Vol 1|Issue 1| 2011 |21-32.

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

International Journal of
Current Pharmaceutical & Clinical Research

www.ijcpcr.com

ANXIOLYTIC AND CNS DEPRESSANT ACTIVITY OF EXTRACTS OBTAINED FROM SEEDS OF *ZIZIPHUS RUGOSA*

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ABSTRACT

*Ziziphus rugosa*is found in India as well as other Asian countries, literature survey revealed that no work has been evaluated for its therapeutic effects against behavioral disorders. In this investigation, the neuropharmacological profile of petroleum ether, chloroform and ethanolic extracts of aerial parts of *Ziziphus rugosa Wild* was evaluated by using models like; potentiation of phenobarbitone-induced sleeping time, loco motor activity, exploratory behavior pattern, elevated plus maze and hole-board apparatus. The observations and results obtained in this study indicated that *Ziziphus rugosa* pet ether extract at doses 100mg/kg and 400mg/kg has given the restrained central activity.

Keywords: *Ziziphus rugosa*, CNS Depressant, Anti-Anxiety, Saponins.

INTRODUCTION

Mental health is defined as a state of well-being in which every individual realize his or her own potential, can cope with the normal stresses of life, can work productively and fruitfully and is able to make a contribution to her or his community. Mental, neurological and behavioral disorders are common to all countries and cause immense suffering. People with these disorders are often subjected to social isolation poor quality of life and increased mortality. These disorders are the cause of staggering economic and social costs. Mental health has gain recognition as a major public health problem only in recent years. Mental disorders contributes a wide spectrum ranging from mild anxiety states to very severe forms of behavioral and thought abnormalities. The causes of mental disorders vary from condition to condition. Despite newer technological understanding there enigma of the causation of mental disorders continues as a complex interaction of the brain, mind and milieu [1].

As the Indian health care scene has inherited a large number of traditional practices, systems and medicines as part of its total health care scenario, medicinal plants which are endogenous to India and referred as wealth of India can be used for their valuable contribution in the central nervous system disorders. Based on the strong traditional knowledge on the use of plants as therapeutic agents and the phytoconstituents represented by the plants, a rational approach is being developed to use medicinal plants as lead for the discovery of active molecules with one of the largest reservoirs of bio-resources [10].

Since herbal remedies are back into prominence today, because of the efficacy of conventional medicines, it is imperative that India develops a concerned, integrated, structural and modern approach in this area and gains a competitive edged in the international market place for the discovery and development of plants based drugs for a variety of diseases for which currently adequate or appropriate remedies are not available [2].

Traditionally it was mentioned that *Ziziphus rugosa* was used as potent sedative and other species also evaluated for CNS activities so present study is designed to evaluate the anxiolytic and sedative property of extracts of *Ziziphus rugosa* Wild. With its probable mechanism of action [3-5]

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e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The seeds of the plant collected from the forest near the MalshejGhat. The sample of *Ziziphus rugosa* Wild was identified and authenticated at Botanical survey of India, Pune.

EXTRACTION[8,9]

Freshly collected seeds of the plant *Ziziphu srugosa* Wild.were washed, shade dried under room temperature for a period of three weeks. The dried plant material was made to a coarse powder and weighted quantity of the powder (400 g) was subjected to hot percolation in a soxhlet apparatus using petroleum ether, chloroform and ethanol, at a temperature range of 40-80^{o}C. Before and after every extraction, the marc was completely dried and weighed. The extracts were concentrated by evaporation of solvent at room temperature. The percentage yield of petroleum ether extract, chloroform extract and ethanolic extract were found to be 1.4 %, 1.7 % and 2.3 % w/w respectively.

PHARMACOLOGICAL ACTIVITY

A) PREPARATION OF DOSAGE FORM

Petroleum ether extract, chloroform extract and methanolic extracts of *Ziziphus rugosa* Wild.*(*seeds) were used for studies and their dosage forms is prepared as follows.

The suspension of petroleum ether extract was prepared by triturating (blending) the accurate weighed quantity of the extract with 2.5% Tween 80 in a glass mortar, with the gradual addition of water for injection to make up the required volume. The same procedure was followed for preparing the chloroform and ethanolic extract.

Vehicles to be administered to respective control groups, were prepared using the same procedure without addition of extract.

The dosage forms of the extract were prepared freshly on the day of experiment and kept at room temperature in air-tight vials. The standard drug dose i.e. for diazepam and phenobarbitone were prepared by using water for injection.

B) ANIMALS

Swiss albino male mice (20-25 g) were obtained from the Animal House of SharadchandraPawar College of Pharmacy, Otur.The animals were housed in groups of six per polypropylene cages and maintained at 24^{o}C $\pm$ 1^{o}C with the relative humidity of 45-55 % and 12:12 h dark light cycle. The experiments were carried out between 10:00 to 17:00 h. The animals had free access to food (standard chew pallets, VRK Nutritional Solutions, Pune) and water *ad libitum*. Food but not water has been withdrawn as per the necessity of experiment. The institutional animal ethics committee (No.1197/c/08CPCSEA) of SharadchandraPawar College of Pharmacy, Otur approved the Pharmacological and acute toxicity protocol.

C) LD[18]DETERMINATION[11]

Acute oral toxicity has been evaluated, for petroleum ether extract, chloroform extract and ethanolic extract.

Procedure

Acute oral toxicity study of pet extract, chloroform extract and ethanolic extract was determined by using nulliparous, non-pregnant female mice. As per the OECD guidelines a stepwise procedure with the use three animals per step was followed. Absence or presence of compound related mortality of the animals dosed at one step will determine the next step i.e.

- No further testing is needed
- Dosing of 3 additional animals, with the same dose
- Dosing of 3 additional animals at the next higher or the next lower dose level.

The mice were fasted overnight and the drug was administrated through oral route at the dose level of 5 mg/kg, 50 mg/kg, 400 mg/kg, 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg, of body weight. The animals were observed individually after dosing once in 30 minutes periodically during the first 2 hrs. The special attention was given during the first four hours and observed for 24 hours.

