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NEUROBEHAVIOURAL SCREENING OF *PTEROSPERMUM ACERIFOLIUM* IN ANIMAL MODELS

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Abstract

Pterospermum acerifolium is common plant in India is considered carminative, stimulant and emmenagogue. In the present study, ethanol extract of bark of *Pterospermum acerifolium* have been evaluated for Neurobehavioural study The CNS activity was assayed in several experimental models for Epilepsy i.e. Threshold Pentylenetetrazole induced seizure, Models for anxiolytic study i.e. Elevated plus maze, Models for nootropic study i.e. Object recognition test, Models for antidepressant study i.e. Forced swim test, Tail suspension test, Models for Muscle relaxant study i.e. Grip-strength test, The ethanol extract significantly and in dose dependent manner reduce the nociception induced by acetic acid. From the present study it was concluded that the herbal drugs can be potentially used to control the state of CNS disorders. Further investigations are, however, necessary to explore mechanism(s) of action involved in these pharmacological activities.

Keywords: *Pterospermum acerifolium,* Neurobehavioural parameters, Epilepsy, Anxiolytic, Muscle relaxant study.

1.1 Introduction

Indian traditional medicine is predicated on phytochemical, pharmacological & allied approaches including instrumental techniques like chromatography, microscopy and others. There is accumulating evidence suggesting medicinal plants are illimitable reservoirs of drugs. Researchers with interest in natural products have intensified their effort to towards scientific evaluation of traditional medicines. There is accumulating evidence suggesting medicinal plants are illimitable reservoirs of drugs. The astonishing structural diversity among their active components makes them a subsidiary source of novel therapeutic compounds India has an antediluvian heritage of traditional medicine. (Mukherjee. et al., 1999) India has an antediluvian heritage of traditional medicine. Materica medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically consequential natural product .Indian traditional medicine is predicated on phytochemical, pharmacological & allied approaches including instrumental techniques like chromatography, microscopy and others. There is accumulating evidence suggesting medicinal plants are illimitable reservoirs of drugs.

Recently available antipsychotics drugs are associated with variety of endocrine, allergic, hematopoietic, autonomic and neurological side effects. So, there is high prevalence of usage of traditional and alternative system of medicines for treatment of psychiatric disorders. In the search for new valuable products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. (Zhang ZJ. Et al.,2004) *Pterospermum acerifolium* is traditionally used to reduce CNS disorders. Generally, plants possess many pharmacological actions, such as Hepatoprotective, antioxidant, anti-inflammatory, anthelmintic, antimicrobial since they contain numerous constituents of active chemicals in it Based on the claim by traditional healers that the plant is effective in the treatment of central nervous system (CNS) diseases. The present project is done to explore the potential of herbal drugs for the treatment of CNS disorders with a view to perform phytochemical investigation and assess Neurobehavioural and Neurochemical screening.

2.0 Experimental

2.1 Plant material

Plants materials *P. acerifolium* bark were collected from the local market of Bhopal, (M.P.) during the month of May –July, 2012. The specimens were identified and authenticated by Dr. Zia ul Hassan, Assistant professor, Department of Botany, Saifia College of Science & Education, Bhopal and their herbarium was deposited. These collected specimens were chosen for the extraction process and assessment of Neurobehavioural activity.

2.2 Extraction

2.2.1 Ethanolic Extraction

The plant materials so collected were cleaned properly and washed with distilled water to remove dust particles and dried in shade. The dried drugs were coarsely powdered and then exhaustively extracted with 50% ethanol in Soxhlet apparatus for 72 h. The ethanolic extracts so obtained were freed of solvent under vacuum. (Yield: 9.33 %)

2.2.3 Qualitative analysis

The Ethanol extracts were screened for the presence of secondary metabolites. Tests was carried out for carbohydrates, reducing sugars, tannins, polyphenols, lipids, flavonoids, ketones, alkaloids, steroids and triterpenes.

3.0 In vivo study

3.1 Animals for experiment

Swiss albino rats were obtained from animal house VNS institute of Pharmacy with due permission from Institutional animal ethical committee (Registration Number. 778/03/c/cpcsa).

