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A STUDY ON LEAVES OF GLYCYRRHIZA GLABRA HAS SELECTED FOR ANXIOLYTIC ACTIVITY ON EXPERIMENTAL ANIMALS IN THE ELEVATED PLUS MAZE, AQUEOUS AND ETHANOLIC EXTRACTS

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ABSTRACT:

Background: Anxiety disorders are marked by excessive fear, often in response to specific objects or situations and in the absence of true danger and they are extremely common in the general population. According to a recent epidemiological study, the lifetime prevalence of any anxiety disorder is 28.8%. Anxiety disorders are associated with impaired workplace performance and hefty light/dark test used.

Results: In the elevated plus maze, aqueous and ethanolic extracts (400mg/kg; p.o) showed an anxiolytic effect by increasing the percentage of time spent in open arms and the percentage of open arm entries as compared to control group.. In the light/dark transition test, aqueous extracts (400mg/kg; p.o) had increased the time spent in light area, latency to enter dark chamber and tunnel crossing. Whereas, to diazepam, had no inhibitory effect on locomotion in these tests, its side effect profile might be superior to the benzodiazepines.

Key words: glycyrrhiza glabra *leaves*; Anxiolytic property; Elevated plus maze; light/dark test.

Introduction:

Animal models of psychiatric diseases attempt to capture various feature of the human condition, from behavioral and physiological ne species for the purpose of studying phenomena occurring in another species. In the case of animal models in human psychopathology one seeks to develop syndromes in animals which resemble those of human in certain ways in order to study selected aspects of human psychopathology". Currently, the third criteria is regarded as having heuristic value because the central nervous processes that lead to anxiety still have to be elucidated; therefore this criterion is regarded as desirable, but not essential. Thus, in an ideal and perfect model one would like to have causative conditions, symptom profiles and treatment response identical to those seen in the human disease state

The anti-anxiety and antipsychotic indicate a qualitative distinction in the clinical use and mode of action of the drug. Pathological models. Therefore, several tests have to be performed to find a spectrum of activities which can be considered to be predictive for therapeutic efficacy in patients.

For *in vivo* studies, most investigators use a battery of anticonvulsive tests, anti-aggressive tests and evaluation of conditioned behaviour. Most of the actions of benzodiazepines are thought to be mediated by potentiation of g-amino-butyric acid (GABA). Two subtypes of GABA receptors ($GABA_A$ and $GABA_B$) have been described. Moreover, specific binding sites for benzodiazepines have been discovered would have an anxiolytic effect. Based in these findings various *in vitro* tests have been developed

Anxiety enables the individual to recognize danger and to deal with an unknown or vague internal or external threat. Fear is a similar alerting signal, but differs from anxiety in that it is regarded as response to a known, definite, non-conflictual threat. Clinicians assessing anxiety distinguish between "normal" and "pathological" anxiety. Normal anxiety is an advantageous response to a threatening situation that accompanies many aspects of daily life. By contrast, pathological anxiety is an inappropriate response to an external or internal stimulus. In light of the high complexity of anxiety disorders and the comorbidity with major depressive disorder, the chance of succeeding in developing comprehensive animal models that accurately reflect the relative influences of contributing factors in human is probably quite poor

METHODOLOGY

4.1 Materials:

4.1.1 Plant material:

The plant *Glycyrrhiza glabra* belongs to the family Fabaceae The description, history, cultivation and constituents of which have been already described. The leaves of the plant are collected from the local area of

Guntur district in the winter. The plant was authenticated by Dr.Satyanarayana Raju (M.Sc., M.Phil., Ph.D.) , plant taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna Dragendroff's reagent.

- Hagers reagent.
- Mayer's reagent.
- Wagner's reagent.

4.1.4 Chemicals:

All chemicals used were of analytical grade.

- Petroleum ether.
- Chloroform.
- Ethanol.

4.1.5 Instrument:

- Elevated plus maze apparatus.
- Social
- Electronic weighing balance.
- Oral feeding needle.

4.1.6 Animals:

Albino male Wister rats and albino mice weighing between 150 to 200g and 18 to 22 gm respectively were procured form registered breeders (1242/PO/BC/08/CPCSEA/GENTOX BIO SERVICES PVT LIMITED, Hyderabad.). The animals were housed under standard conditions of temperature 25 ±

4.2.1 Extraction [83]:

The plants collected were carefully protected and leaves were separated. The leaves were carefully washed with tap water and left to dry for 15 days in the shade at room temperature. Then they were stored in well sealed cellophane bags, so as to prevent from the environmental effects. The shade dried powdered leaves were used for the extraction with Petroleum ether, Chloroform, Ethanol and Distilled water. In each case, the powder weighing approximately 225-250 gm was extracted by adding 1000 ml of the solvents. The duration of extraction varies with the solvent and was found to be between 08-12 h with all the solvents. The extract was filtered and the filtrate evaporated to dryness under reduced pressure using a rotary evaporator.

