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Original Article

ANTI-ANXIETY EVALUATION OF EXTRACTS OF *STIGMA MAYDIS* (CORN SILK)

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ABSTRACT

Objective: The anxiolytic activity of petroleum ether, chloroform and ethyl acetate extracts of *Stigma maydis* was investigated by Elevated Plus Maze, Hole Board and Mirror Chamber Test.

Methods: The study was conducted using elevated plus maze, whole board and mirror chamber test. Female Laca/Balb c was used to carry out the studies. In each experiment, animals were equally divided into five groups; control, given saline solution and Tween 80, standard given diazepam (2 mg/kg i. p.) and test groups were given 250, 500, 750 and 1000 mg/kg of petroleum ether, chloroform and ethyl acetate extracts of *Stigma maydis*. The data were subjected to analysis of variance by taking mean and standard error to the mean using Tukey's post-hoc test.

Results: In Elevated Plus Maze chloroform extract (750 and 1000 mg/kg) of *Stigma maydis* revealed increase in time spent in open arm, frequency and preference to open arm as compared to control, which was almost comparable to diazepam. In Hole Board test decrease in number of head dips as compared to control was observed. In Mirror Chamber Test, the decrease in latency, increase in time spent in the mirror chamber and frequency as compared to control was observed. All of the changes were statistically highly significant.

Conclusion: From our results it can be concluded that the chloroform extract of *Stigma maydis* showed anxiolytic activity at the dose of 750 and 1000 mg/kg.

Keywords: Antianxiety, *Stigma maydis*, Elevated plus maze, Mirror chamber test, Hole-board test.

INTRODUCTION

Anxiety is a feeling of apprehension, uncertainty or tension streaming from the anticipation of an imagined or unreal threat. Anxiety is affecting 1/8 of the total world population and has become an important research area in psychopharmacology in past decades. Symptoms of anxiety are due to the release of stress hormones like adrenaline and cortisol which almost affect every organ of the body [1].

Anxiety disorders are the most common and prevalent behavioral disorders that can result in significant impairment of function and quality of life, and they affect 17.2% of the population in the United State [2]. Primary treatments for anxiety related disorders include selective serotonin reuptake inhibitors (SSRI), Serotonin-norepinephrine reuptake inhibitors (SNRI), benzodiazepines, and the azipirone buspirone and beta adrenergic antagonists [3].

Benzodiazepines, being major class of compounds used for treatment of anxiety [4], present a narrow margin of safety between the anxiolytic effect and unwanted side effects, has prompted researchers to evaluate new compounds specially plant based drugs having less undesirable effects [5].

In the present study *Stigma maydis* also known as corn silk which belongs to family *Graminae* has been evaluated for its anxiolytic potential. Corn silk looks like tuft of hairs. It is yellowish thread like strands of maize (also known as Zea mays. Basically it is made of stigma and styles, the yellowish thread like strands of female flower of maize [6].

It is rich in phenolic compounds, particularly flavonoids [7]. It also consists of proteins, vitamins, carbohydrates, calcium, potassium, magnesium and sodium salts, volatiles oils and steroids such as sitosterol and stigmasterol, alkaloids, and saponins [8].

Corn silk is used for the treatment of various disorders. Traditionally corn silk was used in China, America, Turkey and France. It was used for the treatment of cystitis, gout, kidney stones, malarial and prostate disorders. It has also been used in the treatment of nephritis, kidney stones, malarial and heart disorder [9]. It soothes and relaxes the lining of the bladder and urinary tubules, hence reducing irritation and increasing urine secretion [10].

Other beneficial uses of corn silk include anti-fatigue, hepato protective, anti-hyperlipidemic, anti-diabetic, anti-inflammatory, anti-tumour and anti-cancer [11-17]. In addition, it possesses excellent anti-oxidant capacity [18] and protective effect in nephro toxicity [19]. Flavonoids have been attributed to have its effect on the central nervous system. Since corn silk is rich in flavonoids, therefore it may exert anxiolytic effect. Among the Central Nervous System activities, corn silk has been found to possess anti-depressant activity [20]. But till now, no study is available on the anti-anxiety action of *Stigma maydis* (corn silk).