Acute toxicity studies were conducted by using albino mice of either sex weighing between 20 and 25 gms and healthy adult male albino rats weighing between150 and 200 gms were selected for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25±20C) and maintained on 12-h light: 12-dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water ad libitum.

3.2 Acute toxicity

In an acute toxicity study of *Allium Sativum* plant extract, animals were given single doses of drug. The Swiss albino rats were divided into groups. All animals fed with standard rat pelleted diet (Lipton India Ltd. pellets) and had free access to tap water *ad libitum.* Acute toxicity studies were performed according to the OECD guidelines. The doses selected for the study were 50 mg/kg, 100 mg/ kg, 200 mg/ kg, 300 mg/kg, 400 mg/kg for one day. Three animals were taken for each dose. It was observed that the extract don't produce any significant effect on the behaviour of rats. The animals were observed for 3 hours after dose administration and also after 24 and 48 hours.

3.3 Screening for Neurobehavioural and Neurochemical screening

Screening for Neurobehavioural and Neurochemical screening was carried out in Wistar albino mice of either sex weighing 100-120 g.

3.3.1 Assessment of Epileptic activity

Wistar albino mice (25-35 g) bred in Central Animal facility of the Institute, were used. They were housed under standard conditions, maintained on a 12 h light/dark cycle and had free access to food and water up to the time of experimentation. The mice were acclimatized to the laboratory environment 1 h before the experiments. All experiments were conducted during the light period (08.00-16.00 h). The ethical committee of the Research Centre having following CPCSEA Reg. No.-778/03/c/CPCSEA approved the usage of animals. During the experiments animals were free access to water only. All the protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

3.3.2 Pentylenetetrazol (PTZ)-induced convulsion test

Animal groups

Four groups of mice (n = 10) were used. Group I was administered the vehicle, i.e., normal saline (1 ml/100g body weight) and served as control, Group II received reference standard (diazepam, 2 mg/kg, i.p.) while Groups III and IV were administered extract 100 and 200 mg/kg, p.o., respectively, of the extracts. Two hours later, PTZ was administered (60 mg/kg, i.p.) to all four groups. The animals were observed for 30 min and the onset and duration of convulsion noted. (Kitano. et al., 2005)

3.3.3 Assessment of anxiolytic study

Animal groups

Elevated plus – Maze test:

Animals were randomly allocated to four experimental groups (n= 5 each). Group 1 and 2 were named as negative and positive control and 2 other groups were termed as treated group. (Dhananjaya. et al., 2011) The test groups received ethanolic extract of *P. acerifolium* at the dose of 200 and 400mg/kg body weight respectively. Group 1 received 1% Tween 80 solution. Group 2 got administration of Diazepam (as a standard drug) at 1mg/kg bodyweight. Drug or vehicle was injected intraperitoneally in a volume of 0.5ml/kg. Testswere performed 30 min after injections. (Navarro JF et al., 2006).

3.3.4 Assessment of nootropic study

Object recognition test

A plastic chamber (35cm×35cm×35 cm) was used in low light condition (about 40 lx) during the light phase of the light/dark cycle. The general procedure, as described elsewhere, consisted of three different phases: a habituation phase, an acquisition phase, and a retention phase. On the 1st day (habituation phase), mice were individually subjected to a single familiarization session of 10 min, during which they were introduced in the empty arena, in order to become familiar with the apparatus. On the 2nd day (acquisition phase) animals were subjected to a single 10-min session, during which floor-fixed two objects (A and B) were placed in a symmetric position in the central line of the arena, 10cm from each and 8 cm from the nearest wall (each object occupies approximately 5 cm space by its size). The two objects, made of the same wooden material with the similar color and smell, were different in shape but identical in size. Mice were allowed to explore the objects in the open field. The exploration time on each object was shown (as seconds) to indicate the exploring activity of mice. On the 3rd day (retention phase), mice were allowed to explore the open field in the presence of two objects: the familiar object A and a novel object C in different shapes but in similar color and size (A and C). A recognition index (for retention session), calculated for each mouse, was expressed as the ratio (TC×100)/(TA + TC), where TA and TC are the time spent during retention phase on object A and object C, respectively. The time spent exploring any object (nose pointing toward the object at a distance≤1 cm, but not mounting on the object or playing with the object) was recorded (using stopwatch) by hand. (Shete. et al., 2010)

