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Anxiolytic effect of hydroethanolic extract of *Drymaria cordata* L Willd

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Drymaria cordata hydroethanolic extract (DCHE) at 25, 50 and 100 mg/kg (po) was administered to study anxiolytic effect. Different models for anxiolytic activity viz. Hole board, Open field, Elevated plus maze, Light/dark exploration model were used. In the hole board model, there was dose dependent and significant increase in the numbers of head pokes and the time of head dipping in the treated groups in comparison to the vehicle. In open field test, the number of rearing, assisted rearing and numbers of squares traversed increased significantly. Similarly, in elevated plus maze test, there was significant increase in the time spent and number of entries in open arm as compared to the time spent and number of entries in closed arm in dose dependent manner. In light/dark exploration test, another model for anxiolytic activity, the time spent in lit box , number of crossing and the latency period increased significantly with reduction in time spent in dark box after treatment with DCHE. The presence of phytochemicals *viz.* triterpenes, diterpenes, steroids and tannins might contribute to its anxiolytic activity.

Keywords : Anxiolytic effect, Diazepam, *Drymaria cordata*

Anxiety affects one eighth of the population of the total worldwide population. Benzodiazepines are among the first line of drugs that have been extensively used for the last 45 years to treat several forms of anxiety[1]. Although benzodiazepines have well-known benefits, their side effects are prominent, including sedation, muscle relaxation, anterograde amnesia and physical dependence[2]. Till date, the efficacy of the drugs for these conditions is very limited so the need for newer, better-tolerated and more efficacious treatments is in high demand. Therefore, herbal therapies should be considered as an alternative to complementary medicines. Recently, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly. This has been reflected in the large number of herbs whose psychotherapeutic potential has been assessed in a variety of animal models.

Drymaria cordata L Willd, locally known as *Laijabori* is traditionally used as antidote, appetizer, depurative, emollient, febrifuge, laxative and stimulant. Moreover, the pounded leaf is applied to snake bites[3]. Studies on *Drymaria cordata* exhibited significant antitussive activity when compared with control in a dose dependent manner[4]. Antibacterial[5] and anti-inflammatory[6] activity of *Drymaria cordata* have also been reported. The present study was undertaken to investigate the anxiolytic effect of the hydroethanolic extract of leaves of *Drymaria cordata* as there is no report available on the anxiolytic activity of the plant.

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Materials and Methods

Preparation of extract— The leaves of *Drymaria cordata* L Willd were collected during the month of July-Sept, 2008 from the medicinal garden of the Department of Pharmacology & Toxicology, C.V.Sc. Khanapara, Guwahati and identified by the Botanical Survey of India, Shillong, Meghalaya. A voucher specimen (No AAU/CVSC/PHT/ 07-08/ 02) has also been deposited at the Botanical Survey of India, Guwahati. Fresh leaves of *Drymaria cordata* were cleaned and washed thoroughly with water and rewashed with distilled water. Washed fresh leaves were dried under shade in clean dust free environment, then grinded and stored in air tight container. They were (250 g) soaked in 1000 ml of hydroethanol (50:50) for 72 h in separate beakers. The hydroethanol mixture of leaf powder plant was stirred

every 18 h using a sterile glass rod. The solvent was filtered through muslin cloth and Whatman's filter paper No 1. The filtrate obtained was concentrated in Rotary Evaporator (Equitron) at 50°-60°C under reduced pressure leaving a dark brown residue. The *Drymaria cordata* hydroethanolic extract (DCHE) thus obtained was transferred to a Petri dish and kept over water bath (50°C) until solvent was completely evaporated. It was stored at 4°C for future use. Recovery was 18.06% (W/W).

Source of chemicals— The drugs used in the study were obtained from various sources – diazepam (Calmpose® Ranbaxy, India), Ethanol (Merck India Limited, Mumbai). All chemicals and solvents were of analytical grade.

Phytochemical study— Preliminary phytochemical study was done for qualitative identification of the phytoconstituents[7].