So, the aim of the present study is to evaluate the anti anxiety activity of petroleum ether, chloroform and ethyl acetate extract of *Stigma maydis* (Corn silk) using different animal models of anxiety.

MATERIALS AND METHODS

Collection and authentication of plant material

Plant was procured from Punjab Agricultural University, Ludhiana, India and identified by Dr. Sunita Garg, Chief scientist, Raw Material Herbarium and Museum (RHMD), CSIR-NISCAIR, Delhi, India (Voucher specimen No. NISCAIR/RHMD/Consult/2014/2518/97). A voucher specimen was deposited in the herbarium of the institute.

Preparation of extract

Stigma maydis was dried in shade and powdered. 250 g of dried and powdered *Stigma maydis* was packed into Soxhlet apparatus and extraction was done by hot continuous percolation using solvents petroleum ether, chloroform and ethyl acetate successively. Extracts were dried using rotary evaporator EYELA N-1100 and dried extract were preserved in vacuum desiccator with anhydrous silica gel blue.

Animals

Female Laca/Balb c mice (weighing 15-30g) were taken from Central Animal House of the Punjab University; Chandigarh and kept in polypropylene cages of 5 mice at 22±1 °C on a 12-h light/dark cycle. Water and food were available *ad libitum*. Groups of 5 mice were randomly assigned to different treatment groups and tested in a counterbalance order. Six groups were made with five animals in each group. Control group received vehicle, positive control received

Diazepam (2 mg/kg i. p.) while other group received four petroleum ether, chloroform and ethyl acetate extract doses (250, 500, 750 and 1000 mg/kg respectively).

Drugs and chemicals

Diazepam was obtained from Glaxo Smith Kline and petroleum ether, chloroform and ethyl acetate were procured from (S. D. Fine-Chem Ltd, Mumbai). Diazepam was used in concentration of 0.5 mg in 10 ml of distilled water and all the extracts were dissolved in Tween 80 and 0.9% saline solution and were administered to mice according their weights.

Petroleum ether, chloroform and ethyl acetate extract and Diazepam were given by i. p. route. Control mice received vehicle (Tween 80 and 0.9% saline solution). The effects of the drugs were estimated 45 min after the administration of the dose. The entire tests were carried out between 8:00-14:00. In each experiment, apparatus was cleaned using 5% ethanol before introducing the next animal to preclude the possible cueing effects of odours left by previous subjects.

Dosing protocol

The extract was dissolved in Tween 80 and 0.9% w/v saline solution. Four sets of test doses (250, 500, 750 and 1000 mg/kg respectively) were prepared by suspending dried extract in vehicle. Extracts were given according to the weight of the animal. Diazepam 2 mg/kg suspended in the vehicle was used as a standard anxiolytic drug. Vehicle (Tween 80 and 0.9%w/v saline solution) was used as control.

Animal models for anxiety

A) Elevated plus maze

The plus maze apparatus consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof with a plus maze elevated (25 cm) from the floor was used to observe anxiolytic behaviour of animals. The animals were fasted 18 h prior to experiment. Methanolic extract of *Stigma maydis* was administered by intraperitoneal route to the animals. The dose administration schedule was so adjusted that each mice was having its turn on plus maze after 45 min of administration of dose. Each animal was placed in the centre of the elevated plus maze with its head facing the open arms.

During this 5 min experiment, behaviour of the mice was recorded as a) preference of the animal for its first entry to open/closed arm b) no. of entries into the open/closed arm c) average time spent by the animal in each arm. During the entire experiment each animal was allowed to socialize. Every precaution was taken to ensure that no external stimuli evoked the animal [21].