D) NEUROPHARMACOLOGICAL EVALUATION

a) Potentiation of Phenobarbitone induced sleeping time [5]

This test is used to elucidate CNS- active properties of drugs. Not only hypnotics, sedatives, and tranquilizers but also antidepressants at higher doses can be evaluated as they potentiate the sleeping time. Many of the pharmacological testes are based on the potentiation of sleeping time induced by barbiturates or other sedative agent.

Procedure

The animals were divided into 7 groups each containing 6 mice.

Group I –received vehicle (2.5% Tween 80) and Phenobarb 40 mg/kg,i.p.

Group II - received pet. ether extract 100 mg/kg, p.o. and Phenobarb 40 mg/kg, i.p.

Group III – received pet. ether extract 400 mg/kg, p.o and Phenobarb 40 mg/kg, i.p.

Group IV - received chloroform extract 100 mg/kg, p.o. and phenobarb 40 mg/kg, i.p.

Group V - received chloroform extract 400 mg/kg, p.o. and Phenobarb 40 mg/kg i.p.

Group VI- received ethanolic ether extract 100 mg/kg, p.o.and Phenobarb 40 mg/kg, i.p.

Group VII- received ethanolic extract 400 mg/kg, p.o.and Phenobarb 40 mg/kg, i.p.

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

Each animal was observed and onset of sleep and duration of sleep was recorded. Sleeping time in all cases was measured as the time interval between the loss and regaining of righting reflex.

b) Spontaneous Motor Activity (SMA)[14,15]

Spontaneous motor activity was performed using Actophotometer (Inco, Ambala, India). The central nervous system depressant or stimulant property can be evaluated by considering locomotor activity of the animal after treating with drug. Mice were grouped of six each and treated with vehicle or plant extracts or received diazepam.The treatment schedule is as follows.

Group I - served as control and received vehicle orally. (2.5 % Tween 80).
Group II - served as standard and received diazepam 4mg/ kg, i.p.
Group III - received pet. ether extract 100 mg/kg, p.o.
Group IV - received pet. ether extract 400 mg/kg, p.o.
Group V - received chloroform extract 100 mg/kg, p.o.
Group VI - received chloroform extract 400 mg/kg, p.o.
Group VII - received ethanolic extract 100 mg/kg, p.o.
Group VIII - received ethanolic extract 400 mg/kg, p.o.

The locomotor activity for each animal was automatically recorded for 10 minutes before drug treatment and after the treatment at 30 minute interval for total 90 minutes. Results of the treated groups were compared with those of control group at each time interval.

C) Exploratory Behaviour Pattern [14,18]

The study was carried out using wooden board measuring 40 x 40 cm with 16 evenly spaced holes 8 groups of mice containing 6 in each were used for study and they were treated as follows.

Group I - served as control and received vehicle orally. (2.5 % Tween 80).
Group II - served as standard and diazepam 1mg/kg was given i.p.
Group III - received pet. ether extract 100 mg/kg, p.o.
Group IV - received pet. ether extract 400 mg/kg, p.o.
Group V - received chloroform extract 100 mg/kg, p.o.
Group VI - received chloroform extract 400 mg/kg, p.o.
Group VII - received ethanolic extract 100 mg/kg, p.o.
Group VIII - received ethanolic extract 400 mg/kg, p.o.

The total head dips for before any treatments, 30 minutes after diazepam treatment and 45 minutes after extract treatment were recorded for 5 min by placing the animal on a board with 16 evenly spaced holes. Results were expressed as means for the various treatment groups at different time intervals.

e) Elevated Plus Maze (EPM) [17, 19, 20, 21]

Elevated plus maze is the most simple apparatus to study anxiolytic response of almost all type of antianxiety agents. Exposure of the animals to novel maze alley evokes an approach – avoidance conflict which is stronger in open arm as compared to enclosed arm. Rodents (Rats and mice) have aversion for high and open space and prefer enclosed arm, therefore, spends more time in enclosed arm. Anxiolytic compounds by decreasing anxiety increase the open arm entries of exploration time.

The EPM apparatus consists of two open arms (30 x 5 cm) and two closed arms (30 x 5 x 20 cm) emanating from a common central platform (5 x 5 cm). The two pairs of identical arms were opposite to each other. The entire apparatus was elevated to a height of 50 cm above the floor level. The animals received the treatment as per the following schedule.

Group I - served as control and treated orally with vehicle (2.5 % Tween 80).
Group II - served as standard and diazepam 1mg/ kg was given i.p.
Group III - received pet. ether extract 100 mg/kg, p.o.
Group IV - received pet. ether extract 400 mg/kg, p.o.
Group V - received chloroform extract 100 mg/kg, p.o.
Group VI - received chloroform extract 400 mg/kg, p.o.
Group VII - received ethanolic extract 100 mg/kg, p.o.
Group VIII - received ethanolic extract 400 mg/kg, p.o.

The animals received the treatment 45 min before the start of session. At the beginning of the session, a mouse was placed at the centre of the maze, its head facing the closed arm. It was allowed to explore the maze for 5 min. The time spent in open arm, percent entries in the open and closed arms and total entries were recorded. An entry was defined as the presence of all four paws in the arm. The EPM was carefully wiped, with 10 % ethanol after each trial, to eliminate the possible bias due to the odour of the previous animals.

$$\text{Percent time spent in open arm} = \frac{\text{Time in the open arm}}{\text{Time in the open arm} + \text{time in the closed arm}} \times 100$$

STATISTICAL ANALYSIS[22]

The data obtained were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. $P < 0.05$ was considered statistically significant.The results are expressed in mean $\pm$ SEM of six animals from each group [23].