Animals and treatment regimens — Male Albino Swiss mice (18-22g) of 3-4 weeks of age were used for the study. The animals were housed in colony cages and maintained under standard environmental conditions, 25°±2°C temperature, RH 45-55% and 12:12 h light:dark cycle. They were provided free access to food and water *ad libitum.* The animals were fasted overnight before the experiment. All experiments were carried out during the light period (0800-1600 h). The experiment was conducted in accordance with the ethical rules on animal experimentation, approved by ethical committee, Gauhati Medical College, Guwahati (Registration numbers- 351). The animals were divided into five groups, containing six mice each and given treatment —: Group I, saline-treated animals, served as controls, Group II- animals received the standard drug diazepam, (1mg/kg; ip) as positive control, Group III, IV and V were fed orally with DCHE at a dose of 25, 50 and 100 mg/kg, respectively. Experiment was conducted after 30 min of administration of test drug/ standard/ vehicle to respective group. Initially, a preliminary screening was carried out to select the dose for anxiolytic activity and three oral doses *viz* 25, 50 and 100 mg/kg of DCHE was selected as there was drop in anxiolytic activity beyond 100 mg/kg oral dose.

Hole board test[8]— The hole board apparatus consisted of a wooden box (40×40×25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the base of box. The apparatus was elevated to the height of 25 cm. Mice were fed orally with DCHE (25, 50 and 100 mg/ kg) or vehicle 30 min before they were placed in the apparatus. The numbers of head pokes and the time of head dipping during a 5 min period were recorded.

Open field test[8,9]— The apparatus consisted of a wooden box (60×60×30 cm). The base of the box was divided into 16 squares (15×15 cm). The apparatus was illuminated with 40-W lamp suspended 100 cm above it. Mice were fed orally with DCHE (25, 50, 100 mg/kg), vehicle or diazepam. After 30 min they were placed in one of the corner squares, the number of rearing, assisted rearing (forepaws touching the walls of the apparatus) and the numbers of squares crossed were counted for 5 min.

Elevated plus maze (EPM)[10] — The EPM consisted of two open arms (35×5 cm) crossed with two closed arms (35×5×20 cm). The arms were connected together with a central square of 5×5 cm. The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Mice were treated with DCHE (25,50,100 mg/kg po), diazepam (1mg/kg ip), or vehicle 30 min before being placed individually in the center of the EPM, facing a closed arm. The time spent in both the open and closed arms was recorded for 5 min. The number of entries into open and closed arms were counted during the test. An entry was defined as having all four paws within the arm.

Light/dark exploration test[11]— The apparatus consisted of two boxes (25×25×25 cm) joined together. One box was made dark by covering its top with plywood, whereas a 40- W lamp illuminated the other box. The light source was placed 25 cm above the open box. The mice were placed individually in the center of the lit box and observed for the next 5 min for the time spent in the lit and dark boxes. The mice were orally administered with DCHE (25, 50 and 100 mg/kg), diazepam (1mg/kg; ip) or vehicle 30 min before being placed in the lit box.

Statistical analysis— The statistical analysis of data was done using one-way analysis of variance by using SPSS software (version 11.5). A probability less than 0.01 was considered to be statistically significant.

Results and Discussion

The phytochemical screening of DCHE showed the presence of tannins by ferric chloride and gelatin test; diterpenes, triterpenes by Salkowski's test and Liberman Buchardt's test; and steroids by Salkowski's test and Liberman Buchardts test.

Anxiety may be regarded as a particular form of behavioural inhibition that occurs in response to environmental events that are novel. It has been established that there are lots of plant secondary metabolites being employed in the treatment of psychotic disorders especially for anxiety in traditional medicine practice, most of which directly or indirectly affect the central nervous system, noradrenaline, serotonin, GABA and BZD neurotransmitters activities[12-16]. The traditional knowledge that *Drymaria cordata* is a stimulant, has prompted us to undertake the study to explore anxiolytic activity of DCHE. Hole-board model indicates that head-dipping behaviour is sensitive to changes in the emotional state of the animal and suggest that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behaviour[17]. DCHE at 50 and 100 mg/kg (po), dose showed significant increase in number of head poking and time of head dipping. At 100 mg/kg (po), dose of DCHE, the number of head pokes and the duration of head pokes were more than the standard drug diazepam (Fig. 1).

