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RESEARCH ARTICLE

PHARMACOLOGY

ANXIOLYTIC EFFECT OF ETHANOLIC EXTRACT OF *OXALIS CORNICULATA* .L IN MICE

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ABSTRACT

The aim of the study is to evaluate anxiolytic effect of Ethanolic extract of Oxalis corniculata (200mg/kg and 400mg/kg P.O) on male mice using various paradigms of anxiety. In elevated plus maze, extract (200mg/kg and 400mg/kg) had shown a dose-dependent increase in time spent and number of entries into open arm compared to control group. The number of central squares, peripheral squares crossed and rearings were stepped up significantly in open field paradigm. The treated groups had shown accession in time spent in light compartment, number of crossings, latency compared to control group in light dark exploration test. In Hole board model, number of head dips were minified in mice that received Ethanolic extract of Oxalis corniculata (200mg/kg and 400mg/kg). Further studies are required to find out the exact chemical constituent and mechanism responsible for anxiolytic effect.

KEY WORDS

Anxiolytic, Oxalis, Elevated plus maze, open field, light – dark, hole board.

INTRODUCTION

According to National Comorbidity survey, United States, Women are twice as likely as men to be depressed and 2 to 3 times more prone to anxiety disorders [1]. Anxiety is one of the most common disorders of CNS among the present multiplying health problems across the globe.[2] Anxiety is a state of excessive fear characterized by motor tension, apprehension, sympathetic hyperactivity and vigilance syndromes, leading to impairment of memory, intelligence and psychological function[3]. Literature clearly shows the involvement of 5-HT_{1A} and $GABA_A$ receptors in anxiety treatment. Drugs like Benzodiazepines act on GABA are used as anxiolytics for the past 45 years. Despite anxiolytic effect they share some side effects like sedation, amnesia, muscle relaxant[4], Tolerance development and withdrawal symptoms. Buspirone, a 5-HT_{1A} agonist shows potential anxiolytic effect with less sedation but causes restlessness and diziness[5]. As most of these synthetic drugs are showing potential side effects, research is going across the globe to develop anxiolytics with less side effects and wide safety margins. This made the researchers to work on herbals and their related constituents to develop a potential anxiolytic.

Oxalis corniculata . L commonly known as creeper wood sorrel or procumbent yellow sorrel belongs to the family Oxalidaceae[6]. The entire plant was rich in Vitamin - C and used in scurvy. The plant also contains Tannins, Flavones, glycoflavones, flavonols, palmitic acid, calcium and phenolic acids. Traditionally it is used in treatment of stomach trouble, antidote for scorpion sting, apthae, giddiness, diarrhoea and dysentery. Research did on Oxalis corniculata reveals its antibacterial [7], anti implantation and abortifacient[8], wound healing[9], anticancer[10], antioxidant[11], Alzheimer's and antiepileptic[12] activity. The present study was performed to evaluate anxiolytic potential of Oxalis corniculata as there was no such evidence reported till date.

MATERIALS AND METHODS

Plant material: Whole plant of Oxalis corniculata. L was collected from Narakasur hill top, Guwahati, India during the month of June to September. Authentication of the plant was done by Curator Dr. Ganesh Sharma, department of Botany from Guwahati University and a voucher specimen (NO. 01577) was preserved for further reference.

Preparation of Ethanolic extracts: The whole plant was thoroughly washed, shade dried and then chopped in to course powder using a mixer. Powder (200 gram) was tightly packed in Soxhlet apparatus and subjected for continuous hot percolation employing ethanol as solvent for 48 hrs. The extract was filtered using whatman filter paper No.1 and the filtrate was evaporated until it gets concentrated. The % yield of oxalis corniculata was 20.21 %.

Source of chemicals: Drugs used in the study were procured from various sources. Diazepam was purchased from Sigma Aldrich (Germany) and Ethanol was supplied by Helix India. All drugs and solvents employed were of analytical grade.

Phytochemical screening[13]: The extract was subjected for screening of Flavonoids, Tannins, carbohydrates, glycosides and steroids.