4.2.2 Qualitative chemical test [84, 85]:

Preliminary phytochemical investigation of extract:

Qualitative chemical tests were conducted for petroleum ether, chloroform, ethanolic and aqueous extracts of leaves of *Glycyrrhiza glabra* to identify the various phytoconstituents. The various tests and reagents used are given below and observations are recorded and tabulated in results (Table 2).

Acute oral toxicity study for the formulation was carried out using **OECD guideline 420 (modified, adopted 23rd march 2006).** The test procedure minimizes the number of animals required to estimate the oral acute toxicity of a chemical and in addition estimation of LD_{50}, confidence intervals. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

Principle of the FDP:

The fixed dose procedure is method for assessing acute oral toxicity that involve the identification of a dose level that cause evidence of non-lethal toxicity(termed evident toxicity) rather than a dose level that cause lethality. Evident toxicity is terms describing clear signs of toxicity following administration of test substance, such that an increase to the next highest fixed dose would result in the development of sever toxic signs and probably mortality.

Procedure: As suggested, after acclimatization of animals for 4-5 days, study was carried out as follows:

- Healthy, young adult Albino Swiss female mice (18-25gm), nulliporous and non pregnant were used for this study Food, but not water was with held for 3-4 hours and further 1-2 hours post administration of sample under study.
- Fixed dose level of 5, 50, 500 mg/kg were initially chosen as dose level that would be expected to allow the identification of dose producing evident toxicity.
- During the validation procedure, a fixed dose of 2000mg/kg was added to provide more information on substance of

aqueous and ethanolic extracts, animals were observed individually during the first 30 min and periodically during 24 hours with special attention during the first four hours and daily thereafter for a period of 14 days. Once daily animals were observed principally in relation to changes in skin, fur, eyes and mucous membrane (nasal) and also autonomic symptoms like sedation, lacrimation, perspiration, piloerection, urinary incontinence and control nervous system (ptosis, drawsiness, gait tremors and convulsion).

4.2.2 Elevated plus maze model:

Elevated plus maze (EPM) one of the commonly used animal model (exteroceptive aversion stimulus model) for testing anti anxiety drugs was employed to assess anxiolytic activity of sample under study. EPM is

based on the apparent natural aversion of rodents to open and high spaces animals have tendency to spend more time in enclosed arms than in the open arms [67].

Procedure:

Albino mice of either sex weighing between 18-25 gm were dividing into six experimental groups of six animals each.

Group I - Control (2% gum acacia).

Group II- Standard drug (Diazepam 2mg/kg i.p.)

Group III- Ethanolic extracts dose EEGG1 (200 mg/kg p.o).

Group IV- Ethanolic extracts dose EEGG2 (400 mg/kg p.o).

Group V- Aqueous extracts dose AEGG1 (200 mg/kg p.o).

Group VI- Aqueous extracts dose AEGG2 (400 mg/kg p.o).

Standard drug diazepam was administered 45 min prior to testing and extracts were administered p.o 45 min prior to testing. Anxiolytic activity was measured using the elevated plus maze test [76]. The maze consisted of two open (28 cm x 5 cm) and two closed (28 cm x 5 cm x 14 cm) arms, extending from the central platform (5 cm x 5 cm) and elevated up to the height of 40 cm above the floor. The entire maze was made of clear Plexiglass. Mice were individually placed on the centre of the maze facing an open arm, and the number of entries and the time spend in closed and open arm were recorded during a 5 min observation period. Arm entries were defined as entry of all four

the unfamiliar (neophobia). The exploratory activity reflects the combined result of these tendencies in novel situations. Thus, in the light/dark test, drug induced increase in behaviour in the white part of a two compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of anxiolytic activity [71].

Procedure:

Albino mice of either sex to testing.

Group I - Control- (2% gum acacia).

Group II- Standard drug (Diazepam 2mg/kg i.p.)

Group III- ethanolic extracts dose EEGG1 (200 mg/kg p.o).

Group IV- ethanolic extracts dose EEGG2 (400 mg/kg p.o).

Group V- Aqueous extracts dose AEGG1 (200 mg/kg p.o).

Group VI- Aqueous extracts dose AEGG2 (400 mg/kg p.o).

The apparatus for light/dark transition test consist of two compartments: one light area (27 L x 27 W x 27 H cm),. The following parameter were recorded during 5 min: Latency time for the first crossing to the dark compartment, the number of transition between the light and the dark compartment (tunnel crossing), the total time spent in the light compartment. The apparatus was cleaned thoroughly between trials.

