

EVALUATION OF ANTI ANXIETY ACTIVITY OF ETHANOLIC EXTRACT OF *Sapindusemarginatus* FLOWERS IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

The Anti anxiety activity of Ethanolic extract of *Sapindusemarginatus* flowers was determined by experimental animal models like Elevated plus maze, Despair swim test and Haloperidol induced catalepsy. The plant was collected in the month of March from Erode city and was extracted by Maceration using ethanol as solvent. The anxiolytic activity of the plant was studied using Diazepam (0.5mg/kg, i.p) as standard and with two different doses of extract (200mg/kg & 400mg/kg, Oral) by using elevated plus maze, despair swim test and haloperidol induced catalepsy. The time spent by the test group in the open arm was found significant when compared to the standard in Elevated plus maze with both the doses of extract. The immobility time (reaction time) was significantly reduced in the test group when to compare to that of the standard in Despair swim test. The animals in the test group had a failure to correct an externally imposed unusual posture over a prolonged period of time in Catalepsy. The above studies have been concluded that the ethanolic extract of *Sapindusemarginatus* flowers exhibit anxiolytic effect in experimental rats. So it supports the use of *Sapindusemarginatus* flowers as anxiolytic agents. Further investigations should be made to elucidate the active constituent of responsible for the activity.

Keywords: Anxiolytic activity; Phytochemical analysis; Catalepsy; Despair swim test.

INTRODUCTION

Anxiety is a psychological and physiological state which is characterized by somatic, emotional, cognitive and behavioural components. Anxiety affects most of the population nearly one-eighth of the total population world-wide (Eisenberg *et al.*, 1998). Anxiety is a state of excessive fear, characterized by motor tension, sympathetic hyperactivity, and vigilance syndromes. Anxiety may interfere with intelligence, psychomotor function as well as memory. Anxiety associated with chronic pain among medical patients in both developed and developing countries (Pine *et al.*, 1999). Benzodiazepines, being major class of compounds used for treatment of anxiety (Lader *et al.*, 1991), present a narrow margin of safety

between the anxiolytic effect and unwanted side effects, researchers concentrated to discover new compounds specially plant based drugs proved lesser undesirable effects.

Sapindusemarginatus belongs to sapindaceae commonly found in south India. The fruits of *Sapindusemarginatus* are commonly used for hair problems and also in preparation of shampoos (Herbtime. wordpress. com/2010), seeds of *Sapindusemarginatus* contain anti-inflammatory oil which is traditionally used to purify the blood (Nair *et al.*, 2005). It also exhibits various pharmacological effects like Antihyperlipidemic activity (Otway *et al.*, 1967), Larvicidal activity (Koodalingam *et al.*, 2009; Arunagirinathan Koodalingam *et al.*, 2011). It also showed pharmacological effects on central

nervous system like sedative as well as CNS depressant action (Srikanth *et al.*, 2009) with the methanolic extracts of leaves.

Thus the present study was undertaken to evaluate anti-anxiety activity of ethanolic extract of *sapindusemarginatus* flowers in experimental animal models.

MATERIALS AND METHODS

MATERIALS

The flowers of the plant were collected in the month of march from Erode city and it was authenticated by the pharmacognosist Dr. R. Duraisami, Professor and head, Department of pharmacognosy, Nandhacollege of pharmacy, Erode.

Preparation of plant extract

The ethanolic extract of the plant was prepared according to the methodology of Indian Pharmacopoeia. The flowers were dried in shade and the dried flowers were subjected to pulverization to get coarse powder. The coarse powder material was subjected to maceration with ethanol. The extract was concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°C). Then the extract was stored in a refrigerator in air tight containers. The extract was analyzed for phytochemical screening of compound and pharmacological activity.

Preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different chemical groups present in the extract and qualitative phytochemical analyses were done by using the standard procedures.

Experimental animals

Experimental animals are adult male Swiss albino rat from our own breeding stock were used. They were housed in groups in polypropylene cages (11cm × 17cm × 28cm) under controlled conditions of light (12 h light-dark cycle) and temperature (20±22°C). The animals were allowed a standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), and the experimental procedures were in accordance to the guidelines of IAEC (688/02/C/CPCSEA).