***Animals**:* Male albino Swiss mice (25-30g) of 4-6 weeks of age were selected from animal house of National Institute of Pharmaceutical Education and Research, Guwahati. They were bedded in polypropylene cages (12×14×15 cms). Each cage housed 6 mice. Cages were maintained in a well ventilated room under standard and controlled environmental conditions (25±2ºc, 55-60RH, 12:12h light: dark cycle). Ethical committee clearance was done by institutional animal ethics committee of CPCSEA (Registration no. 351/3/1/2001).

***Acute Toxicity**:* The acute toxicity studies were done according to OECD guidelines No.423. The animals were alive even at 2000mg/kg dose. Hence 1/5 and 1/10 doses (200 and 400mg/kg) were selected for anxiolytic study.

***Treatment**:* The mice were divided into four groups with 6 per group and were given respective treatment. Group-1 served as control received Normal saline , Group-2 served as positive control received standard Diazepam (1mg/kg i.p.), Group- 3,4 were administered oral dose of 200mg/kg , 400mg/kg dose of EEOC. After 45 min of respective treatment, mice were individually subjected for anxiety paradigms.

Elevated plus maze [14,15]***:*** Elevated plus maze was made of 2 open arms (35×5cms) and 2 closed arms (35×5×20 cms). They were arranged perpendicular to each other with a small central square (5× 5) between arms. The maze was hiked up to 25cms from the floor in a dimly lit room. All the four groups were given respective treatment and after 45 min, mice were individually placed in centre square facing either one of the open arms. The number of entries into open arm, closed arm, latency time, time spent in open and closed arm were recorded for period of 5 min. An entry into an arm is noted when the mice with its four paws cross the demarcation of respective arm.

Open field test[16]***:*** A dark colored wooden box (60×60×30cms) with its floor carved into 16 equal sized squares (15×15cms) and 40W lamp appended 100cms above for illumination was used. Before dropping the individual mice in one of the corner of the box (i.e. 45min prior) respective groups were administered with respective treatment (Normal saline, diazepam1mg/kg i.p, EEOC 200mg/kg and 400mg/kg) and then record number of central squares crossed, peripheral squares crossed and number of rearing for 5min.

Light - Dark exploration test [17] *:* In this test two wooden boxes (25×25×25cms) of dimensions attached to each other were used . one box was made dark by covering with plywood and other was illuminated with 40W lamp hung 25 cms above .The mice were individually placed in centre of the light box and observed for 5 mins. The observations were noted as time passed in light, dark and latency, along with number of crossings. The mice were administered with respective treatment (Normal saline, diazepam 1mg/kg i.p, EEOC 200mg/kg and 400mg/kg orally) 45 min before test.

Hole Board test [17]***:*** Mice were placed in a black Perspex box (50 x 50 cm, walls 30 cm high) with 16 equally spaced holes (2.5 cm diameter, 10 cm apart from each other) in the floor and the box was raised to a height of 25cms from the ground. Mice were given respective doses (Normal saline, diazepam 1mg/kg i.p, EEOC 200mg/kg and 400mg/kg) 45 before carrying out the test. The number of head dips was recorded for duration of 5 min.

***STATISTICAL ANALYSIS**:* The obtained results were analyzed by using SPSS software. The entire data was illustrated as Mean ± SEM values and was analyzed by one way ANOVA. Whenever ANOVA was considerable, Tukey Kramer multiple comparison test was applied for further comparison between control Vs treated groups. The level of statistical significant consider was $P<0.05$.

RESULTS

Elevated plus maze: The effect of treatments on the behavior of mice in EPM were tabulated in Table.1 and depicted in figure no.1and 2. ANOVA was found to be statistically significant at P<0.05. Even though it was followed up by Tukey Kramer multiple comparison test, contrast with control group, the Diazepam (1mg/kg), EEOC (200mg/kg, 400mg/kg) had shown significant increase in time spent in open arm (q = 37.7 P<0.001, q =13.1 p < 0.01, q = 18.5 P<0.001). There was decrease in latency time of group received EEOC (400mg/kg) (q=8.27 p< 0.001) and increase in Diazepam (1mg/kg), EEOC (200mg/kg) group (q=0.47 p >0.05, q= 3.76 p>0.05) compare to control group. Diazepam (1mg/kg), EEOC (200mg/kg, 400mg/kg) groups significantly increased the number of open arm entries (q=13.4 p <0.001, q= 6.12 p <0.01, q= 10.2 p< 0.001) compared to control group.

