SCREENING OF *XANTHIUM STRUMARIUM* (L) ROOT EXTRACTS FOR DEPRESSENT ACTIVITY

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ABSTRACT

The research work deals with the screening of ethanol and chloroform extracts of the root of *Xanthium strumarium* L. for central nervous system depressant activity. The plant *X. strumarium* has been widely reported to have several medicinal properties in traditional form of medicine. The beneficial properties are sedative, demulcent, analgesic, styptic, sudorific, anodyne, antibacterial, antifungal, antispasmodic, bactericide, bitter, depressant, hemostat, anti-rheumatic and for the treatment of scrofulous tumors. Since root of *Xanthium strumarium* is used as CNS depressant for the treatment some CNS disorders, we made an attempt to study its CNS depressant activity. The different activities studied were potentiation of diazepam induced sleep, test for locomotor activity and effect on muscle coordination, anti-aggressive and anti-anxiety activities. The result of the study reflected that ethanol extract of the root (175mg/kg, p.o) decreased locomotor activity, produced muscle relaxation and showed anti-anxiety and anti-aggressive activity.

KEY WORDS: *Xanthium strumarium*, sedative, muscle relaxant, anti-anxiety, anti-aggressive.

1.INTRODUCTION

Advance in science and technology has contributed to an enormous improvement in the quality of life of humankind. However, modern life stress, associated trials and tribulation are responsible for the surge in incidence of variety of psychiatric disorders. Path breaking research in psychopharmacology has flooded the market place with drugs for specification. For instance, benzodiazepines (diazepam, nitrazepam, lorazepam and alprazolam etc) are the most frequently prescribed synthetic drugs for variety of conditions particularly anxiety, depression, epilepsy and insomnia. But these psycho- neural drugs have very serious side effects like chronic use benzodiazepines causes deterioration of cognitive functions, physical dependence and tolerance. Besides addiction liabilities, benzodiazepines adversely affect the respiratory, digestive and immune system of body and the chronic treatment with benzodiazepines often prove more harmful in the longer run (Dhawan,2003).

In the present investigation revivification is given to the plant products and there is great hope that drugs of plant origin will have significantly lesser side effects than that observed with synthetic drugs while having comparable efficacy.

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A variety of naturally occurring drugs such as *Thymus linearis, Lactuca serola*, *Papaver somniferum* (opium) and *Atropa belladonna* were tested for psychopharmacological effects and were found to be effective in the treatment of psychiatric disorders (Evan,1998). The plant *X. strumarium* is an erected shrub growing mostly in the warmer of the world in marshy places and reported to contain xanthanolides like Xanthinosine, Xanthatin, epixanthatin, dihydroxanthatin and Sequeterpienes and phenolic acids like 1, 3, 5-tri-0-caffeoylquinicacid (Alberto,1993; Saxena and Mandal,1994; Agharkar,1991).There are very few reports on the pharmacological activities of the plant. As there are no scientific reports about the depressant activity of the plant *X. strumarium* but traditionally it is in the practice that the root extracts is used for the treatment of various CNS disorders. Considering the above facts it is worthwhile to evaluate the *X. strumarium* root extracts for CNS depressant active.

2.MATERIALS AND METHODS

Experimental animals

Healthy Swiss Albino mice of either sex, weighing 20-30 g were procured from the animal house of S.J.M.College of Pharmacy, Chitradurga, India. The animal house was well ventilated and animals had 12 ± 1 h day and night schedule. The animals were housed in large spacious hygienic cages during the course of the

experimental period and room temperature was maintained at 25 ± 1°C. The animals were fed with standard rat feed (Hindustan Lever Ltd; Bangalore) and water. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approval no. SJMCP /IAEC /PhD/ PH.CHEM /05/2007-08).

Chemicals

Pentobarbitone sodium was obtained from Sigma-Aldrich, USA, Diazepam was procured from Cipla, Ahmadabad (India), Chloroform, chlorpromazine and Diethyl ether were purchased from a reputed companies.

Preparation of extracts:

Various extracts of the plant material were prepared by successive solvent extraction method as described below.