Experimental design

The rats were divided into four groups and six rat in each group.

Group I: Control (Normal saline 1ml/kg, p.o)

Group II: Standard (Diazepam 0.5mg/kg, i.p) (Reshmafarooq *et al.*, 2008)

Group III: Test I (Ethanolic extract of *S. emarginatus* 200mg/kg, p.o)

Group IV: Test II (Ethanolic extract of *S. emarginatus* 400mg/kg, p.o) (Suneetha *et al.*, 2013)

Elevated plus maze

The elevated plus-maze comprised two open (50 cm×10 cm×25 cm) and two enclosed (50 cm×10 cm×40 cm) arms that radiated from a central platform (10 cm×10 cm) to form a plus sign. The maze was constructed of black painted wood. A slight raised edge on the open arms (0.25 cm) provided additional grip for the animals. The plus-maze was elevated to a height of 50 cm above floor level by a single central support. Four 25W red fluorescent lights arranged as a cross at 100 cm above the maze were used as the source of illumination¹⁶. The experiment was conducted during the dark phase of the light cycle (9:00–14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. The number of entries into, each of the two types of arm, were counted during a 10 min test period. The percentage open arm entries used as indices of anxiety. A rat was considered to have entered an arm when all four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioral recordings were carried out with the observer unaware of the treatment the rat had received (Gerhard Vogel *et al.*, 2002).

Despair swim test

Swiss albino rats weighing 160-180g are used. They are brought to the laboratory at least one day one day before the experiment and are housed separately in polypropylene cages with free access to food and water. Swiss albino rats are individually forced to swim inside a vertical plexy glass cylinder (height: 40 cm, diameter : 18cm, containing 15cm of water maintained at 25°C). Rats placed in the cylinder for first time initially highly active, vigorously, swimming in circles, training to climb the wall or diving to the bottom. After 2, 3 minutes actively begins subside and to the interspersed with phases of immobility or floating of increasing length. After 5-6 minutes immobility reaches where the rats remain immobile for approximately 80% of the time. After 15 minutes in the water the rats are removed and allowed to dry in a heated enclosure (32°C) before being returned to their home cages. They are again placed in the cylinder 24 hours later and the total duration of immobility is measured during a 5minute test. Floating behavior during this 5minute period has been found to be reproducible in different

groups of rat. An animal is judged to be immobile when it remains passively in the water in a slightly hunched but upright position, its nose just above the surface. The extract (200 & 400mg/kg) and standard (diazepam 0.5mg/kg) are administered and evaluate the action at 0 hr, 1hr, 2hr and 3hrs (Furuzan *et al.*, 2000).

Haloperidol induced Catalepsy in rats

Swiss albino rats were divided into four groups contain six in each. The rats were administered with vehicle, i.e, water (p.o). After 30min later the rats were injected with Haloperidol 1mg/kg (i.p), both forepaws of rats were placed wooden bar elevated 6cm above the ground. The duration for which the rats retain the forepaws n the elevated bar was noted at 0, 30, 60, 90 and 120 minutes. The cutoff time was 300 minutes. After administration of extract (200 &400 mg/kg) and standard drug Diazepam (0.5mg/kg), haloperidol was administrated 1mg/kg (i.p) after 45 minutes. Then, both forepaws of rats were placed wooden bar elevated 6cm above the ground. The duration for which the rats retain the forepaws n the elevated bar was noted at 0, 30, 60, 90 and 120 minutes (Cosall *et al.*, 1974).

RESULTS

Phytochemical analysis

The phytochemical analysis which shown that the ethanolic flower extract of *Sapindusemarginatus* contains alkaloids, carbohydrates, phenols, flavonoids, saponins fixed oils & fats.