Animals were grouped into five (n=5). Group A was taken as Control (0.9% saline+Tween 80). Group B was treated with Diazepam (standard drug). Groups C1 C2 C3 C4, D1 D2 D3 D4, E1 E2 E3 E4 were treated with test doses of the petroleum ether, chloroform and ethyl acetate extract (250, 500, 750 and 1000 mg/kg respectively).

B) Mirror chamber test

The mirror chamber consisted of wooden chamber (40x40x30.5 cm) having a mirror (30x30x30 cm) enclosed within it. Placement of the mirrored cube into the centre of the container forms a 5 cm that completes surrounding the mirror chamber. The animals were placed individually into the chambers of the mirrors at a fixed corner.

During the 5 min experiment following parameters was noted a) latency to enter mirror chamber b) no. of entries into the mirror chamber c) total time spent in the mirror chamber [22].

Animals were grouped into five (n=5). Group A was taken as Control (0.9% saline+Tween 80). Group B was treated with Diazepam (standard drug). Groups C1 C2 C3 C4, D1 D2 D3 D4, E1 E2 E3 E4 were treated with test doses of the petroleum ether, chloroform and ethyl acetate extract (250, 500, 750 and 1000 mg/kg respectively).

C) Hole-board test

The whole 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 a height of 25 cm. Mice (n = 5) were treated with test doses of the prepared extract (250, 500, 750and 1000 mg/kg, i. p. respectively), diazepam (2 mg/kg, i. p.) and normal saline 45 min before they were placed in the apparatus. The numbers of head dips and the time of head dipping during a 5 min period were recorded [23].

Animals were grouped into five (n=5). Group A was taken as Control (0.9% saline+Tween 80). Group B was treated with Diazepam (standard drug). Groups C1 C2 C3 C4, D1 D2 D3 D4, E1 E2 E3 E4 were treated with test doses of the petroleum ether, chloroform and ethyl acetate extract (250, 500, 750 and 1000 mg/kg respectively).

Statistical analysis

All the values were expressed as mean±SEM. Statistically significant difference between the groups were calculated by the application of one way analysis of variance (ANOVA) followed by Tukey's, post-hoc test. The groups treated with extract (test groups) were compared with the respective control (vehicle) group; P<0.05 was considered statistically significant.

RESULTS

Elevated plus maze test

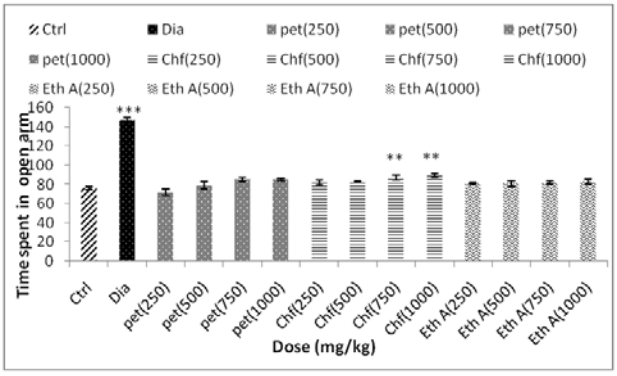

Fig. 1: Ctrl=control (saline solution p. o.), Dia=Diazepam (2 mg/kg i. p.), pet=petroleum ether extract (250, 500, 750 and 1000 mg/kg i. p.), Chf= chloroform extract (250, 500, 750 and 1000 mg/kg i. p.), Eth A= ethyl acetate extract (250, 500, 750 and 1000 mg/kg i. p.). Results are expressed as mean±SEM (n=5); *** P<0.001 and ** P<0.01 as compared to control