The powdered material of roots of *Xanthium strumanium* L was refluxed successively with the solvents petroleum ether (40^0-60^0, E- Merck, Mumbai, India), Chloroform (50^0 – 70^0, E-Merck, Mumbai, India) and Ethanol (E- Merck, Mumbai, India) in a soxhlet extractor (Fig- 1) for 48 hrs in batches of 350g each. Every time, before extracting with the next solvent the marc was dried. After extraction with ethanol lastly, marc was kept in closed jar in distilled water for 48 hrs with occasional shaking, then distilled water obtained by pressing the marc with tincture press.

Fig-1: Soxhlet extractor

Fig-2: Rotary flash evaporator

All the extracts were concentrated in vacuum using rotary flash evaporator (Fig-2) (Buchi-Flawil, Switzerland). The solvents were removed completely over the water bath and finally desiccator dried. The extracts so obtained from each of the solvents were labeled, weighed and the yield was calculated in terms of grams percent of the weight of the powdered roots.

Acute toxicity studies as per OECD Guideline 425

In the assessment and evaluation of the toxic characters of the substance, determination of acute oral toxicity is usually an initial step. It provides information of health hazards likely to arise from a short-term exposure by the oral route. Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24h. Data from an acute study may serve as a basis for classification and labeling. LD_{50} (medium lethal dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral route. The LD_{50} value expressed in terms of test substance per unit weight of test animal (mg/kg). It is initial step in establishing a dosage regimen in sub chronic and other studies and may provide initial information on the mode of toxic action of a substance.

The concept of the up and down (UDP, stair case method) was first designed by Dixon and Mood (Dixon,1991; Dixon and Mood). In this method animals of a single sex, usually females, with the first animal

receiving a dose just below the best estimate of the LD_{50}. Depending on the out come for the previous animal, the dose for the next is increased or decreased, usually by the factor of 3.2. This sequence continues until there is a reversal of the initial outcome (i.e., the point where an increasing dose results in death rather than survival or decreasing dose result in survival rather than death) then, additional animals are dosed following the up-down principle until a stopping criterion is met. If there is no reversal before reaching the selected upper (2000 or 5000 mg/kg) limit dose, then a specific number of animals are dosed at the limit dose. The option to use an upper limit dose of 5000 mg/ kg should be taken only when justified by a specific regulatory need.

Healthy Wister rats weighing between 150-180 g were used to carry out acute toxicity studies by the 'staircase' method. All successive of *Xanthium strumarium*, roots in 0.5% Tween 80 was administered orally by gavages in graduated doses to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects were made at 0,1,2,4 and 24 h for any mortality. Observations include changes in skin and fur, mucous membranes and also muscle spasm, convulsion, motor activity and behavioral patterns.

Selection of the extract

The chloroform and ethanol extract of the root of *X. strumarium* were evaluated for sedative –hypnotic activity in pentobarbitone induced sleep test. The extract, which potentiated the sedative-hypnotic activity of pentobabitone was chosen for further study (Singh,2000).

Test for locomotor activity

The spontaneous locomotor activity of each mouse was recorded individually for 10 min using Actophotometer. The ethanol and chloroform extract of *X. strumarium* was administered at two doses (100and 200mgs) 60 min before the test and chlorpromazine (3mg/kg, i.p), used as standard was given 30min before the test. The control group was treated with 2%w/v tween 80 orally, 60 min before the test (Kulkarni,1987).

Muscle co-ordination test

This test was carried out using inclined plane and rotorod apparatus.

Inclined plane

The plane consists of two rectangular plywood boards connected at one end by a hinge. One board is the base; the other is the movable inclined plane. Two plywood side panels with degrees marked on their surface are fixed on the base. A rubber mat with ridges 0.2 cm in height is fixed to the inclined plane, which was set at 65 degrees. Swiss albino mice were taken and divided into 6 groups, each group comprised of 6 animals. The two doses of each ethanol and chloroform (100 and 200mgs/ kg body weight) were administered orally, the standard was treated with diazepam (4mg/kg) intra-peritonealy and control group received Tween 80 (2%w/v) orally. The test was carried out 30, 60 and 90 min after administration of drugs and vehicle. The animals were placed at upper part of the inclined plane and were given 30sec to hang on or fall off (Tatarezynska,2004).