Elevated plus maze

Table 1.1 shows the result for number of entries to closed arm and 1.2 shows the number of entries to open arm. Administration of the standard drug diazepam (0.5 m/kg) significantly increases number of entries in open arm and reduce the number of entries in closed arm. Plant extract treated rats exhibited significant increase in entries in the open arm and reduction in the entries to closed arm at a dose of 400mg/kg when compared to normal control.

Despair swim test

Table No:2 shows the result for anti anxiety activity of ethanolic flower extract of *Sapindusemarginatus* by using despair swim test. In despair swim test apparatus, the ethanolic extract of flowers of *Sapindusemarginatus* at a dose of 400 mg/kg p.o. significantly decreased the immobility time compared to normal control. The magnitude of the antidepressant effects of 400 mg/kg p.o. of ethanolic extract of *Sapindusemarginatus* was

comparable to that of the result obtained with the treatment of Diazepam 0.5mg/kg,i.p.

Haloperidol induced Catalepsy.

The Figure No:1 shows the results obtained from the anti anxiety study using haloperidol induced catalepsy. Haloperidol is a neuroleptic agent wcich induce catalepsy in rodents like rats. The two doses of extract 200 mg/kg and 400 mg/kg were compared with normal control and Diazepam treated groups and observed with the time intervals of 0 min, 30 min, 60 min, 90 min and 120 minutes. The dose at 400mg/kg shown significant effect compared to normal control group. It reduces the time interval of catalepsy with the drug haloperidol and was acting like dopaminergic antagonist.

DISCUSSION

Anxiety is a psychological and physiological state wcich is characterized by somatic, emotional, cognitive and behavioural components (Humphery Rang *et al.*, 2007). Anxiety disorders are due to involvement of GABAergic (Naga kishore *et al.*, 2012) and adrenergic and dopaminergic systems play main role in it. Synthetic drugs like diazepam etc are used widely, because of their unwanted effects researchers are concentrating in natural sources nowadays. The present study demonstrates that the evaluation of antianxiety activity of ethanolic flower extract of *Sapindusemarginatus* in various experimental animal models. The preliminary phytochemical tests shown that the ethanolic flower extract of *Sapindusemarginatus* contains alkaloids, carbohydrates, phenols, flavonoids, saponins fixed oils & fats.

Elevated plus Maze is a widely used method to evaluate psychomotor performance and emotional aspects of rodents. with respect to our findings, the extract at a dose of 400mg/kg cause an increase in the number of entries into the open arm and decrease in the entry to closed arm. The number of arm entries, but decrease in time spent in closed arm reflects plants anxiolytic property (Naga kishore *et al.*, 2012). It is well established that the despair swim test (forced swim test) is sensitive to the test wcich enhance adrenergic transmission, particularly in rats. In this antidepressants or antianxiety drugs which reduce immobility but increase swimming instead of climbing. The present result showed that ethanolic flower extract of *Sapindusemarginatus* was effective in producing significant anti depressant and antianxiety effects in animal models.

Catalepsy in rat is defined as a failure to correct an externally imposed, unusual posture over period of time (Winters *et al.*, 1972).

Neuroleptics have an inhibitory action nigrostratal dopamine system induced catalepsy (SandeepGoyal et al., 2010). In this the dose at 400mg/kg shown significant effect compared to normal control group. There is a reduction in the time interval of catalepsy with the drug haloperidol and was acting like dopaminergic agonist

The effects are due to the action of phytochemicals present in the plant extract. Flavonoids have shown anti-anxiety activity in various studies (Paladini et al., 1999). Further, the anxiolytic effect of flavonoids has been attributed to its effect on central nervous system (Medina et al, 1997) and benzodiazepine receptors. (Wolfman et al., 1994). Therefore, flavonoids of ethanolic flower extract of

Sapindusemarginatus may be responsible for the anti-anxiety activity.

Finally, it is concluded from the present study that the ethanolic flower extract of *Sapindusemarginatus* exhibits anti-anxiety activity at the dose of 400 mg/kg in rat using elevated plus maze and despair swim test models. The studies are under progress to isolate bioactive constituent(s)/ fraction from *Sapindusemarginatus* flower responsible for anti-anxiety activity.