The open field model showed that administration of DCHE increased rearing, assisted rearing and number of squares traversed significantly which was dose- dependent (Fig. 2). The elevated plus maze (EPM) is a well-established animal model for testing anxiolytic drugs. The EPM test is based on a premise where the exposure to an EPM evoke an approach-avoidance conflict that is stronger than evoke by the exposure to an enclosed arm[18]. The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed by the increased time spent and entries into the open arm. The primary index is spatiotemporal in nature: it is reduced by anxiolytic drugs and can be increased by anxiogenic compounds[19]. Administration of DCHE in mice significantly increased the number of entries in open arm along with increase in duration of time spent as compared to the vehicle treated control group (Fig. 3). The light-dark test may be useful to predict the anxiolytic-like activity of drugs. Transitions have been reported to be an index of activity exploration because of habituation over time and the time spent in each compartment to be a reflection of aversion[20]. The administration of DCHE showed dose dependent and significant increase in the time spent in lit box, number of crossings and the time of latency with decrease in time spent in the dark box (Fig. 4).

Mechanism of anxiolytic action of plants may be by interaction with some of the natural endogenous mediators in the body as reported by various workers[21-22]. There could also be a linkage in the interaction of the plant extract with serotonergic pathway[23-25]. Effect of most of the anxiolytic agents is to enhance the response to GABA, by facilitating the

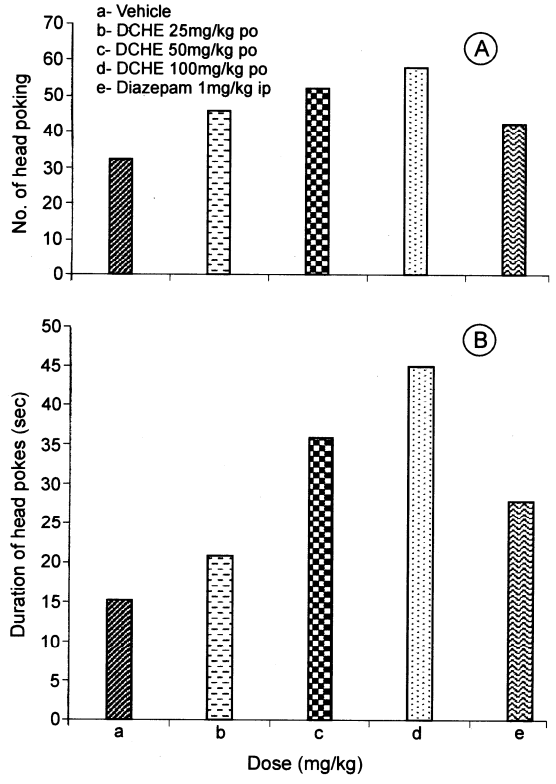

Fig. 1— Effect of DCHE on (A) Number of head pokes; and (B) Duration of head pokes in Hole board test.

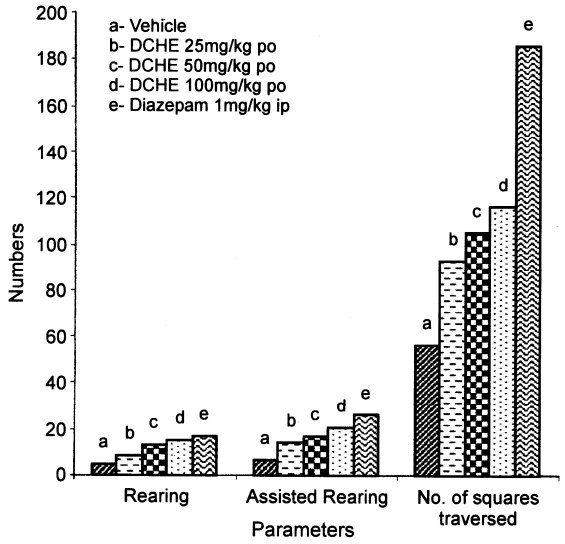

Fig. 2—Effect of DCHE on the number of rearing, assisted rearing and squares traversed in open field test