Rotarod

The rotarod apparatus consists of a metal rod (3cm diameter) coated with rubber attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75cm in length and is divided into 6 sections by metallic discs, allowing the simultaneous testing of 6mice. The rod is in a height of about 50 cm above the tabletop in order to discourage the animals from jumping off the roller. Cages below the section serve to restrict the movement of the animals when they fall from the roller. Swiss albino mice underwent a pretest on the apparatus. Only those animals, which had demonstrated their ability to remain on the revolving rod (20 rpm) for 5min, were used for the test. The animals were treated in the same way as mentioned under inclined plane test (Allmark and Bachinski,1949).

Anti- anxiety activity

The Anti- anxiety activity was evaluated using staircase and elevated plus maize test.

Staircase test

Staircase consists of five identical steps 2.5 high, 10 cm wide and 7.5 cm deep. The internal height of the walls is constant along whole length of the staircase. The drugs and treatments were same as mentioned under inclined plane. The animals were placed on the floor of the box with its back to the staircase. The number of steps climbed and the number rears were counted over a 3min period. A step was considered to be climbed only if the mouse had placed all four paws on the step. In order to simplify the observation, the number of steps descended was not taken into account. After each step the box was cleaned in order to eliminate any olfactory cues, which might modify the behavior of the next animal (Rakotonirina,2001).

Elevated plus maze

The apparatus consist of two open arms (5×10cm) and two closed arms (5×10×15cm) radiating from a platform (5×5 cm) to form a plus –sign figure. The apparatus situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arm edges were 15cm in height. The drugs and treatments were as same as mentioned under inclined plane.

The animal was placed at the center of the maze, facing one of the closed arms.

During 5 min test period the following measures are taken:

- The number entries into open arms
- The number entries into closed arms
- Time spent in the open arms

Arms entry was counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room, with observations made from an adjacent room (Nishikava,2004).

Anti-aggressive activity

This was carried out using isolation induced aggressive test. Male albino mice weighing about 12-20 gm are kept isolated in small cages for a period of 6weeks. Prior to the administration of the drug, the aggressive behavior of the animals was tested. The male mouse being accustomed to live together with other animals was placed into the cage of an isolated mouse. The aggressive behavior of the isolated was recorded. The following parameter was used to assess aggressive behavior.

- Hitting the tail on the bottom of the cage
- Screaming (piercing noise) and
- Biting

After these initial tests, the animals were subjected to different drug as mentioned under inclined plane. The aggressiveness was studied again after 60 and 120 min after drug or vehicle administration (Oliver and Mos,2000).

Statistical analysis

Results were expressed as Mean±SEM. The difference between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Dunnett's test and were considered statically significant when $P<0.05$.

Test for locomotor activity

Ethanol extract at the dose of 175 mg/kg p.o and diazepam at the dose of 4 mg/kg body weight decreased the locomotor activity significantly ($P<0.01$) where as, low dose of ethanol extract (75mg/kg p.o) did not show a significant reduction in the locomotor activity (Table 2).

Test for muscle coordination

Inclined plane

The number of animals falling from inclined plane after 60 and 90 min of treatment were significantly more in diazepam (4mg/kg i.p) treated and alcohol extract treated groups (175mg/kg) when compared to control group ($P<0.01$). Low dose of test drug was ineffective (Table 2).

Rotorod test

This test, alcohol treated group (175mg/kg) significantly reduced the time spent by the animals on revolving rod when compared to control ($P<0.05$). The standard drug (diazepam) also showed significant effect when compared to control ($P<0.01$). Low dose of drug (75mg/kg) did not show any significant effect (Table 2).

Anti-anxiety test

Staircase test

The statistical summary of the rearing and number of steps climbed is presented in Table 3. After 60 and 90 min of treatment, a reduction in anxiety-linked behavior was indicated by a reduction in number of rearing and sedation that was evaluated by number of steps climbed. High dose of alcohol extract (175mg/kg p.o) and standard drug (diazepam 4mg/kg, i.p) significantly reduced the number of rearing as well as the number of steps climbed ($P<0.01$). Low dose of alcohol extract (75mg/kg, p.o) did not produce a significant decrease in the number of rearing or the number of steps climbed.