3.3.5 Assessment of antidepressant study

Forced Swimming Test (FST)

Mice of either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1°C. The immobility time, defined as the absence of escape oriented behaviors, such as swimming, was scored during 6min with the help of stop-watch, as described previously by (Eckeli *et al*., 2000). All the mice of either sex were divided in five different groups. The first group assigned as control receiving only vehicle (NaCl 5 mL kg-1). The other groups received acute dose of extracts. The total duration of immobility was recorded during the last 6 min of the 10 min period, where the activity in the two first minutes is discarded (Porsolt, 1981). Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect. For the next exposure of crude extracts, FST of repetitive doses of crude extracts were assessed after 3 days of treatment within 30 min after the last dose of administration. During the test session, the immobility time was recorded. The mice were considered immobile when neither hind leg was moving; the mice were slightly hunched forward. Another reason for choosing this animal model is the correlation which is observed between results in this model and clinical potency, which is not found in any other models

(Willner, 1984; Shah et al., 2006). A different interpretation of the FST holds that rats or mice, in this behavioral paradigm, learn to be immobile. Immobility is considered to be an adaptive response to the situation consisting of animals learning to keep their heads out of water with a minimum of energy expenditure (Parra *et al*., 1999).

Tail Suspension Test (TST)

The total duration of immobility induced by tail suspension test was measured according to the method described by Steru *et al*. (1985). Mice both acoustically and visually isolated were suspended 70 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The total immobility period was scored manually during 6 min test session with the help of stopwatch. Immobility was defined as the absence of any limb or body movements, except for those caused by respiration or when they hung passively and completely motionless. The parameter obtained was the number of seconds spent immobile. Parameter used was the number of seconds spent immobile. (Cryan. et al., 2005)

3.3.6 Assessment of Muscle relaxant study

Grip strength test:

A Rota-rod (Inco- Ambala, Instruments and chemicals Pvt. Ltd. Model town, Ambala City -03) was used to measure the grip strength in mice. The instrument (a horizontal rotation device) was set at a rate of 16 revolutions per minute5,6. The animals were placed on the rotating rod and fall off time i.e, when the animal falls from the rotating rod, was recorded, which was taken as grip strength. Diazepam (26 mg/kg i.p.) was used as the standard drug. Grip strength in all the groups was measured before and at 30 minutes, 1 h, 2 h, 4 h, and 6 h of the administration of the dose. (Rogers. et al., 1997)

4.0 Results and discussion

4.1 Qualitative analysis

The preliminary phytochemical screening of extracts revealed the presence of various phyto-constituents. The ethanolic extract of *P.acerifolium* revealed the presence of alkaloids, glycosides, sterols, flavonoids, tannins, phenolic compounds, carbohydrates, fats and oils.

4.2.1 Pentylenetetrazol (PTZ)-induced convulsion test

On the basis of phytochemical investigations, the pharmacological screening was performed on ethanolic extracts of selected plants on Pentylenetetrazol (PTZ)-induced convulsion test.Table 6.5 shows that the extract increased the onset of pentylenetetrazol-induced seizures but reduced the duration of the convulsions.

Table 1: Effect of *P.acerifolium* on pentylenetetrazol (PTZ)-induced seizures in mice

Groups	Onset of convulsion (min)	Duration of convulsion (min)
Vehicle(control)	1.01 ± 0.11	28.2 ± 0.2
Standard (Diazepam)	0 ± 0†	0 ± 0 †
Etoh (100mg/kg)	4.94 ± 0.17#	6.9 ± 0.1#
Etoh (200mg/kg)	1.59 ± 0.13*	16.1 ± 0.1#

†P < 0.001; #p < 0.01; *p < 0.05

The increase in the onset of seizure was statistically significant, $p < 0.01$) and $p < 0.05$) for extract doses of 100 and 200 mg/kg, respectively, and highly significant ($p < 0.001$) for the standard drug, diazepam; with regard to their duration reduction effect, the difference was statistically significant at $p < 0.01$ for both doses. However, the clonus phase was completely abolished only at the lower dose of the extract and also with diazepam.