Open field test*:* The results of tests were given in Table no.2 and depicted in figure no. 3. ANOVA was significant (p<0.05) and for further comparison Tukey kramer test was employed. The Diazepam (1mg/kg), EEOC (400mg/kg) significantly increased the number of central crossings (q=10.9 p<0.001, q=8.23 p<0.001) compare to control group. Diazepam (1mg/kg) and EEOC (400mg/kg) had shown a considerable increase in number of rearing (q=8.06 p<0.001, q=7.5 p<0.001) compared to control group.

Light – Dark exploration test*:* Light - dark test measures were summarized in Table no. 3 and figure no.4 and 5. ANOVA was significant at p<0.05 and further comparison was done by Tukey Kramer multiple comparison tests. The Diazepam (1mg/kg), EEOC (400mg/kg) significantly increased time spent in light arena (q = 7.17 p < 0.001, q = 3.96 p < 0.05). The number of crossings were considerably increased in Diazepam (1mg/kg), EEOC (400mg/kg) compared to control group (q=9.3p<0.001, q=6.13 p<0.01). The latency was found to be not significant by using ANOVA (P>0.05).

Hole board test: The results were tabulated in Table no.4 and depicted in graph no.6 and analyzed by using ANOVA and found to be significant (p<0.05). Further comparison was done by Tukey Kramer multiple comparison test. In this test there was a significant decrease in the number of head dips of Diazepam (1mg/kg), EEOC (400mg/kg) compared to control group (q=8.64 p<0.001, q=6.06 p< 0.01).

Table no.1
(EPM)

Groups (n= 6)	Time spent in sec (Mean ± SEM)			No .of entries (Mean ± SEM)	
	Open arm	Closed arm	Latency	open arm	closed arm
Control	28.17±2.3	223.67±10.8	48.17±11.2	2.17±0.3	27.17±8.3
Diazepam (1mg/kg)	136.5±4.1	103.67±4.3	45.5±2.8	13.7±0.7	28.1±5.4
EEOC(200mg/k)	65.83±2.1	162.5±3.8	71.67±2.5	7.17±0.4	38.33±10.1
EEO(400mg/kg)	81.5±2.5	118.5±3.2	99.83±4.1	10.5±1.4	53.33±14.2

Table no.2
(Open Field)

Group (n= 6)	NO. of crossings (Mean ± SEM)		
	Periphery Square	Centre square	Rearing
Control	74.33 ±1.6	14.67±1.3	7.83± 0.9
Diazepam (1mg/kg)	110.17 ±5.3	40.83 ±4.2	32 ± 2.3
EEOC(200mg/kg)	65.3 ±10.1	10±1.1	16 ± 2.4
EEOC(400mg/kg)	139.5 ±14.6	36 ± 6.1	30.33 ± 4.9

Table no.3
(Light-Dark test)

Group (n= 6)	Time spent in sec (Mean± SEM)			No.of crossings (Mean± SEM)
	Light	Dark	Latency	
Control	93.17±14.5	194.17±12.7	9.83±1.4	11.33±1.0
Diazepam(1mg/kg)	162.33±3.5	125.33±3.8	14.5±1.7	25.33±1.6
EEOC(200mg/kg)	115.33±6.6	171.17±9	11.17±1.8	14±1.8
EEOC(400mg/kg)	131.3±10.1	155.67±7.9	10.1±1.9	20.5±1.4

Table no.4
(Hole board test)

Group (n=6)	No. of Head dips (Mean ± SEM)
Control	43.83 ±2.3
Diazepam (1mg/kg)	19.83 ±1.6
EEOC(200mg/kg)	36.67 ±3.6
EEOC(400mg/kg)	27.0 ±3.1