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print-ISSN 2248 – 9134

RESULTS

Table1. Qualitative chemicalevaluation of *Ziziphus rugosa* Wild

Sr.No	Test / Reagents used	Pet. ether extract	Chloroform extract	Ethanol extract
1	Carbohydrate Molisch' test	-	-	+
2	Glycosides a) Legal's Test b) Borntrager's Test	+ +	- -	- -
3	Alkaloids a) Wagner's Test b) Dragondorff's Test c) Mayers Test d) Hanger's reagent.	- - - -	+ + + +	- - - -
4	Phytosterols a) Salkowaski Test b) Libermenn's Test	+ +	+ +	+ +
5	Saponins	+	-	-
6	Tannins a) Gelatin Test b) Ferric Chloride Test	- -	- -	+ +
7	Saponins a) Shinoda's Test	+	-	-
8	Proteins a) Biuret Test b) Ninhydin Test	+ +	- -	- -

Table 2.

LD[18]**Determination for Pet ether extract of *Ziziphus rugosa* Wild.**

Group	Dose	Number of animals dead	% Animals dead
I	5 mg/kg	0	0
II	50 mg/kg	0	0
III	100 mg/kg	0	0
IV	200 mg/kg	0	0
V	400 mg/kg	0	0
VI	1000 mg/kg	0	0
VII	2000 mg/kg	0	0
VIII	4000 mg/kg	0	0

Table 3.

LD[18]**Determination for Chloroform extract of *Ziziphus rugosa*(seed) Wild.**

Group	Dose	Number of animals dead	% Animals dead
I	5 mg/kg	0	0
II	50 mg/kg	0	0
III	100 mg/kg	0	0
IV	200 mg/kg	0	0
V	400 mg/kg	0	0
VI	1000 mg/kg	0	0
VII	2000 mg/kg	0	0
VIII	4000 mg/kg	0	0

e-ISSN 2248 – 9142
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Table 4. LD[18]**Determination for Ethanolic extract of *Ziziphus rugosa* Wild.**

Group	Dose	Number of animals dead	% Animals dead
I	5 mg/kg	0	0
II	50 mg/kg	0	0
III	100 mg/kg	0	0
IV	200 mg/kg	0	0
V	400 mg/kg	0	0
VI	1000 mg/kg	0	0
VII	2000 mg/kg	0	0
VIII	4000 mg/kg	0	0

Table 5Effect of *Ziziphus rugosa* Wild on Phenobarbitone induced sleeping time

Group	Treatment	Onset of action (min)	Duration of Action (min)
I	Control Phenobarbitone 40 mg/kg	6.5 ± 0.28	98.26± 1.7
II	Pet. Ether extract 100mg/kg + Phenobarbitone40 mg/kg	7.0 ± 0.40	110.25± 1.5*
III	Pet. Ether extract 400 mg/kg + Phenobarbitone 40 mg/kg	6.5 ± 0.28	149.34± 1.7**
IV	Chloroform extract 100 mg/kg + Phenobarbitone 40 mg/kg	6.75 ± 0.47	95.75 ± 1.5
V	Chloroform extract 400 mg/kg + Phenobarbitone 40 mg/kg	5.5 ± 0.8	98.35 ± 2.75
VI	Ethanolic extract 100mg/kg + Phenobarbitone 40 mg/kg	5.28 ± 0.25	97.23 ± 2.7
VII	Ethanolic extract 400mg/kg + Phenobarbitone 40 mg/kg	4.25 ± 0.25	94.25 ± 1.2

Each value represents the mean ± SEM (n=6)
** Values are significantly different at $p < 0.01$
The data obtained were analyzed using one way analysis of variance (ANOVA) followed byDunnett's't'Stest. $P < 0.05$was considered statistically significant.

Table 6. Effect of *Ziziphus rugosa* Wild on Spontaneous motor activity

Group I	Treatment	Experimental Mean Time (min)			
		0	30	60	90
I	Vehicle Control	162.3 ± 1.256	168.5 ± 6.556	166.2 ± 3.790	163.5 ± 1.85
II	Diazepam 4mg/kg	169.3 ± 4.470	56.17 ± 1.352**	26.12 ± 1.401**	21.83 ± 0.792**
III	Pet ether extract 100 mg/kg	163.23 ± 1.65	169.3 ± 4.379	146.8 ± 2.522*	140.7 ± 2.985*
IV	Pet ether extract 400 mg/kg	168.4 ± 6.24	150.2 ± 2.951**	91.67 ± 1.470**	83.17 ± 0.915**
V	Chloroform extract 100 mg/kg	165.3 ± 3.169	160.5 ± 1.776	162.3 ± 1.256	164.0 ± 1.732
VI	Chloroform extract 400 mg/kg	164.4 ± 2.033	162.3 ± 2.092	161.5 ± 1.335	165.5 ± 1.544
VII	Ethanolic extract 100 mg/kg	164.7 ± 2.033	166.5 ± 3.713	164.3 ± 2.89	164.8 ± 5.203
VIII	Ethanolic extract 400 mg/kg	162.2 ± 1.701	161.7 ± 1.453	169.8 ± 4.400	167.3 ± 3.451

Each value represents the mean ± SEM (n=6)
* Values are significantly different at $p < 0.05$
** Values are significantly different at $p < 0.01$
The data obtained were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. $P < 0.05$ was considered statistically significant.

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

Table7. Effect of *Ziziphus rugosa* Wild on Exploratory behavior (Head dip test)

Group	Treatment	Head dips in 5 min	
		Before treatment	**After treatment**
I	Vehicle (Control)	22.67 ± 0.171	23.83 ± 1.19
II	Diazepam 1mg/kg	24.83 ± 1.44	41.00 ± 0.77**
III	Pet ether extract 100 mg/kg	23.67 ± 0.98	35.83 ± 0.83**
IV	Pet ether extract 400 mg/kg	24.00 ± 1.45	10.33 ± 0.98
V	Chloroform extract 100 mg/kg	23.00 ± 0.73	22.50 ± 1.38
VI	Chloroform extract 400 mg/kg	22.67 ± 0.71	19.67 ± 0.66
VII	Ethanolic extract 100 mg/kg	21.83 ± 0.69	25.33 ± 0.71
VIII	Ethanolic extract 400 mg/kg	23.75 ± 0.966	22.00 ± 0.73

Each value represents the mean ± SEM (n=6)
* Values are significantly different at $p < 0.05$
** Values are significantly different at $p < 0.01$
The data obtained were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. $P < 0.05$ was considered statistically significant.