5. RESULTS

5.1 Preliminary Phytochemical testing of *Glycyrrhiza glabra* leaves extracts:

The investigation of the preliminary phytochemical qualitative examination of various extracts of *Glycyrrhiza glabra* shows the presence of different constituents in Table. 2

Table.2. Preliminary Phytochemical testing of *Glycyrrhiza glabra* leaves extracts:

S. No	Chemical Test		Chloroform extract	Alcohol extract	Aqueous extract
1	**Tests for steroids**				
	a. Salkowski test		-	+	+
	b. Liebermann Burchard test		-	+	+
2	**Tests for saponin**				
	a. Foam test				
3	**Tests for steroidal saponin**		+	-	-
	a. Salkowski test				
	b. Liebermann-Burchard test				
	Tests for Alkaloids		+	+	-
	(a) Mayer's test		-	+	+
4	(b) Dragendroff's test				
	(c) Wagner's test				
	(d) Hager's test		-	+	+
	Tests for carbohydrates		-	-	-
	(a) Molisch's test		-	-	-
5	**Test of reducing sugar**		-	+	-

	(a) Fehling's test				
6	(b) Benedict's test		-	-	-
	Test for Monosaccharides				
	(a) Barford's test		+	+	+
7			-		-
	Tests for Flavonoides				
	(a) Shinoda test				
8	(b) Ferric		-	+	-
	(a) Ferric chloride test				
	(b) Gelatin test		+	-	+
	(c) Bromine water test		-	+	+
	(d)Vanillin hydrochloride test		+	+	+
	Tests for Cardiac Glycosides		-	+	+
9	(a) Baljet test				
	(b) Legal's test				
	(c) Kellar-Killiani test		-	+	+
	(d) Lugal's test		+	+	+
	Tests for Proteins		-	+	+
	(a) Ninhydrin reagent test		-	+	+
10	(b) Xanthoprotein test				
	(c) Biuret test				
	(d) Millon's test			+	+
	Test for Amino acids		-	+	+
	(a) Ninhydrin test		-	+	-
	(b) Test for tyrosine		+	+	-
9	(c) Test for tryptophan		-	-	-
			-	-	-
			-	-	-
			-	-	-
			-	-	-
10					
			-	-	-
			-	-	-

'+' = Present

'-' = Absent

5.3 Acute toxicity study:

Acute toxicity studies for ethanolic, aqueous extracts of *Glycyrrhiza glabra* were conducted as per OECD guidelines 420 using albino Swiss mice. Each animal was administered aqueous and ethanolic extracts by oral route. The animals were observed for any changes continuously for the

on EPM:

The behavioural effects of EEGG1, EEGG2, and diazepam, on the behaviour of mice in the elevated plus maze test were summarized in Table 3 and Figure. - 7 to 9.

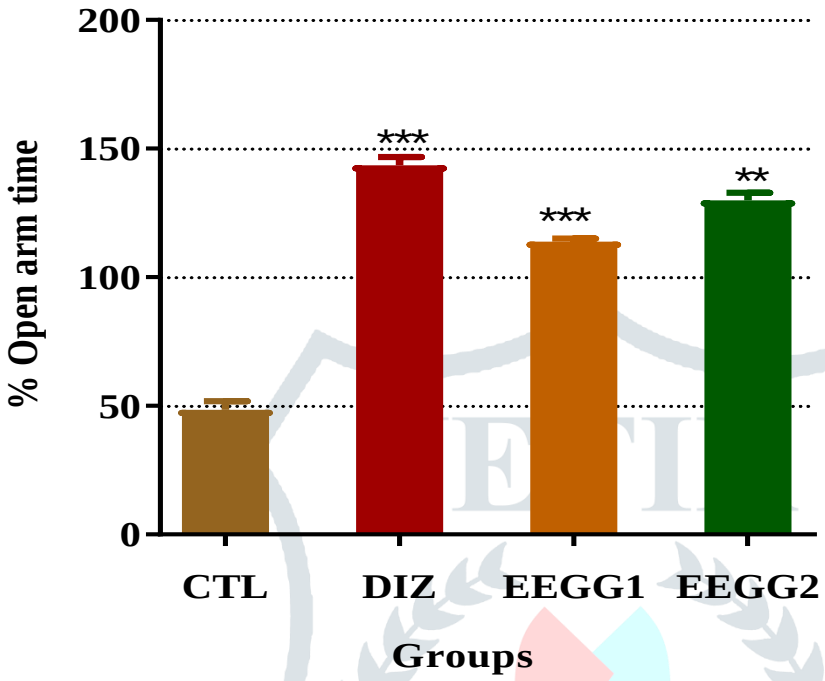
Diazepam has increased the percentage of time spent and of arm entries in open arms significantly ($P < 0.001$, Figure.ure.7 and 9), whereas in closed arm it has decreased significantly ($P < 0.001$, Figure.8) as compare to control group. It was seen that the EEGG1 has increased percentage of time spent in open arm significantly ($P < 0.05$, Figure.ure.7) with no entry in to open arm as compare to control.