Elevated plus maze

High dose of alcohol extract (175mg/kg p.o), significantly increased the time spent in open arms ($P<0.05$) and number of entries into closed arms ($P<0.01$) but did not affect significantly the number of entries into open arms when compared with control. The standard drug (diazepam4mg/kg, i.p) showed a significant decrease in the number of entries into closed arms ($p<0.01$) and open arms ($p<0.05$) and also significantly increase the time spent in open arms ($p<0.01$). Low dose of ethanol extract (75mg/kg, p.o) did not show any difference in activity compared to control (Table 3).

Anti- aggressive activity

Both alcohol extract (175mg/kg, p.o) and diazepam (4mg/kg, i.p) produced marked inhibition of aggressiveness in isolated rat as indicated by a significant decrease in screaming, hitting the tail on the bottom, and biting. Low dose did not show any significant effect (Table 4).

3.Results and Discussion

The study showed that ALE (175mg/kg,p.o) posses sedative, anti-anxiety, muscle relaxant and anti aggressive activity The ALE-2, potentiated the sleep induced by pentobarbitone that it poses some sleep inducing property. The study on the spontaneous motor activity showed that frequency and the motor activity could be attributed to the sedative effect of the extract (Rakotonirina,2001).

Inclined plane method was originally developed for testing curare-like agents. Later on, it has been used by many authors for testing compounds for muscle relaxing activity of both centrally acting and peripheral acting muscle relaxants (Allmark and Bachinski,1949). ALE-2 (175mg/kg,p.o) made the animals unable to stay on inclined plane during 30 sec period. ALE-2 also reduced the time spent on the revolving rod by mice in the rotorod test, a test mainly used to screen centrally acting muscle relaxants (Rakotonirina,2001).This represented that ALE-2 may have muscle relaxant activity, which could be due to CNS depressant activity. The mouse staircase was used for the assessment of anxiety (number of rearing) and sedation (number of rearing) and sedation (number of steps ascended). Greater number of rear indicates anxiety like behavior and lesser number of steps ascended indicated increased sedation (Deborah,2005).The present investigation successfully detected the anxiolytic-like effects of ALE-2 and diazepam; both significantly decreased the number of rearing and number of steps ascended compared to control. This showed that ALE-2 has both anxiolytic and sedative properties.

The sedative, muscle relaxant and anxiolytic effects of ALE-2 could be due to the interaction of sequeterpinoids (chemical constituent of the plant) with GABA/ Benzodiazepine receptors complex in brain.

Elevated plus-maze test is used to evaluate psychomotor performance and emotional aspects of rodents (Nogueira and Vassilief,2000). The result showed that ALE-2 significantly increased the time spent on the open arms and decreased the number of entries into open and closed arms. This type of effect is observed with the drugs that act on GABA/benzodiazepine receptors complex as well with drugs that stimulate gluco\corticoid production and release in the adrenal cortex (Nishikava,2004), after administration of 5-HT_{1B} receptor antagonists and $5HT_{1A}$ agonists (Millan,1997). Therefore, with the present data, it is difficult to predict the precise mechanism for the anxiolytic activity of the root extracts of *X. strumarium*.

ALE-2 showed inhibition of aggressiveness in isolated mice. The serotogeninergic system is implicated in aggressive states and it has been hypothesized that decreasing in serotonergic activity may encourage aggressive behavior (Mifczek,1995). Since, both antianxiety and anti-aggressive effects are seen with $5HT_{1A}$ antagonists, it is assumed that ALE-2 may also interact with the 5-HT_{1A} receptors.

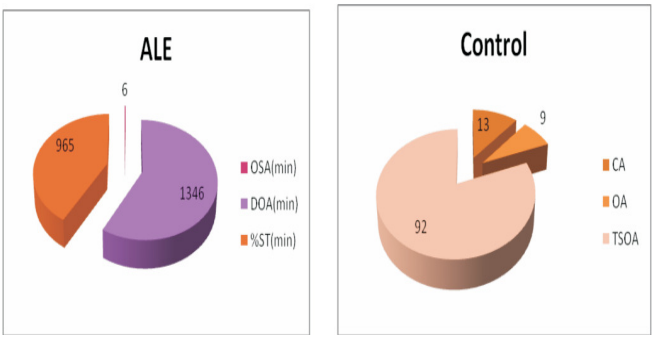
To conclude, the ethanol extract (ALE-2) of *X. strumarium* possess sedative, anti-anxiety, muscle relaxant and anti-aggressive properties. The result of the present study substantiates the traditional use of the root of *X. strumarium* for the treatment of sleeplessness.