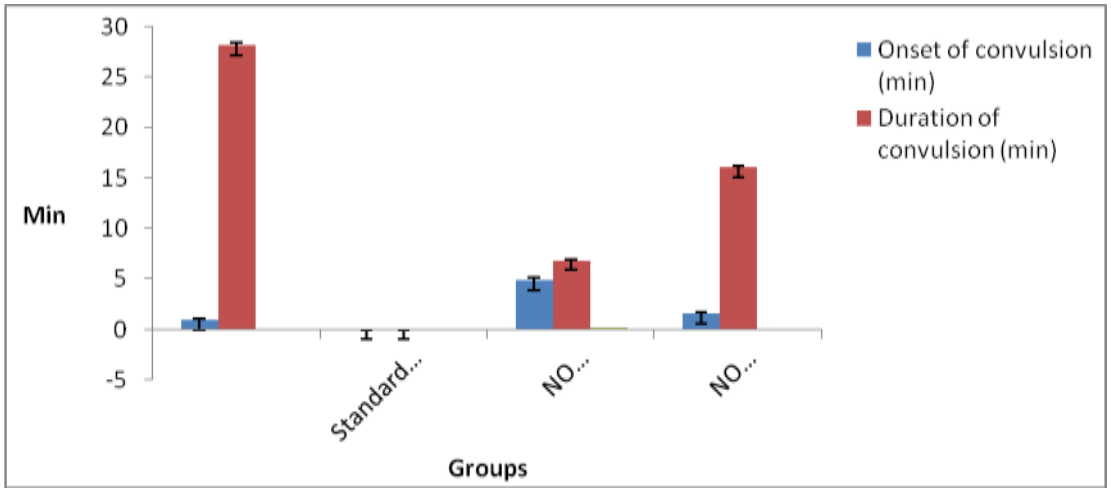

Figure 1: Effect of *P. acerifolium* on pentylenetetrazol (PTZ)-induced seizures

4.2.2 Assessment of anxiolytic study

Elevated plus – Maze test:

From the experiment it was observed that mice taken ethanol at dose of 200 and 400 mg/kg body weight, stayed more time in open arm of Elevated plus Maze apparatus in comparison to standard and negative control group. Moreover they were also stayed less time in closed arm of Elevated plus Maze apparatus in comparison to standard and negative control group. The value obtained from these extract were statistically significant (p<0.05).From the observed result, it could be concluded that ethanolic extract may have anxiolytic activity.

Table 2: Effect of *Pterospermum acerifolium* on Elevated plus Maze experiment

Mean ± SD (counts/5minutes)

Animal Group	Open arm Duration	Frequency of open arm	Closed arm Duration	Frequency of Closed arm
Vehicle(control)	2.6	0.8	257.8±11.39398	8.4±1.719
Standard (Diazepam)	2.4	0.2	261.8±11.39398	7.6±1.719
Etoh (200mg/kg)	16	3.2	207.6±11.39398	13.2±1.719
Etoh (400mg/kg)	8.2	2.6	203.2±11.39398	14.4±1.719