TABLE 8. Effect of *Ziziphus rugosa* Wild on Elevated plus maze

Group	Treatment	No.of entries in open arm	No of entries in closed arm	Time spent in open arm(sec)	Time spent in closed arm (sec)	Total entires
I	Control	2.5 ± 0.25	7.75 ± 0.75	14.5 ± 1.44	287.25 ± 1.10	10.25 ± 0.85
II	Diazepam 1mg/kg	20.25 ± 0.85**	11.40 ± 0.40	96.25 ± 2.28**	205.5 ± 2.90**	31.25 ± 1.25**
III	Pet ether extract 100 mg/kg	13.75 ± 0.62**	10 ± 0.91	80.5 ± 3.75**	225.5 ± 3.5**	24.25 ± 0.62**
IV	Pet ether extract 400 mg/kg	1.66 ± 0.21	3.66 ± 0.55	8.66 ± 0.44	288.20 ± 0.70	3.2 ± 1.25
V	Chloroform extract 100 mg/kg	8.25 ± 0.85**	7.25 ± 0.62	70.25 ± 1.75**	230 ± 2.32**	13.75 ± 1.25
VI	Chloroform extract 400 mg/kg	8.25 ± 0.85**	7.25 ± 0.62	70.25 ± 1.75**	230 ± 2.32**	13.75 ± 1.25
VII	Ethanolic extract 100 mg/kg	4.40 ± 0.40	8 ± 0.70	13.5 ± 1.32	287 ± 1.57	12 ± 0.91
VIII	Ethanolic extract 400 mg/kg	2.00 ± 2.5	6.83 ± 0.65	13.33 ± 1.32	285.33 ± 1.35	8.8 ± 0.65

Each value represents the mean ± SEM (n=6)
** Values are significantly different at $p < 0.01$
* Values are significantly different at $p < 0.05$
The data obtained were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. $P < 0.05$ was considered statistically significant.

Table 9. Effect of *Ziziphus rugosa* Wild on percent closed arm entries and percent time spent in open arm

Group	Open arm entries	Percentage closed arm entries	Percentage time spent in open arm
I	24.54 ± 2.42	75.45 ± 2.43	4.8 ± 0.94
II	64.78 ± 0.29**	35.22 ± 0.29**	31.84 ± 1.6**
III	56.71 ± 2.32**	43.29 ± 2.32**	26.56 ± 2.43**
IV	13.33 ± 5.22	97.31 ± 5.22	2.95 ± 0.57
V	33.25 ± 2.74	66.75 ± 2.75	4.49 ± 0.89

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

Group	Open arm entries	Percentage closed arm entries	Percentage time spent in open arm
VI	33.03 ± 2.98	66.97 ± 2.98	4.42 ± 1.34
VII	33.52 ± 4.67	66.22 ± 4.67	4.97 ± 0.60
VIII	34.49 ± 2.56	40.50 ± 2.57	4.54 ± 2.15

Each value represents the mean ± SEM (n=6)
** Values are significantly different at $p < 0.01$
The data obtained were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. $P < 0.05$ was considered statistically significant.