Table I: **Effect of ethanol and chloroform extracts in pentobarbitone–induced sleep**

Pentobarbitone 40mg/kg, i.p) 30 min Post treatment of the vehicle and drugs	on set of action (Min)	Duration of action(Min)	Percentage Sleeping time
Control (Vehicle 6ml/kg, p.o)	6.16±0.13	139.43±0.43	100
Chloroform extract (100mg/kg, p.o)	6.68±0.32	114.30±0.38	81.97
Ethanol extract (100mg/kg, p.o)	6.34±0.43	1346±0.28***	965.35

All values are Mean±SEM, ***P<0.001 When compared with control

Figure-1: Effect of ethanol and chloroform extracts in pentobarbitone–induced sleep



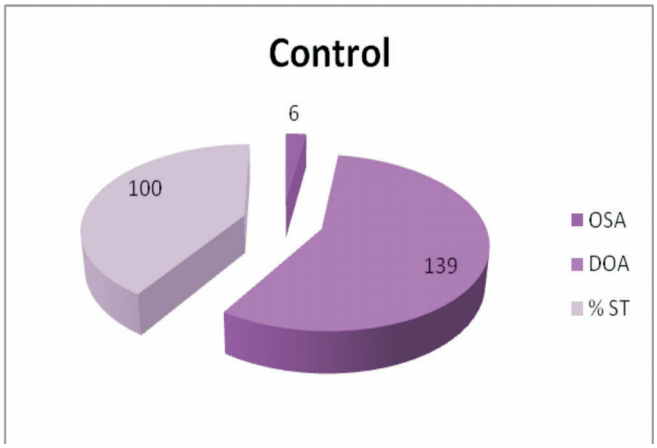
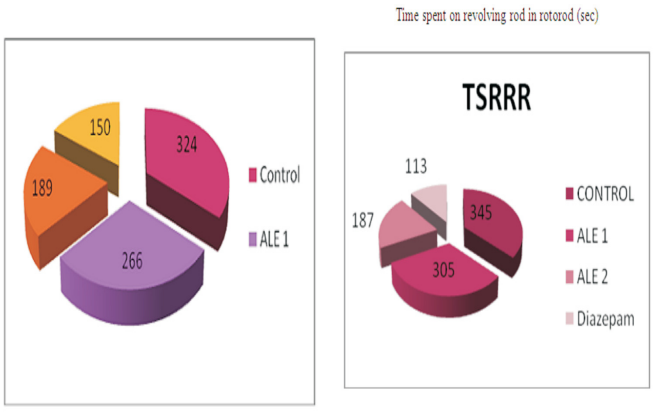


Table II: Effect of alcohol extract on locomotor activity in Actophotometer and muscle coordination in inclined plane and rotator.

Groups	Actophotometer Score in 10min	No of animals falling down within (30sec) from inclined plane			Time spent on revolving rod in Rotator (sec)
		30min	60min	90min	
Control Vehicle 6ml/kg,p.o	324.45±12.43	0±0	0±0	0±0	345.43±21.65
Ethanol Extract (75 mg/kg,p.o)	265.65±14.54	0±0	0±0	0.27±0.17	305.34±24.25
Ethanol Extract (175mg/kg p.o)	189.16±12.46	0±0	0.96±0.13**	0.65±0.27**	187±32.76*
Diazepam 4mg/kg p.o	149.54±37	0.78±0.25**	0.93±0.37**	0.98±0.36**	113±12.67**

Figure-2:-Effect of alcoholic extract on locomotor activity in Actophotometer and muscle coordination in inclined plane and rotator Actophotometer in 10 mins.



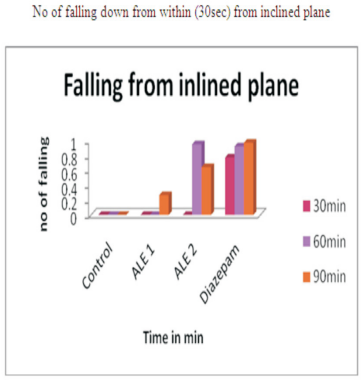


Table III: Effect of alcohol extract and diazepam in stair case test and elevated plus-maze test.