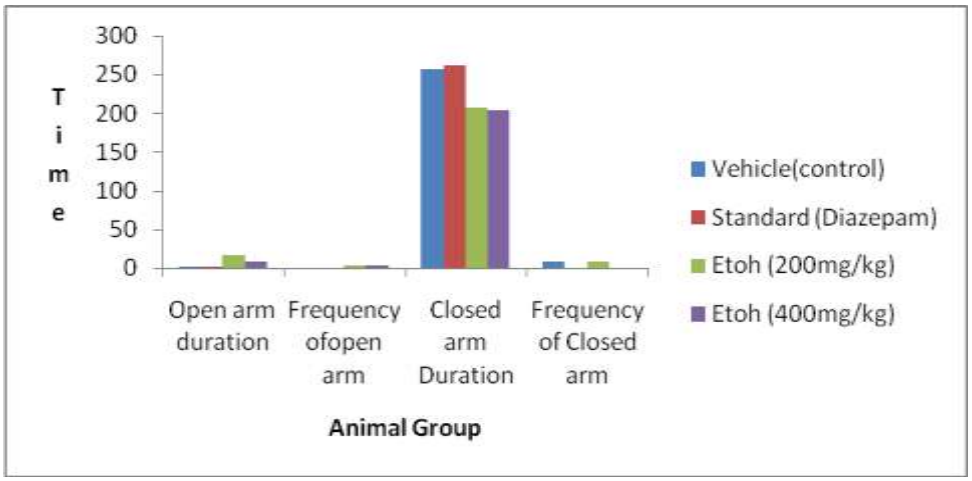

Figure 2: Average time duration in different arms and their frequency in Elevated Plus

4.2.3 Assessment of nootropic study

Object recognition test

The increase in Recognition Index (RI) indicates nootropic effect. The RI of control group was 51.58 ± 1.8 %. Scopolamine (0.3mg/kg) shown significant ($p< 0.05$) decrease in the RI to 30.60 ± 3.2 %. Piracetam (100mg/kg) has significantly ($p<0.01$) increased the RI to 73.8913±2.6 % and also exhibited significant ($p < 0.01$) antagonism of the amnesic effect of scopolamine. The extract in the doses 10 mg/kg increased the RI to 53.70 ± 3.6 % ($p < 0.05$) respectively. Table 6.7

Table 3: Effect of *Pterospermum acerifolium* on object recognition test

Animal Group (Dose in mg/kg)	**Trial-1**	**Trial-2 (sec)**		**Recognition Index**
	Time spent (sec)	**Time spent familiar object**	**Time spent new object**	
Control	20.00±3.05	10.10±0.47	11.10±1.27	51.58±1.84
Scopolamine(0.3)	31.11±3.84	31.98±20.98**	14.45±2.48	30.60±3.24*
Piracetam (100)	36.80±4.04	15.80±2.60	42.80±2.27**	73.89±2.65**
Ethanolic (10)	22.46±5.09	20.45±2.96	24.30±4.58	53.70±3.69

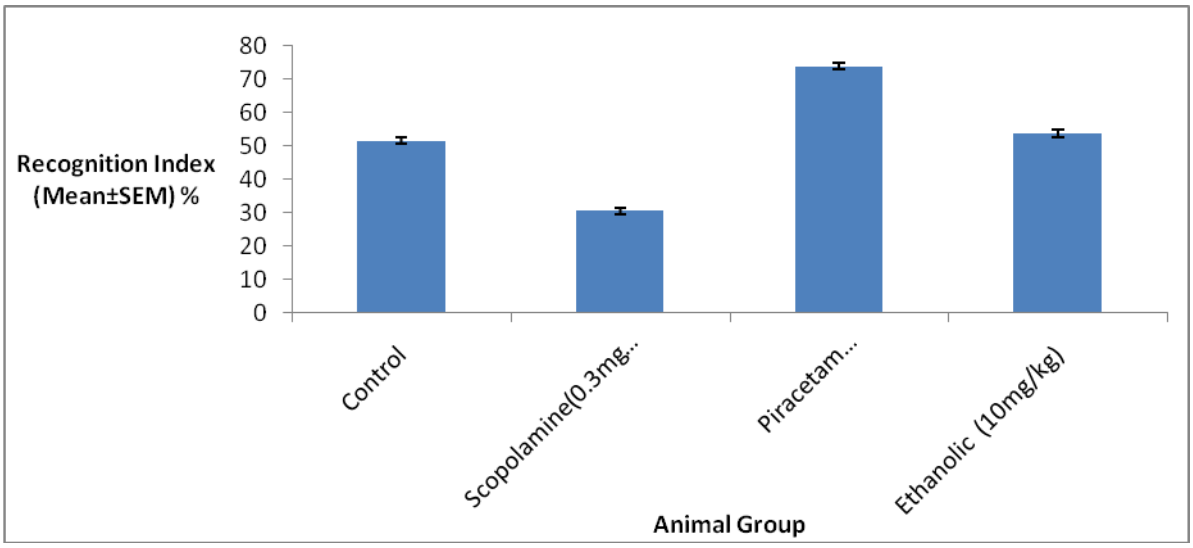

Table 3: Effect of *Pterospermum acerifolium* on object recognition test

4.2.4 Assessment of antidepressant study

Forced Swimming Test (FST)

According to FST results from the first day was shown in Table 6.8. A significant ($p<0.05$) decrease in the duration of immobility was seen with two standard drug haloperidol and fluoxetine and The results obtained after a single administration of the extract suspension showed that the immobility time of mice decreased dose dependently which mice were more active in both employed models and it means that the antidepressant effect was stronger. However, for all two doses administered there were differences compared to the control, that is, they led to reduction of immobility time.