Fig 1 – Effect of *Ziziphus rugosa* Wild on Phenobarbitone induced sleeping time

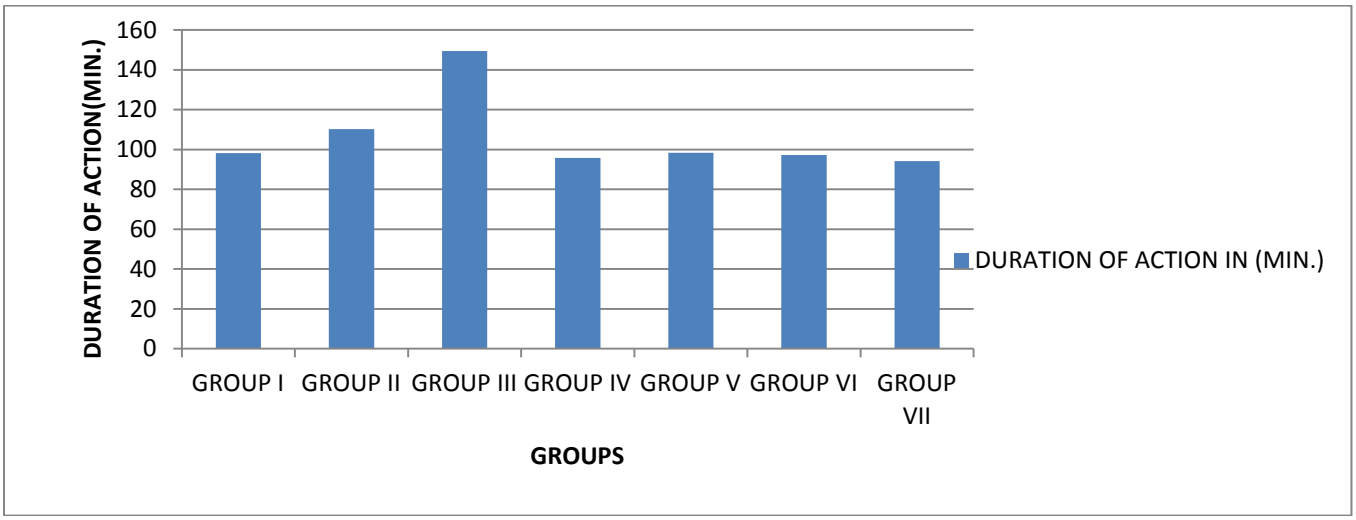
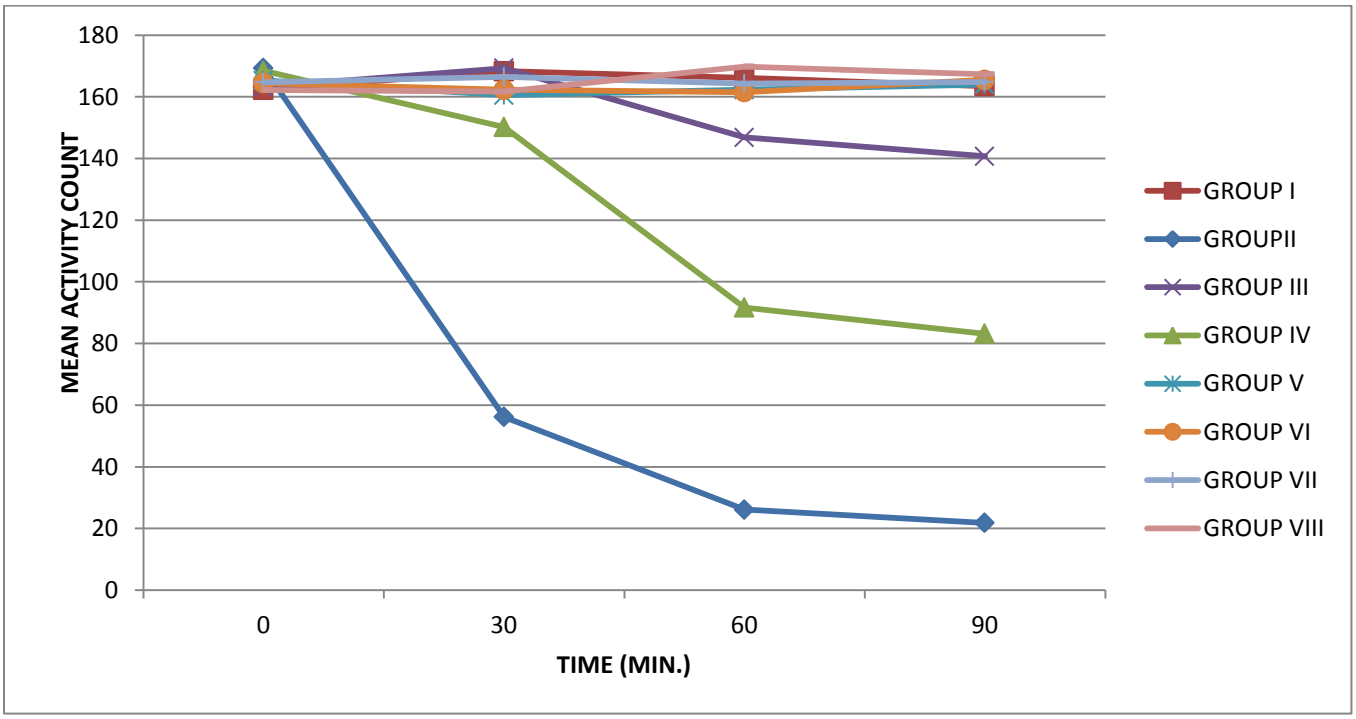


Fig. 2- Effect of *Ziziphus rugosa* Wild on Spontaneous motor activity



e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

Fig.3- Effect of *Ziziphus rugosa* Wild on Exploratory behavior (Head dip test)