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Table 1: Elevated plus maze
Table 1.1: Number of entries to closed arm

S.No	Treatment Group	Number of entries to closed arm
1	Control (Normal saline 1ml/kg.i.o)	9.46±0.81
2	Standard (Diazepam 0.5mg/kg i.p)	4.25±0.25**
3	Test-I (Eth. extract of <i>S. emarginatus</i> 200mg/kg)	5.58±0.17*
4	Test-II (Eth extract of <i>S. emarginatus</i> 400mg/kg)	4.51±0.29**

The table represents anti anxiety activity of *Sapindusemarginatus* in number of entries to closed arm in elevated plus maze. All values are mean ±SEM (n=6); *p< 0.05, **P < 0.01 when compared to control

Table 1.2: Number of entries to open arm

S.No	Treatment Group	Number of entries to open arm
1	Control (Normal saline 1ml/kg.i.o)	5.23±0.25
2	Standard (Diazepam 0.5mg/kg i.p)	13.73±0.46**
3	Test-I (Eth. extract of <i>S. emarginatus</i> 200mg/kg)	9.33±0.38*
4	Test-II (Eth extract of <i>S. emarginatus</i> 400mg/kg)	12.36±0.57**

The graph represents anti anxiety activity of *Sapindusemarginatus* in number of entries to open arm in elevated plus maze. All values are mean ±SEM (n=6); *p< 0.05, **P < 0.01 when compared to control

Table 2: Despair swim test

S.No	Treatment Group	Pre-treatment	1hr	2hr	3hr
1	Control(NS 1ml/kg)	127.5±1.78	127.8±2.97	130.5±2.57	127.3±3.05
2	Standard (Diazepam 0.5mg/kg.i.p)	125.3±3.67	90.5±1.738*	86.7±1.91**	79.5±0.76**
3	Test-I (ESE 200mg/kg.p.o)	110.3±1.36	100.3±1.78	91.3±1.28	86.0±1.53*
4	Test-II (ESE 400 mg/kg, p.o)	107.8±5.87	94.2±1.70	88.8±2.09**	81.6±0.91**

The table represents anti anxiety activity of *Sapindusemarginatus* in despair swim test. All values are mean ±SEM (n=6); *p< 0.05, **P < 0.01 when compared to control

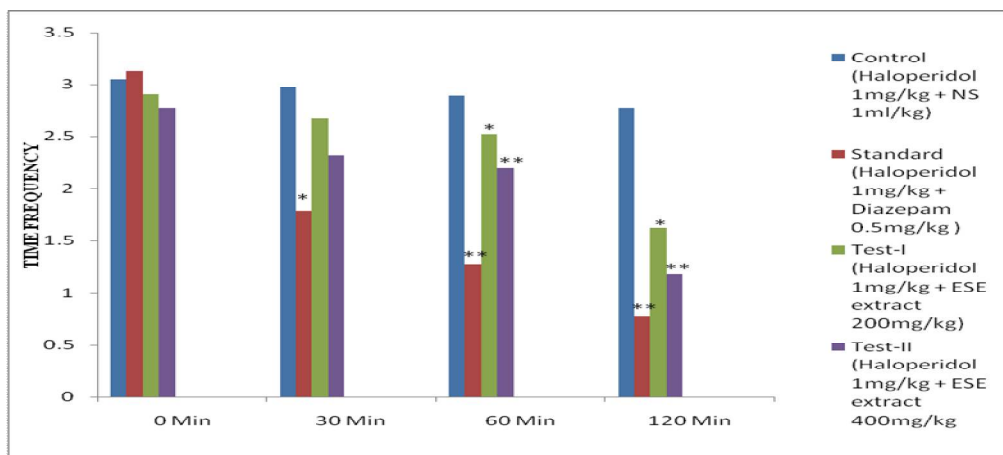


Fig. 1: The graph represents anti anxiety activity of *Sapindusemarginatus* in haloperidol induced catalepsy. All values are mean \pm SEM (n=6); * $p < 0.05$, ** $P < 0.01$ when compared to control

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