Table 4: Effect of *Pterospermum acerifolium* on Forced Swimming Test (FST)

Animal Group (Dose in mg/kg)	Immobility time (s) at first day	Change (%)	Immobility time (s) at third day	Change (%)
Control(0)	376.0 ± 31.5	-	385.0 ± 52.8	-
Haloperidol(1)	105.7 ± 4.5***	-71.84	74.8 ± 8.2***	-80.62
Fluoxetine(10)	201.7 ± 6.1***	-46.25	200.0 ± 7.0***	-48.06
ET(50)	380.3 ± 21.7ns	0.79	235.3 ± 41.8*	-38.17

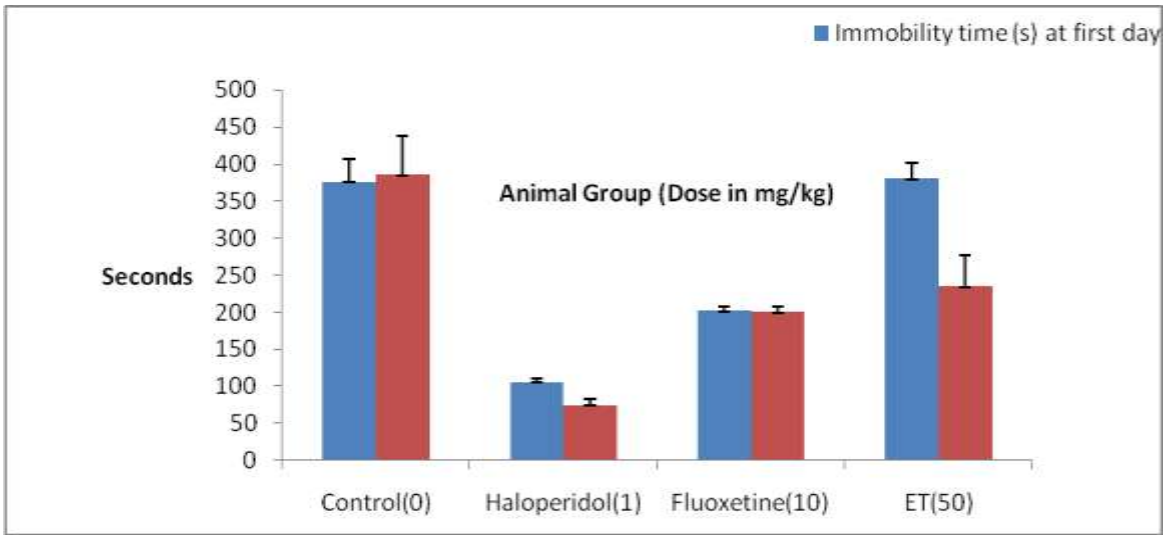

Figure 4: Effect of *Pterospermum acerifolium* on Forced Swimming Test (FST)

Table 5: Effect of *Pterospermum acerifolium* on Tail suspension test (TST)

Animal Group (Dose in mg/kg)	**Immobility time (s) at first day**	**Change (%)**
Control(0)	325.3 ± 30.4	42.39
Haloperidol(1)	63.8 ± 15.1***	-80.39
Fluoxetine(10)	97.8 ± 11.5***	-69.97
ET(50)	187.0 ± 9.5***	-42.39

a Mean±SD, n = 3; Statistically significant difference compared to the control group of animals: *** p<0.05, ** p<0.01, * p<0.001 and ns = Data are not different significant compared to control (vehicle)