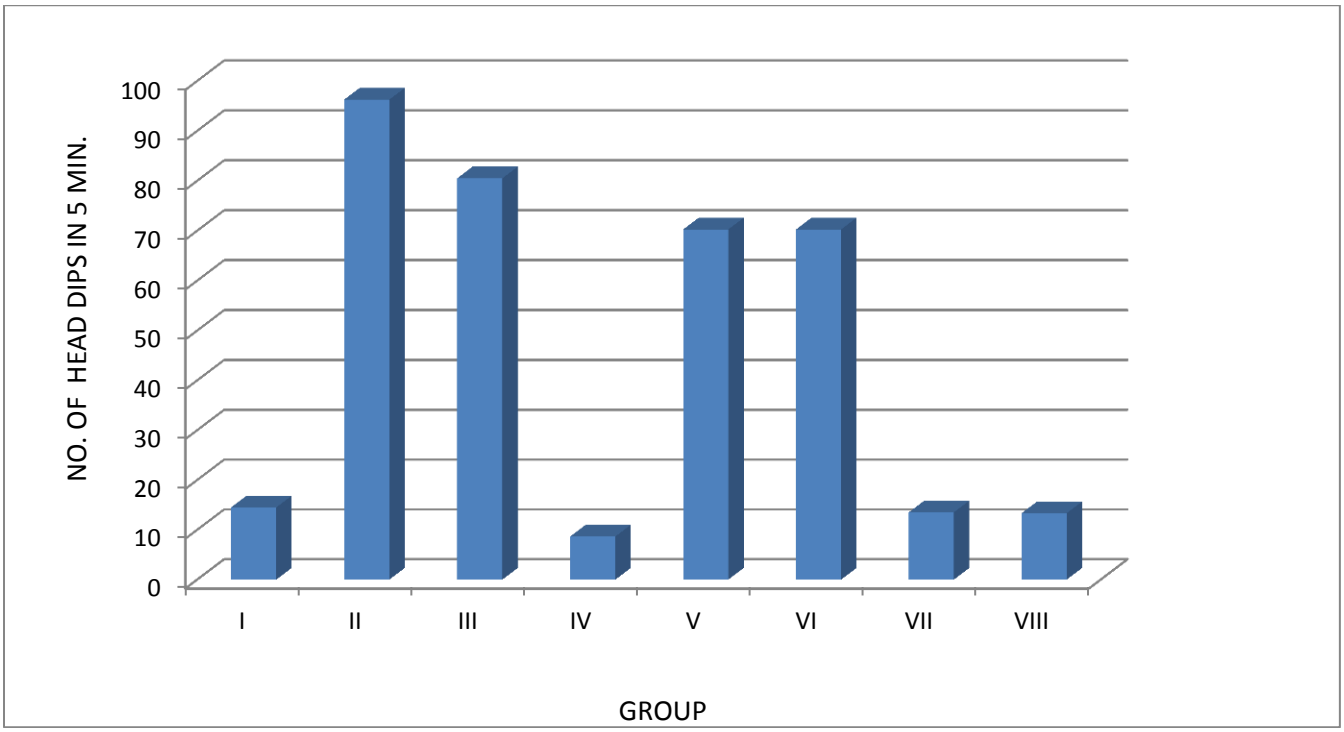
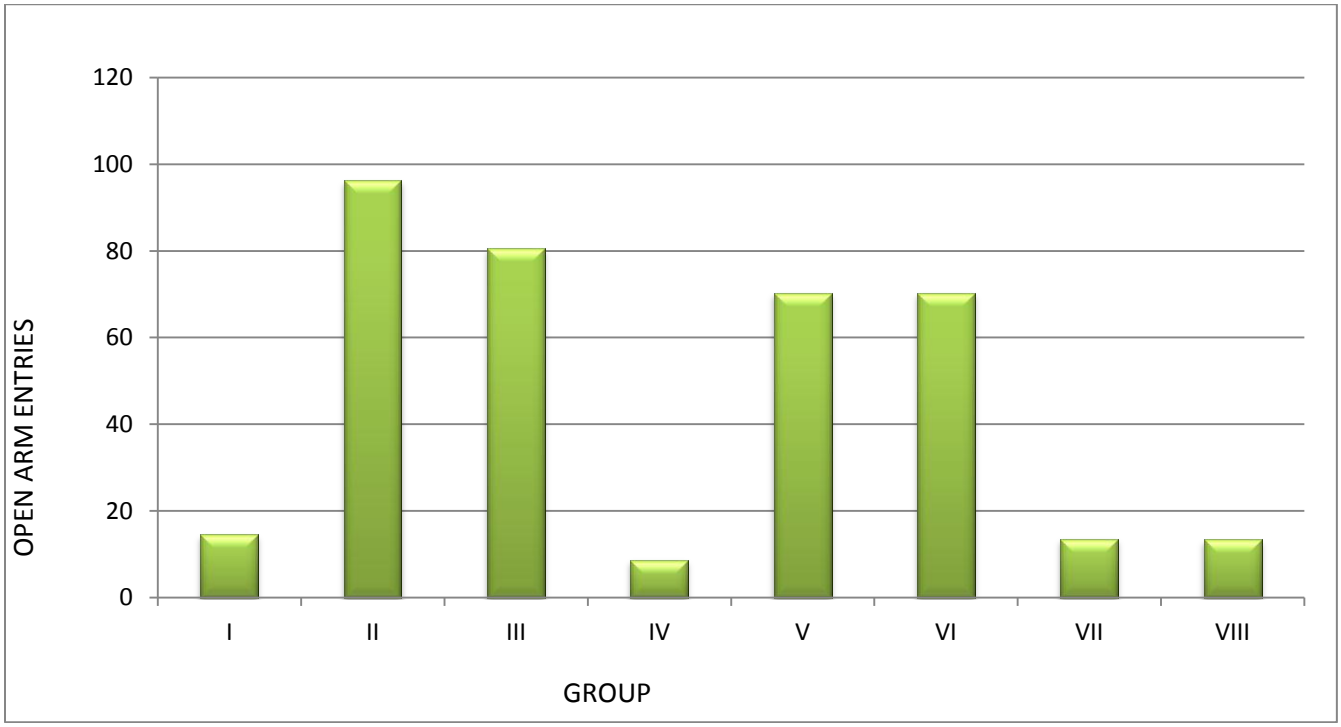