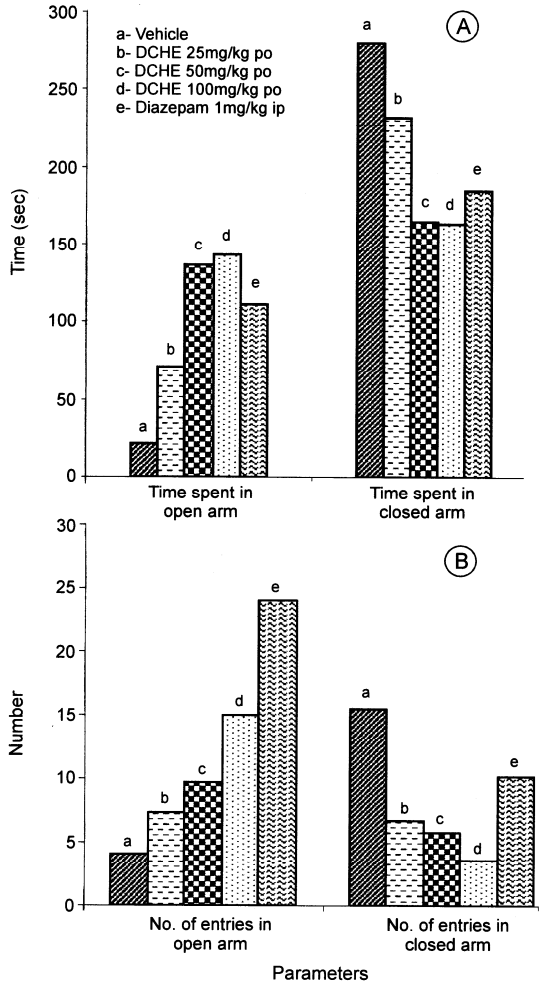

Fig. 3— Effect of DCHE on the (A) Time spent in open arm and closed arm; and (B) Number of entries in open and closed arm in elevated maze test.

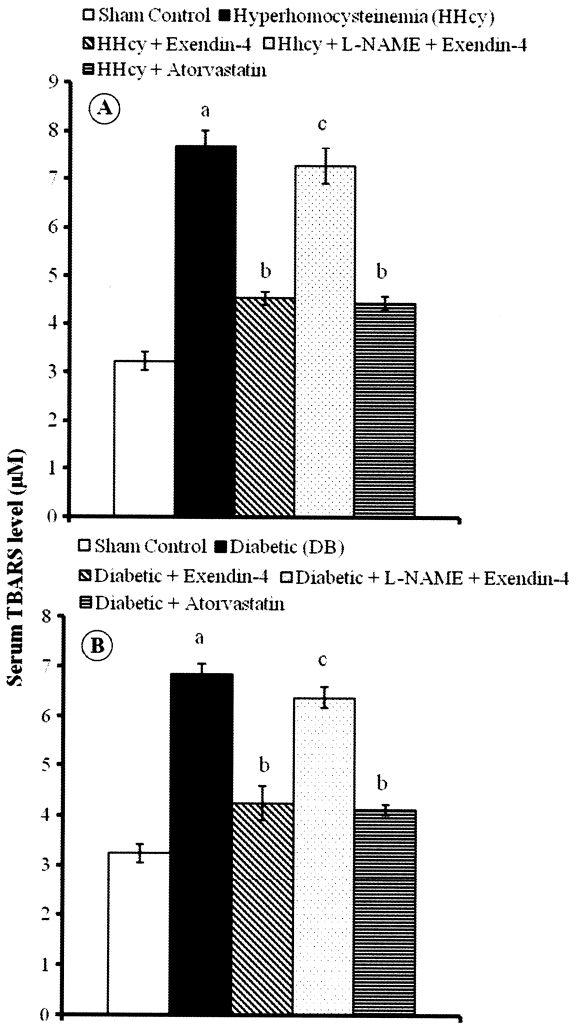

Fig. 4— Effect of DCHE on (A) Time spent in lighted box, dark box and latency time; and (B) Number of crossing in light/dark exploration test.

opening of GABA-activated chloride channels. Thus, the present study showed that DCHE possesed potent anxiolytic activity which was evidenced by all the models as described above. Effects were dose dependent, optimum effect was observed at 100 mg/kg (po) which was significantly higher than vehicle treated control group.

Therefore it was concluded that, DCHE used in this study might affect certain mediators to reduce anxiety. Phytochemical screening of DCHE showed the presence of tannins and triterpenes, which might also be involved in inhibition of some mediators to reduce anxiety as described above. Further in depth study is needed to understand the mechanism of action at biochemical and physiological level.

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