A. **EPM**

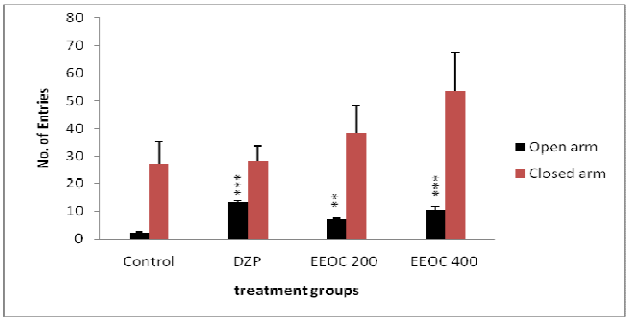

Fig .1

Effect of Normal saline (control), Diazepam 1mg/kg (DZP), EEOC 200mg/kg , EEOC 400mg/kg on the number of entries in open and closed arm in elevated plus maze (EPM). (P<0.001), (** P<0.01) (n=6 in each group)***

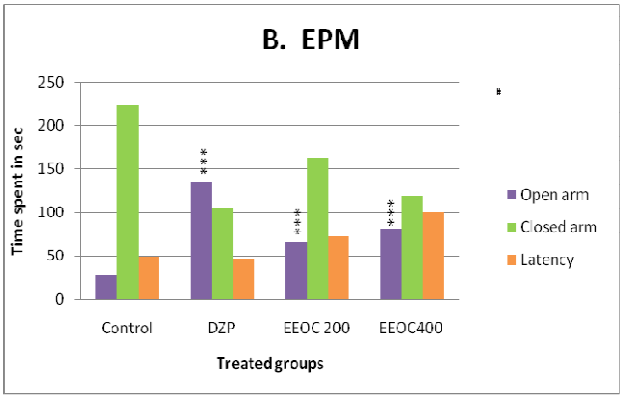

Fig .2

Effect of Normal saline (control), Diazepam1mg/kg (DZP), EEOC (200mg/kg), EEOC (400mg/kg) on the time spent in open and closed arm and latency in elevated plus maze (EPM). (P<0.001). (n=6 in each group)***

Open field test

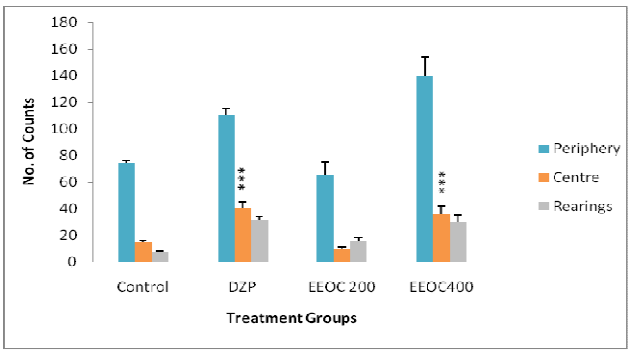

Fig .3

Effect of Normal saline (control), Diazepam 1mg/kg (DZP), EEOC (200mg/kg), EEOC (400mg/kg) on number of rearing, number of peripheral and central squares crossed in open field. (P<0.001) (n=6 in each group)***

A) Light - Dark test

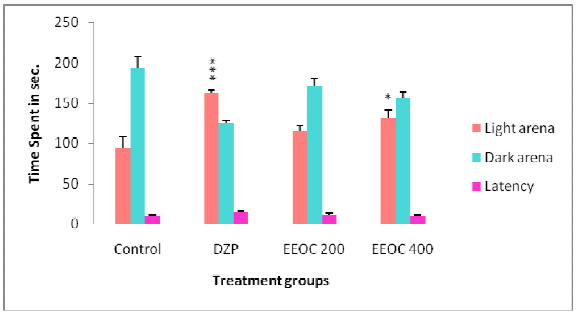

Fig .4

Effect of Normal saline (control), Diazepam 1mg/kg (DZP), EEOC (200mg/kg), EEOC (400mg/kg) on time spent in light, dark arena, latency in light dark test. (P<0.001), (* P<0.05) (n=6 in each group)***