Fig. 4- Effect of *Ziziphus rugosa* Wild on open arm entries



e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

Fig. 5- Effect of *Ziziphus rugosa* Wild on time spent in open arms

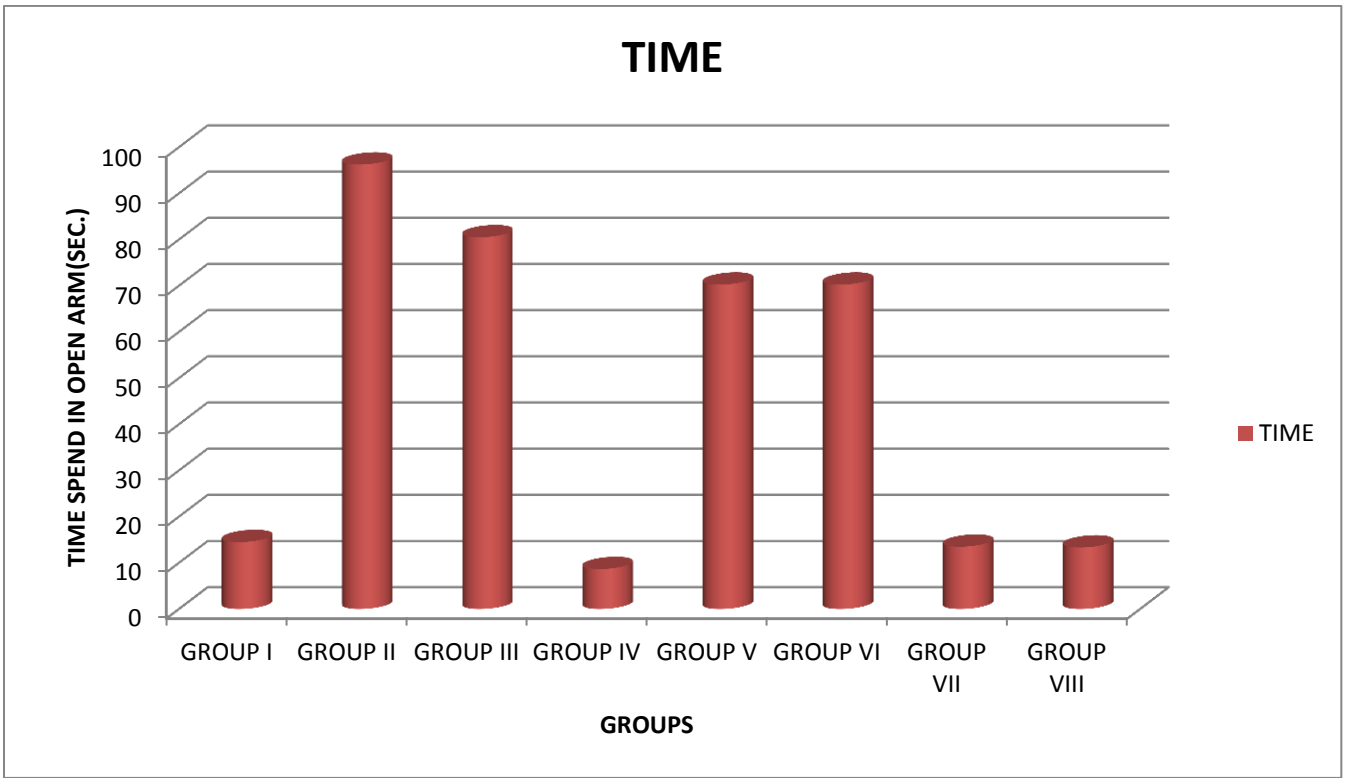
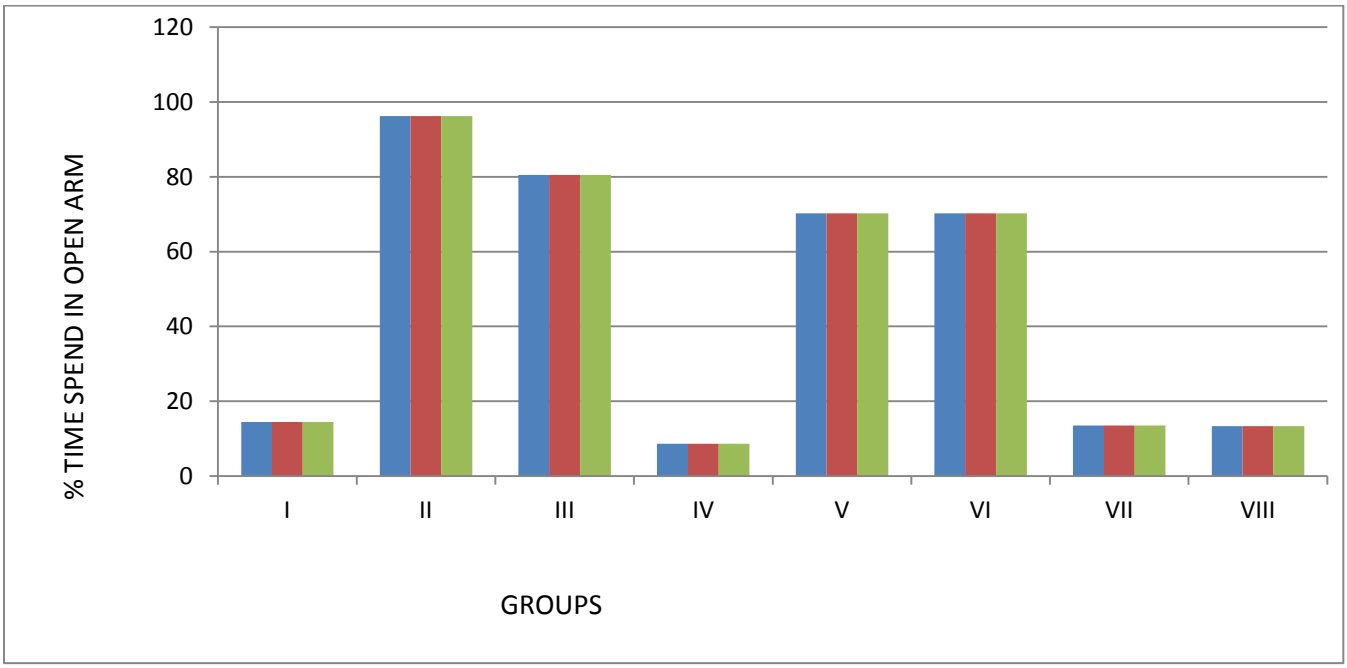


Fig.6 - Effect of *Ziziphus rugosa* Wild on percent time spent in open arm



e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

DISCUSSION

Preliminary Phytochemical Tests

Phytochemical screening were carried out with petroleum ether, chloroform and ethanol extracts of *Ziziphus rugosa* Wild. Results were tabulated in Table 1.
The petroleum ether extract showed the presence of saponins,tannins, etc. Chloroform extract showed the presence of alkaloids, phytosterols. In ethanolic extract phytosterols, carbohydrates, tannins and flavonoids were found to be present. The colour reactions also indicated the presence of saponins in petroleum ether extract.

Acute oral Toxicity

Acute oral toxicity was evaluated for petroleum ether, chloroform and ethanol extract. The administration of various doses of these extract of *Ziziphus rugosa* to the dose of 4000 mg/kg body weight has not produced any signs of toxicity and mortality. So we used lower dose as 100 mg/kg and higher dose 400mg/kg for the study. Results were tabulated in Table 2.

Potentiation of Phenobarbitone induced sleeping time

Petroleum ether extract of *Ziziphus rugosa* showed significant potentiation of sleeping time at dose level (400 mg/kg) and also show little effect at a dose (100 mg/kg) while the chloroform extract and ethanolic extract at 400 mg/kg does not showed significantly ($p < 0.01$) potentiation of the phenobarbitone induced sleeping time.
The pet ether extract increased the duration of sleeping in dose dependent manner, potentiated sleeping time significantly ($p < 0.01$) when compared to vehicle treated group and the effects were dose dependent. Results were given in Table 5 and Fig 1.