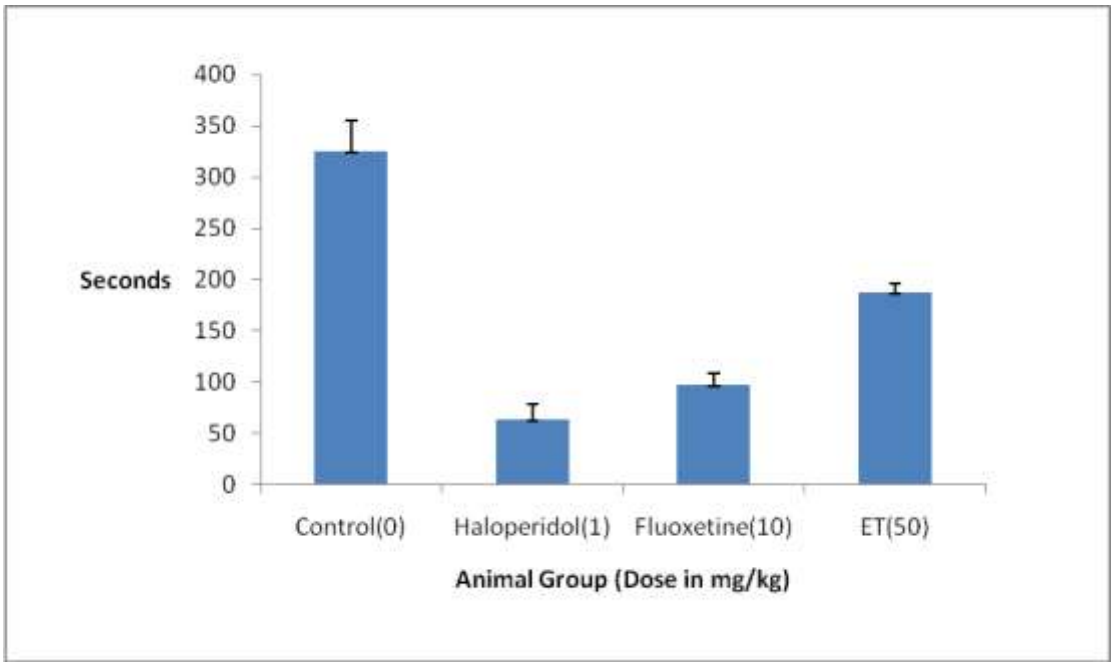

Figure 5: Effect of *Pterospermum acerifolium* on Tail suspension test (TST)

4.2.5 Assessment of Muscle relaxant study

Grip Strength Test

Grip strength in seconds

Group	Treatment	Dose (mg/kg)	0 hr	30 min	1 hr	2 hr	4 hr	6 hr
1	Control	--	357.17 ± 11.620	369.33 ± 19.416	378.23 ± 12.456	380.50 ± 12.132	376.33 ± 11.071	360.17 ± 19.511
2	Diazepam	26	369.50 ± 18.936	* 201.17 ± 13.893	* 154.17 + 13.946	** 124.23 ± 11.923	** 100 ± 33 ± 4.310	** 102 ± 33 ± 4.310
3	Ethanolic extract	200	331.50 ± 12.233	* 308.17 ± 10.778	* 325.23 ± 9.127	ns 360.01 ± 11.923	358.50 ± 10.987	337.23 ± 6.940

Values are mean ± SME; n=6 in each group; *significantly different at $p<0.05$;** significantly different at $p<0.01$; ns-Non.significant