B) Light – Dark test

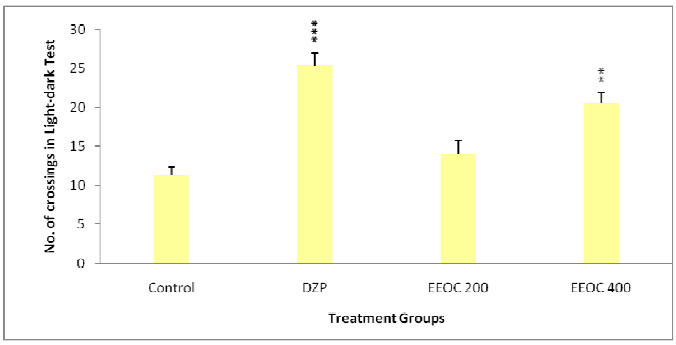

Fig. 5

Effect of Normal saline (control), Diazepam 1mg/kg (DZP), EEOC (200mg/kg), EEOC (400mg/kg) on number of crossings in light dark test. (P<0.001), (**P<0.01) (n=6 in each group)***

Hole board test

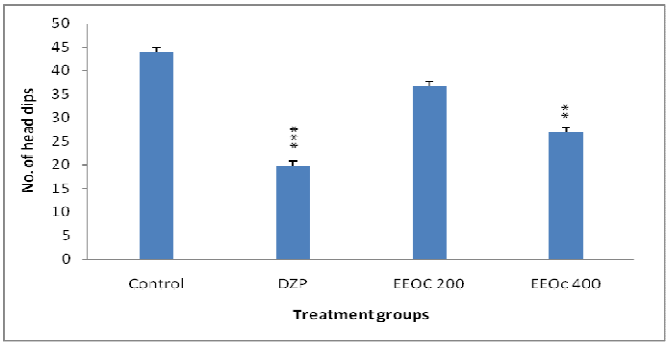

Fig. 6

Effect of Normal saline (control), Diazepam 1mg/kg (DZP), EEOC (200mg/kg), EEOC (400mg/kg) on number of head dips in Hole board test. (P<0.001), (** P<0.01). (n=6 in each group)***

DISCUSSION

Animals whenever subjected to unknown environment exhibits a particular form of behavioral inhibition, termed as anxiety. Traditional medicine has well established documentation regarding the usage of phytochemicals especially secondary metabolites in the treatment of psychotic disorders, most of which directly or indirectly affect the CNS, serotonin, GABA and BZA Neurotransmitter activities[18-22].

The Elevated plus maze is the prime apparatus that is responsible for finding the anxiolytic potential of a drug. The test is based on assumption in which encounter to an open arm maze elicits an approach avoidance conflict that was considerably stronger than elicited by exposure to a closed arm[23]. The anxiolytic effect is considered when there is a decrease aversion to open arm entry and time spent in open arm [24]. Oral administration of EEOC (200mg/kg, 400mg/kg) had shown a significant increase in open arm entry and time spent in open arm in dose dependent manner.

In the Open field test anxiety was induced in rodents by forced confrontational situations where they prefer to move along peripheral squares of the apparatus (Thigmotaxis) [25] instead of central squares. Anxiolytics make the animal preferably to move in central squares and increase time spent in centre squares. In the present study doses of EEOC (200mg/kg, 400mg/kg) significantly increased the central square crossings in dose dependent manner.

Light-Dark box was widely employed paradigm for screening of anxiolytics. It is carved in such a way to observe trend of rodents to analyze a novel environment when confronted with aversive properties of brightly illuminated area. Anxiolytics tend to increase the time spent in light [26], reduce the latency to enter light arena and increase the number of crossings between two compartments. There was a negative view on ~~the~~ number of crossings between light and dark arena. Increase in number of crossings was reported by Crawley and some authors, whereas some authors mentioned no considerable changes by anxiolytics. The most reproducible and convenient parameter for anxiolytic action was time spent in light arena rather than number of crossings between two compartments[27, 28].