Spontaneous motor activity

The pet ether extract at (100 mg/kg), show decrease in locomotor activity on small scale. Pet ether extract at a dose of 400 mg/kg showed significant ($p < 0.01$) decrease in locomotor activity within 60 min. When the mice treated with pet ether extracts (100 mg/kg and 400 mg/kg), the locomotion was reduced with increase in the dose compared to control. Diazepam showed significantly decrease ($p < 0.01$) in locomotor activity after 30 min of its treatment. Whereas the chloroform and ethanotic extract have no significant effect on locomotar activity in mice. Results are given in Table 6 and Fig 2.

Exploratory Behaviour (Head dip test)

The effect of pet ether, chloroform andethanolic extract of *Ziziphus rugosa* at dose level of 100 mg/kg and 400 mg/kg on the number of head poking in the head dipapparatus were shown in Table 6 and Fig 3. During the test interval of 5 min, the pet ether extract increase the number of Head dipping at a dose 100 mg/kg. Diazepam a well-established anxiolytic also exhibited increase in number of head –dipping. Pet ether extract at dose of 400 mg/kg significantly ($p < 0.01$) reduced the head dipping., while at dose of 100 mg/kg significantly ($p < 0.01$) increases the head dipping. Chloroform and ethanolic extract did not showed significantly ($p < 0.01$) reduced the head dipping at dose of 400 mg/kg.

Elevated plus maze (EPM)

In the EPM, the behavior, which as observed, confirmed the anxiolytic activity of diazepam as reported previously[19]. The ethanolic extract and chloroform extract at 400 mg/kg did not showed any significant effect. The pet ether extract at 100 mg/kg significantly ($p < 0.01$) increased time spent in open arm and percent open arm entries. The pet ether extract at 100 mg/kg showed marked increase in time spent in open arm than effect of extract at 400 mg/kg. The total arm entries and percent open arm entries also were increased significantly in the pet ether extract of *Ziziphus rugosa* Wild. at a doses of 100 mg/kg and reduce at 400 mg/kg ($p < 0.01$). The pet ether extract at a higher dose of 400 mg/kg, showed a significant ($p< 0.01$) decrease in time spent in the open arm, without any change in entries in open arm, entries in closed arm and total entries. Results are given in Table 7, 8 and Fig 4, 5, 6.

Insomnia, seizures, anxiety and mental health problems in general and senile neurological disorders in particular, are widely prevalent in modern fast-paced life with a multitude of stressful conditions.

It is now becoming exceedingly apparent that available psychotherapeutics does not properly meet therapeutic demands of a vast majority of patients with mental health problems, and that herbal remedies remain to be the ultimate therapeutic hope for many such patients in the world. However, till now, very little attention has been paid to develop structurally and /or functionally novel CNS active drugs from psychoactive medicinal plants.

The present study demonstrated that the pet ether extract of *Ziziphus rugosa* Wild. At (400 mg/kg dose) produced central inhibitory effects in mice. The extracts of *Ziziphusrugosa* significantly reduced spontaneous motor activity in mice. The decrease in spontaneous motor activity gives an indication of the level of excitability of the central nervous system and this decrease may be closely related to sedation resulting from depression of the central nervous system[16].

The pet ether extract significantly prolonged phenobarbitone-induced sleep. The prolongation of phenobarbitone induced sleeping time may be attributed to an action of extracts on central mechanisms involved in regulation of sleep. While chloroform and ethanolic extract did not shown any central effect at both dose levels

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

(100 mg/kg and 400 mg/kg). Thus suggesting that the pet ether extract at dose (400 mg/kg) might be acting as a mild neurosedativedrug [16].

The pet ether extract at 400 mg/kg dose levels produced a significant decrease in exploratory behavior pattern as shown by the result on head-pocking in the Head –dip test. This decrease in head dipping by plant extracts also reveals sedative behavior. However at a dose 100mg/kg increases the head dipping reveals anxiolytic effect. The pet ether extract at a dose of 100 mg/kg increased the time spent and entries in the open arm. Results are suggesting that the increase in total arm entries is due to an increase in open arm entries rather than closed arm entries. At 400 mg/kg dose of pet ether extract it was found that the time spent in open arm was decreased without any change in open arm entries, closed arm entries and total entries. This prominent effect shown by higher dose of pet ether extract may be due to sedative effect of the extract rather than anxiolytic effect [19].

Sedation and anxiety are primarily mediated in the CNS by the $GABA_A$ receptor complex, which is also involved in other physiological and neurological disorders such as epilepsy, depression, Parkinson syndrome and Alzheimer's disease. Diverse drugs such are used in these pathologies might modify the phenomena of GABA system at the level of the synthesis of GABA mediators, release or re-uptake or metabolism [6].

Many saponins were found to be ligands for the $GABA_A$ receptors in the central nervous system, which led to the hypothesis that they act as benzodiazepine- like molecules. This is supported by their behavioral effects in animal models of anxiety and sedation [7].

However, along with saponins, number of other chemical constituents like sugars, stigma sterol and β-sitosterol also present in *Ziziphus rugosa*. Therefore further studies are planned to establish the exact mechanism of CNS depressant and anxiolytic activity of Pet. ether extract of seeds of *Ziziphus rugosa* by using agonists and antagonists.

CONCLUSION

In conclusion, we can say that the pet ether extracts given the central activity due to presence of saponins and this fact also supported by the chloroform as well as ethanolic extracts which did not show any central effect and saponins also were not found in it. However, along with saponins other constituents also present in *Ziziphus rugosa*. Therefore, it will be interesting to isolate the active chemical constituents and to determine their mechanism of action.

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