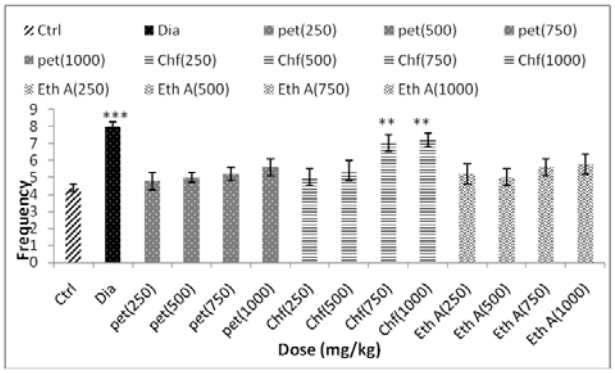

Fig. 2: Ctrl=control (saline solution p. o.), Dia=Diazepam (2 mg/kg i. p.), pet=petroleum ether extract (250, 500, 750 and 1000 mg/kg i. p.), Chf= chloroform extract (250, 500, 750 and 1000 mg/kg i. p.), Eth A= ethyl acetate extract (250, 500, 750 and 1000 mg/kg i. p.). Results are expressed as mean±SEM (n=5); *** P<0.001 and ** P<0.01 as compared to control

Hole board test

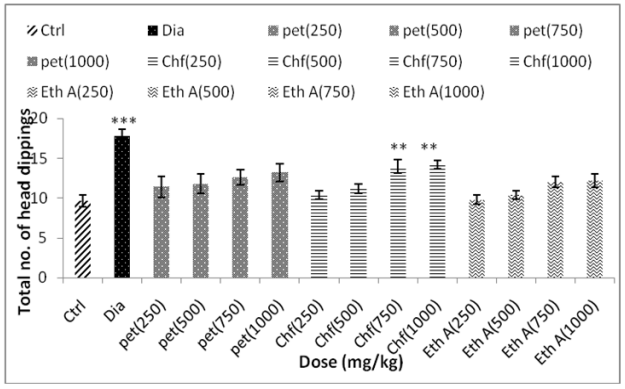

Fig. 3: Ctrl=control (saline solution p. o.), Dia=Diazepam (2 mg/kg i. p.), pet=petroleum ether extract (250, 500, 750 and 1000 mg/kg i. p.), Chf= chloroform extract (250, 500, 750 and 1000 mg/kg i. p.), Eth A= ethyl acetate extract (250, 500, 750 and 1000 mg/kg i. p.). Results are expressed as mean±SEM (n=5); *** P<0.001 and ** P<0.01 as compared to control

Mirror chamber test

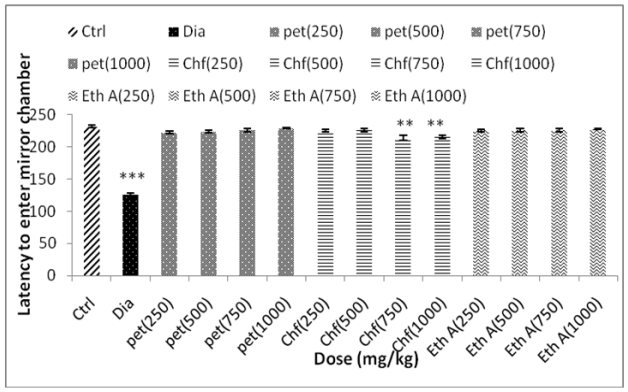

Fig. 4: Ctrl=control (saline solution p. o.), Dia=Diazepam (2 mg/kg i. p.), pet=petroleum ether extract (250, 500, 750 and 1000 mg/kg i. p.), Chf= chloroform extract (250, 500, 750 and 1000 mg/kg i. p.), Eth A= ethyl acetate extract (250, 500, 750 and 1000 mg/kg i. p.). Results are expressed as mean±SEM (n=5); *** P<0.001 and ** P<0.01 as compared to control