Groups	Stair case test		Elevated plus maze test		Time spent in open arms
			No of entry into		
	No of climbing's	No of rearing	closed arms	open arms	
Control (Vehicle 6ml/kg, p.o)	22.36±0.98	10.04±0.43	12.98±1.32	9.23±1.09	92.45±6.65
Ethanol extract (75mg/kg, p.o)	14.76±1.12	8.54±0.53	8.97±1.65	7.34±0.56	108.54±6.34
Ethanol extract (175mg/kg,p.o)	9.34±0.75	6.38±0.59	6.79±1.12	7.56±0.43	153.23±8.56
Diazepam (4mg/kg,p.o)	7.25±0.64	4.97±0.72	6.12±0.78	5.35±0.68	187.67±12.67

All the values are Mean ± SEM, n=6, *P<0.05, **P<0.01 when compared with control

Figure-3:- Effect of alcoholic extract and diazepam in stair case test and elevated plus-maize test.

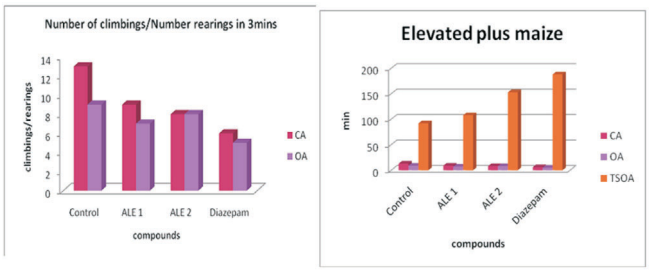
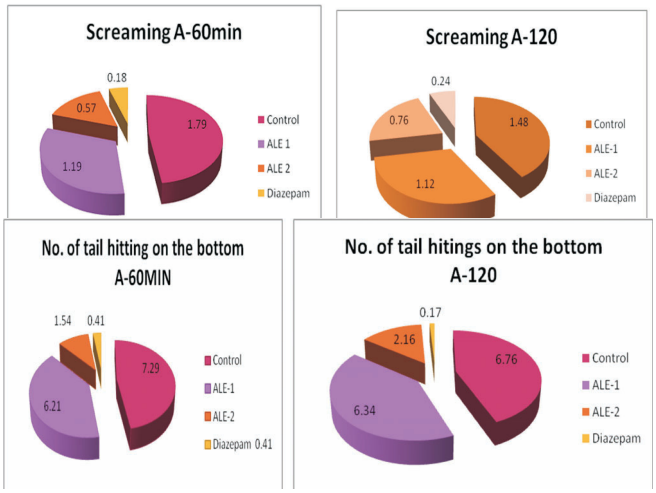


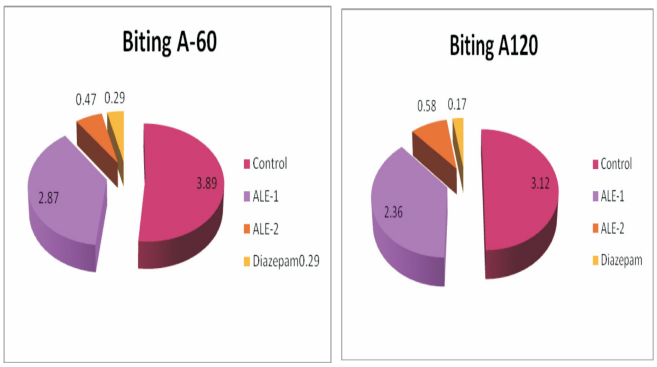
Table-4:- Effect of alcoholic extract and diazepam on isolation induced aggression