The Hole Board model was another apparatus used in the measurement of head dipping behavioral responses of rodents to an unfamiliar environment. Decrease in the number of head dips was measured and groups that received EEOC (200mg/kg, 400mg/kg) had shown significant decrease in head dip counts.

Various investigators reported that phytochemicals might interact with natural endogenous mediators in the physiological system to exert anxiolytic action[30]. Most of the anxiolytic agents exert their action by opening of activated GABA- chloride channel. In conclusion, our study reveals that anxiolytic effect of Ethanolic extract of Oxalis corniculata on mice was evidenced in all the models described above. In addition anxiolytic effect was presumed to be due to one of the lipo-soluble principle that might potentiate the GABA action or might interact with natural endogenous mediators. The optimum effect was observed at 400mg/kg significantly higher than control group. Further studies are to be carried out in order to identify the exact principle responsible and also mechanism of anti-anxiety at molecular level.

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REFERENCES

1. Cesar Augusto Bruning, Marina Prigol, Juliano A, Roehrs, cristina Wayne Nogueira, Gilson Zeni, Involvement of serotonergic system in the anxiolytic effect caused by m-trifluoromethyl-diphenyl diselenide in mice, Behav Brain Res, 205(2009)511-517.
2. Hyun-Sook Yu, Seok-Yong Lee, Choon-Gon Jang, Involvement of $5HT_{1A}$ and $GABA_A$ receptors in the anxiolytic like effects of *Cinnamomum cassia* in mice , Pharmacol, Biochem and Behav, 87(2007)164-170.
3. Pine DS, Wasserman GA, Workman SB, Memory and anxiety in prepubertal boys at risk for delinquency, J American Academic Child and Adolescent Psych, 38 (1999) 1024–1031.
4. Chandana C Barua, Bhaben Buragohain, Achinta G Barua, Prabodh Borah, Mangala Lahkar, Anxiolytic effect of hydroethanolic extract of *Drymaria cordata.* L. wild, Indian J Exp Biol, 47(2009)969-973.
5. Rang H P, Dale M M, Ritter J M, Flower R J, in pharmacology, sixth edition, (Churchil Livingstone, London)2007, 546.
6. Yalla Reddy K, Mohana Lakshmi S, Saravana Kumar A, Surendar Angothu, Effect of *oxalis corniculata* on corticosterone induced Memory impairement in male albino mice, internat J Pharm and Ther, 1(2010)19-24.
7. Unni B G, Archana Borah, Wann S B, Singh H R, Basabrani Devi, Minakshi Bhattacharjee, Phytochemical and Antibacterial Study of Traditional Medicinal Plants of North East India on *Escherichia coli,* Asian J Exp Sci *23 (2009) 103-108.*
8. Sharangouda K, Patil S B, Antiimplantation and abortifacient activities of *oxalis corniculata* in albino rats, Nigerian J nat prod med*, 11(2007) 58-60.*
9. Taranalli A D, Tipare S V, Kumar S, Wound healing activity of *oxalis corniculata* whole plant extracts in rats*,* Indian J Pharmaceut sci, *66 (2004) 444-446.*
10. Kathiriya A, Das K, Kumar EP, Mathai K B, Evaluation of Antitumor and Antioxidant Activity of *Oxalis Corniculata* Linn. against Ehrlich Ascites Carcinoma on Mice, Iran J Cancer Prev*,* 3(2010)157-165.
11. Yalla Reddy K, Mohana Lakshmi S, Surendar Angothu, Antioxidant properties of methanolic extract of *Oxalis corniculata*, Internat J phyto pharmacol, 1 (2010) 43-46.
12. Senthil Kumar K K , Rajkapoor B, Study on phytochemical profile and anti-epileptic activity of *oxalis corniculata* L, Internat J Biol & Pharmaceut Res*, 1 (2010) 34-37.*
13. Harborne J B, in phytochemical screening : Guide to modern techniques of plant analysis, 2nd edition (Chapman and Hall, Newyork)1991, 653.
14. Sei wei chen , Wei xi kong , Yi Jing Zhang, Yu Lei Li, Xiao Juan Mi, Xiao Shuo Mu, Possible anxiolytic effects of *taurine* in the mouse elevated plus maze, Life sci *,* 75(2004) 1503-1511.
15. Nogueira E, Rosa G J M, Haraguchi M, Vassilief V S, Anxiolytic effect of *Rubus brasilensis* in rats and mice, J Ethno pharmacol*,* 61(1998)111-117*.*
16. Helli´on-Ibarrola M C, Ibarrola D A, Montalbetti Y, Kennedy M L, Heinichen O, Campuzanoa M, Tortoriello J, Fern´andez S, Wasowski C, Marder M, De Limad T C M, Morae S, The anxiolytic-like effects of *Aloysia polystachya* (Griseb.) Moldenke (Verbenaceae) in mice, J Ethno pharmacol*,* 105 (2006) 400–408.
17. Xiu-Yan Wei, Jing-Yu Yang, Jin-Hui Wang, Chun-Fu Wu*,* Anxiolytic effect of saponins from *Panax quinquefolium* in