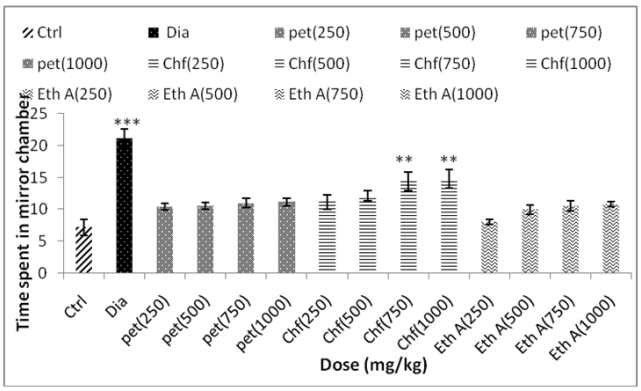

Fig. 5: Ctrl=control (saline solution p. o.), Dia=Diazepam (2 mg/kg i. p.), pet=petroleum ether extract (250, 500, 750 and 1000 mg/kg i. p.), Chf= chloroform extract (250, 500, 750 and 1000 mg/kg i. p.), Eth A= ethyl acetate extract (250, 500, 750 and 1000 mg/kg i. p.). Results are expressed as mean±SEM (n=5); *** P<0.001 and ** P<0.01 as compared to control

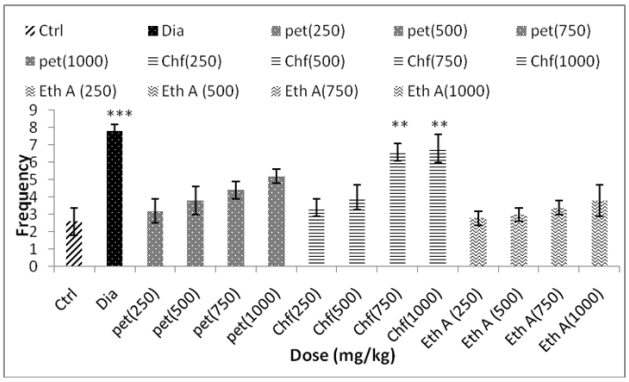

Fig. 6: Ctrl=control (saline solution p. o.), Dia=Diazepam (2 mg/kg i. p.), pet=petroleum ether extract (250, 500, 750 and 1000 mg/kg i. p.), Chf= chloroform extract (250, 500, 750 and 1000 mg/kg i. p.), Eth A= ethyl acetate extract (250, 500, 750 and 1000 mg/kg i. p.). Results are expressed as mean±SEM (n=5); *** P<0.001 and ** P<0.01 as compared to control

DISCUSSION

Plants had been used for healthcare and medicinal purposes long before it was recorded in history [24].

Present study was conducted to evaluate the potential of petroleum ether, chloroform and ethyl acetate extracts of *Stigma maydis* as anxiolytic through behavioural assay systems i.e. Elevated Plus Maze, Hole Board and Mirror Chamber Test. The Elevated Plus Maze is considered to be a valid animal model of anxiety because it uses natural stimulus that is the fear of a new, brightly light open space and the fear of balancing on a relatively narrow raised platform [25]. Moreover, it is known that anxiolytic agents increase the frequency of entries and the time spent in open arms of the Elevated Plus Maze [26]. In the present study, chloroform extract (750 and 1000 mg/kg) markedly increased the frequency of entries and the time spent by the animals in the open arms.

The Hole-Board test provides a simple method for measuring the response of an animal to an unfamiliar environment [27]. It has been showed that head-dipping behaviour was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behaviour. In the present study, chloroform (750 and 1000 mg/kg) increased head-dip counts. Animal species exhibit approach avoidance conflict upon placement of mirror. The extended latency to enter mirror chamber is a parameter of anxiety analogy. Chloroform extract at the dose of 750 and 1000 mg/kg showed decreased latency to enter mirror chamber, increase no. of entries and time spent in mirror chamber [21].

Flavonoids and Diazepam are structurally similar. The anxiolytic effects of chloroform extract of *Stigma maydis* may be related to their flavonoid content. This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors [28, 29]. However, further studies are needed to explore the exact mechanism of action.

CONCLUSION

From our research studies it can be concluded that chloroform extract of *Stigma maydis* after acute dosing possess anxiolytic activity at the dose of 750 and 1000 mg/kg. However, no effect was observed for petroleum ether and ethyl acetate extracts. Further studies can be conducted for the bioactive constituent and to ascertain an exact mechanism of action.

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CONFLICT OF INTERESTS

Declare None.

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