculated to be 0.5 mg/kg. Similarly, phenobarbitone at the dose level of 0.5, 5, and 20 mg/kg bodyweight administered i.p. 60 min, the percentage inhibition of the CNS activity by

Research Letters

phenobarbitone was found to be 11.11%, 25.68%, and 70.31%, respectively. From the dose response curve the effective dose (ED_{50}) for the CNS depressant activity was calculated to be approx. 12 mg/kg. The *intraperitoneal* administration of vehicle (5% Tween 80) at a dose of 10 ml/kg did not reduce locomotor activity significantly. Further, the synergistic activity of pet-ether extract (250 mg/kg) with phenobarbitone (12 mg/kg) was checked [Figure 1] and it was observed that 1 h after administration of the drugs, the percentage inhibition of CNS depressant activity of the combination was calculated to be 59%, which was found slightly higher than the percentage inhibition by pet-ether extract and phenobarbitone individually at their effective doses when compared with control group (vehicle). Similarly, the synergistic activity of pet-ether extract (250 mg/kg), diazepam (0.5 mg/kg) was checked [Figure 2] and it was observed that after one hour administration of drugs the percentage inhibition of CNS depressant activity of the combination was calculated to be 75%, which was much higher than the percentage inhibition by pet-ether extract and diazepam individually at their effective doses when compared with control group (vehicle).

The petroleum ether extract of *A. paeoniifolius* was found to have CNS depressant activity. Phenobarbitone and diazepam are well known CNS depressant drugs and also showed CNS depressant activity in a dose-dependant manner.^[8] A significant synergistic effect of the petroleum ether extract with diazepam was found whereas there was little synergistic effect with phenobarbitone. Both of the phenobarbitone and diazepam exert their CNS depressant effect by acting on the $GABA_A$ receptor. The $GABA_A$ receptor has 5 subunits (α , 2β , γ , δ). The α subunit has the benzodiazepine-binding sites and the barbiturate-binding sites are located on the β subunit.^[9] The drugs phenobarbitone and diazepam bind with their respective binding sites and give their effect. From Figures 1 and 2, it was concluded that pet-ether extract has more synergistic activity of the CNS depression with diazepam than phenobarbitone. As the petroleum ether extract showed synergistic effect with diazepam but not with the phenobarbitone, the components present in the petroleum ether extract may bind with the α subunit and facilitate the GABA mediated Cl^- channel opening, thus hyperpolarizes the cell and show CNS depressant action. Diazepam is a benzodiazepine receptor agonist. So the extract has agonistic activity with benzodiazepine receptor, which might similar to that of diazepam. Further investigations are needed for better understanding of molecular mechanism of action and signal transduction of the components present in petroleum ether extract of *A. paeoniifolius* regarding CNS depressant activity.

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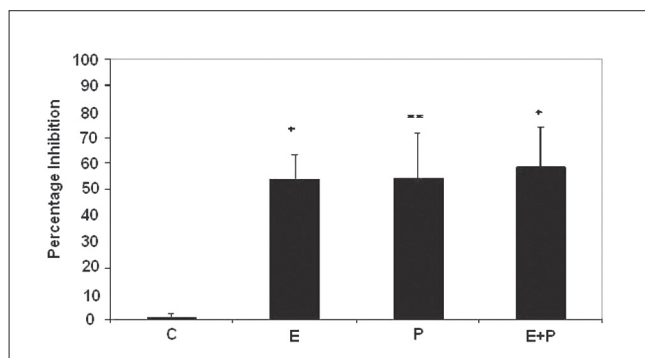


Figure 1: Synergistic activity of petroleum ether extract with phenobarbitone C = Control (Tween 80), E = Petroleum ether extract (250 mg/kg), P = Phenobarbitone (12 mg/kg), and E+P = Petroleum ether extract (250 mg/kg) and Phenobarbitone (12 mg/kg). The data is expressed as Mean $\pm$ SD of n = 6 observations * P < 0.05 and ** P < 0.01 as compared to control; ANOVA followed by Dunnett's test

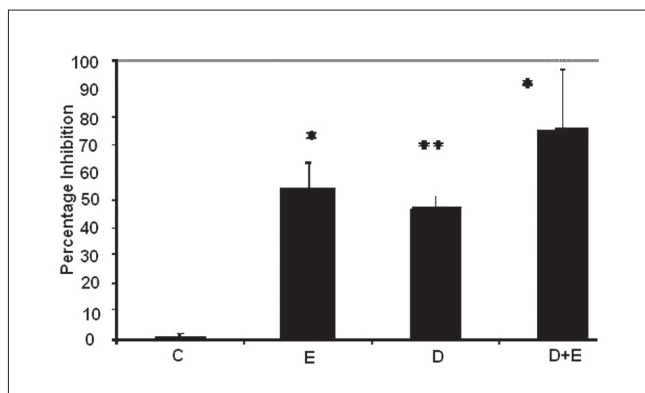


Figure 2: Synergistic activity of petroleum ether extract with diazepam. C = Control (Tween 80), E = Petroleum ether extract (250 mg/kg), D = diazepam (0.5 mg/kg) and D+E = Diazepam (0.5 mg/kg) and Petroleum ether extract (250 mg/kg). The data is expressed as Mean $\pm$ SD of n = 6 observations. * P < 0.05 and ** P < 0.01 as compared to control; ANOVA followed by Dunnett's test

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