mice, J Ethno pharmacol, *111 (2007) 613–618*

18. Wolfman C, Viola H, Paladini A, Dajas F, Medina J H, Possible anxiolytic effects of chrysin, a central BZP receptor ligand isolated from *Passiflora coerulea*, Pharmacol Biochem Behav, 47 (1994)4.
19. Viola H, Stein de M L, Wolfman C, Apigenin, a component of *Matricaria reticutita* flowers is a central benzodiazepine receptor - ligand with anxiolytic effects, planta Med, 61(1996)216.
20. Salgueiro J B, Ardenghi P, Dias M, Ferreira M B, Izquierdo I, Medina J H, Anxiolytic natural and synthetic flavonoid ligands of the central benzodiazepine receptor have no effect on memory tasks in rats, pharmacol Biochem Behav, 58 (1997)891.
21. Paladini C, Marder M , Viola H, Wolfman C , Wasowski C, Medina J H, flavonoids and the central nervous system : from forgetten factors to potent anxiolytic compounds, J pharm pharmacol, 51(1999)526.
22. Dhawan K , Dhawan S, Chhabra S, Attenuation of benzodiazepine dependence in mice by a tri-substituted benzoflavone moiety of passiflora incarnate Linneaus: Anon-habitat forming anxiolytic, J pharm pharmaceu sci, 6(2003)222.
23. Montgomery K C , The relation between fear induced by novel and exploratory behavior, J compare Physiol Psych 48 (1955)254-260.
24. Pellow S, Chopin P, File S E, Briley M , Validation of open: closed arm entries in an elevated plus maze as a measure of anxiety in the rat, J Neuro sci Meth,14(1985)149-167.
25. Kumar S, Sharma A, Anti-anxiety activity studies of various extracts of *Turnera aphrodisiaca* Ward, J Herb Pharmacother, 5(2005) 13-21.
26. Imaizumi M , Suzuki T, Machida H, Onodera K, A fully automated apparatus for a light – dark test measuring anxiolytic or anxiogenic effects of drugs in mice , JPn J psych pharmacol, 14 (1994)83-91.
27. Young R, Johnson DN, A fully automated light/dark apparatus useful for comparing anxiolytic agents, Pharmacol Biochem Behav, 40(1991)739-743.
28. Lepicard E M, Joubert C, Hagneau I, Perez-Diza F, Chapouthier G, Differences in anxiety – related behavior and response to diazepam in BALB/cAByJ and C57BL/6J strains of mice, pharmacol Biochem Behav, 67(2000)739-748.
29. De Angelis L, Bertolissi M, Nardini G, Interaction of caffeine with benzodiazepines: behavioral effects in mice. Arch Int Pharmacodyn Ther, 255(1982) 89 -102.
30. Contarino A, Dellu F, Koob G F, Smith G W, leek, Vale W, Gold L H, Reduced anxiety-like and cognitive performance in mice lacking the corticotrophin – releasing factor 1, Bran Res, 835 (1999)9