Groups	Screaming		No. of Hitting the Tail on the bottom		Biting	
	After 60Min	After 120Min	After 60Min	After 120Min	After 60Min	After 120Min
Control vehicle 6ml/kg,p.o)	1.79±0.21	1.48±0.13	7.89±0.69	6.76±0.46	3.89±0.32	3.12±0.23
ALE 75mg/kg, p.o)	1.19±0.17	1.12±0.21	6.21±0.43	6.34±0.97	2.87±0.67	2.36±0.76
ALE(175mg/kg p.0)	0.57±0.43*	0.76±0.26*	1.54±0.43**	2.16±0.43*	0.47±0.13**	0.58±0.35**
Diazepam (4mg/kg p.o)	0.18±0.11**	0.24±0.17**	0.41±0.21**	0.17±0.11**	0.29±0.15**	0.17±0.03**

All values are Mean ±SEM, n=6,*P<0.01 when compared with control at the same time interval

Figure-4:- Effect of alcoholic extract and Diazepam on isolation Induced aggression





REFERENCES

Dhawan K, Dhawan S, Attenuation of benzodiazepine dependence in mice by a tri-substituted benzoflavone moiety of *Passiflora incarnata Linneous,* A non habit forming anxiolytic, J.Pharma Pharmceu.Sci., 2003, 215-222.

Evan WC, Trease and Evans Pharmacognosy, 14thEdn, Harcourt Brace and Company, India, 1998.

Alberto M.J, Sanz C.J.F, Xanthnolides from Xanthium, Absolute configuration of xanthanol, isoxanthanol and their C-4 epimers, Phytochemistry Oxford, 34(6),1993, 1569-1576.

Saxena V.K and Mondal S.K, A Xanthanolide from *Xanthium strumarium,* Phytochemistry Oxford, 35(4), 1994,1080-1082.

Agharkar S.P, Medicinal plants of Bombay presidency, Pbl.Scientific Publishers, Jodhpur, India, 1991, 230.

Dixon W.J, Staircase Bioassay:the Up and Down method, Neuro Sci.Bio-behav.Rev.,15, 1991, 47-50.

Dixon W.J and Mood A.M, A method for obtaining and analyzing sensitivity data, Amer.Statist.Assoc., 43, 109-126.

Singh J, Khosla P, Srinivastava P.K, Nimodipine potentials anesthetic effects of ethanol, pentobarbitone and ketamine in rats, Indian J.Pharmacol., 32(8), 2000, 206-209.

KulkarniS.K, Handbook of experimental pharmacology, VallabhaPrakashan, New Delhi, India, 1987.

Tatarezynska E, Klodzinska A, Stachowicz K, Chojnacka-Wojcik E, Effects of selective 5-HT$_{1B}$ receptor against and antagonists in animal models of anxiety and depression, Behav.Pharmacol., 15(8), 2004, 523-524.

Allmark M.G, Bachinski W.M, A method of assay for curare using rats, J.Am.Ph.Ass., 38, 1949, 43-45.

Rakotonirina V.S, Bum E.N, Rakotonirena A, Bopelet M, Sedative properties of the decoction of the rhizome of Cyperus anticulatives, Fitoterapia, 72, 2001, 22-29.

Nishikava H, Hata T, Funakami Y.A, role for corticotrophin-releasing factor in repeated cold stress-induced anxiety-like behavior during forced swimming and elevated plus-maze test in mice, Biol.Pharm.Bull., 27(3), 2004, 3521-356.

Oliver B, Mos J, Rodents models of aggressive behavior and serotogenic drugs, Progr. Neuro Psychol., 32, 2000, 206-209.

Deborah A, Gries, Geoge A, Condouris, Shey Z, Houpt M, Anxiolytic-like action in mice treated with nitrous oxide and oral triazolam or diazepam, Life Sci., 76, 2005,1667-1674.

Nogueira E, Vassilief V.S, Hypnotic, anticonvulsant and muscle relaxant effects of Rubus lorasiliensis. Involvement of GABA$_A$ system, J.Ethanopharmacol., 70, 2000, 275-280.

Millan M.J, Veiga S, Goument B, et.al., S15535, A novel benzodioxopiperazine ligand of serotonin (%-5HT)$_{1A}$ receptor II, Modulation of hippocampal serotonin release in relation to potential anxiolytic properties, J.Pharmacol.Exp.Ther., 282, 1997, 148-161.

Mifczek, Weerts E, Vivian J.V, Rapoport D.M, Aggression, anxiety and vocalization in animals, GABA$_A$ and 5-HT anxiolytivcs, Psychopharmacology, 121, 1995,38-56.