Table 6: Effect of *Pterospermum acerifolium* on Grip Strength Test

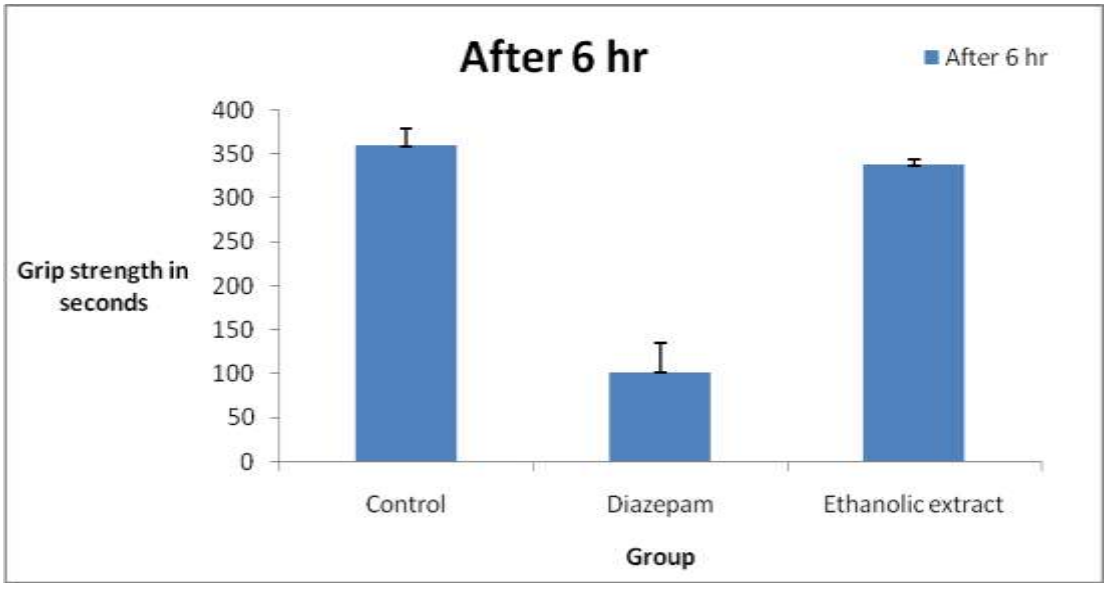

Figure 6: Effect of *Pterospermum acerifolium* on Grip Strength Test

5.0 Conclusion

The preliminary phytochemical screening of extracts revealed the presence of various phyto-constituents. The ethanolic extract of *P.acerifolium* revealed the presence of alkaloids, glycosides, sterols, flavonoids, tannins, phenolic compounds, carbohydrates, fats and oils. The pharmacological screening was performed on ethanolic extracts of selected plants to confirm the Neurobehavioural and Neurochemical screening of *P.acerifolium* ethanolic extract the extract increased the onset of pentylenetetrazol-induced seizures but reduced the duration of the convulsions. The increase in the onset of seizure was statistically significant, $p < 0.01$) and $p < 0.05$) for extract doses of 100 and 200 mg/kg, respectively, and highly significant ($p < 0.001$) for the standard drug, diazepam; with regard to their duration reduction effect, the difference was statistically significant at $p < 0.01$ for both doses. However, the clonus phase was completely abolished only at the lower dose of the extract and also with diazepam.

From the experiment it was observed that mice taken ethanol at dose of 200 and 400 mg/kg body weight, stayed more time in open arm of Elevated Plus Maze apparatus in comparison to standard and negative control group. Moreover they were also stayed less time in closed arm of Elevated plus Maze apparatus in comparison to standard and negative control group. The value obtained from these extract were statistically significant ($p<0.05$).From the observed result, it could be concluded that ethanolic extract may have anxiolytic activity.

The increase in Recognition Index (RI) indicates nootropic effect. The RI of control group was 51.58±1.8 %. Scopolamine (0.3mg/kg) shown significant ($p< 0.05$) decrease in the RI to 30.60 ± 3.2 %. Piracetam (100mg/kg) has significantly ($p<0.01$) increased the RI to 73.89 ±2.6 % and also exhibited significant ($p < 0.01$) antagonism of the amnesic effect of scopolamine. The BF in the doses 10 mg/kg increased the RI to 53.70 ± 3.6 % ($p < 0.05$) respectively.

According to FST results from the first day was shown in Table 6.8 A significant ($p<0.05$) decrease in the duration of immobility was seen with two standard drug haloperidol and fluoxetine and The results obtained after a single administration of the extract suspension showed that the immobility time of mice decreased dose dependently which mice were more active in both employed models and it means that the antidepressant effect was stronger. However, for all two doses administered there were differences compared to the control, that is,

they led to reduction of immobility time. However, ET exhibited highest decrease of duration immobility time in TST (77.61%) followed by haloperidol as a positive control (80.62%).

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