The studies with that of EEGG2 shows significant results as compare to control group.EEGG2 and has significantly increased the percentage time spent and arm entry in open arms whereas in closed arm it has decreased as compare to control group.

EEGG 2 (400 mg/kg)	129	64	8.0

Table No.3 - Effects of ethanolic extracts of GG from elevated plus maze test in mice.

Figure- 7 Percentage of open arm time in 5-min EPM.



5.4.2 Aqueous extracts of GG on EPM:

The effects of Aqueous extract of AEGG1, AEGG2 and diazepam on the behaviour of mice that were summarized in Table 4 and Figure.10 to12.

Diazepam has increased the percentage of time spent and of arm entries in open arms is significant whereas in closed arm it has decreased significantly as compare to control group.

The AEGG1 has shown insignificant in percentage of time spent and arm entry in open and closed. The AEGG2 has shown increase in percentage of time spent and of arm entry in open arms significantly

Table No. 4 - Effects of Aqueous extracts of AEGG from elevated plus maze test in mice.

Treatment	% open arm time	% closed arm time	% open arm entry
Control	55.6	226	5.03
Diazepam (2 mg/kg)	149.3	62	9.8
AEGG1 (200 mg/kg)	110.33	87	5.75
AEGG 2 (400 mg/kg)	119	83.66	6.50

Effects of aqueous of AEGG from elevated plus maze test after acute treatment with 200 mg/kg and 400 mg/kg extract in mice.

Figure 10- Percentage of open arm time in 5-min EPM.

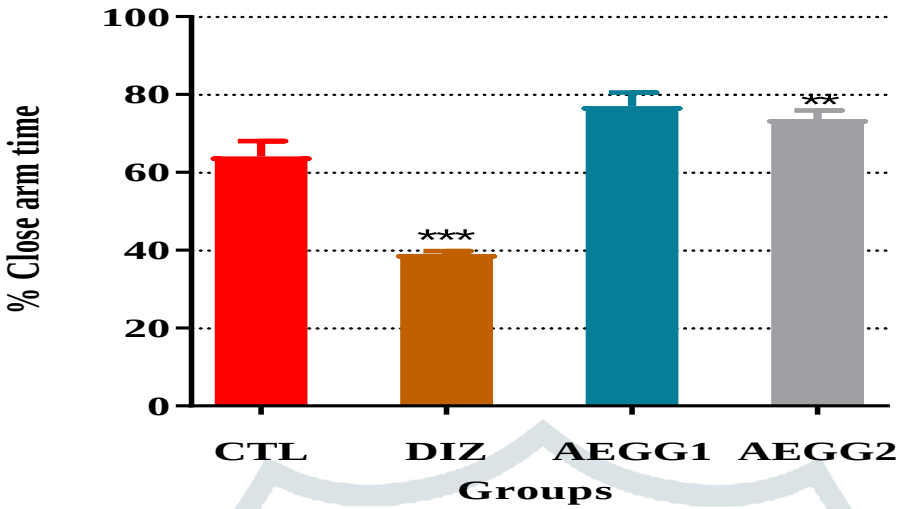
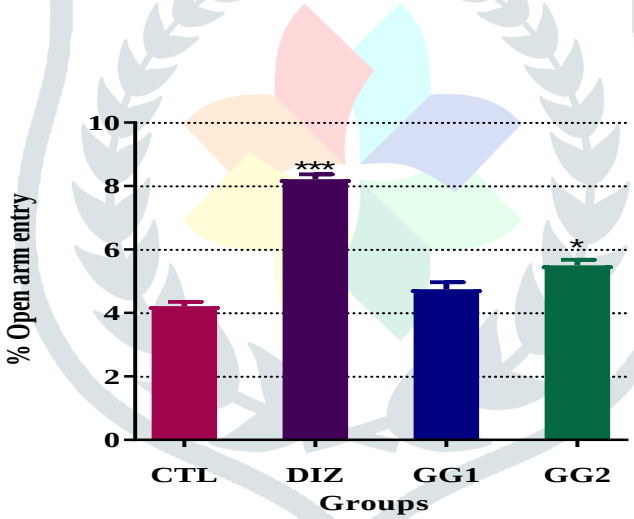


Figure 11- Percentage of closed arm time in 5-min EPM.



5.5 The Light/Dark transition test:

5.5.1 Aqueous extract of AEGG on L/D test:

Results of the light/dark test of shows in table 5 and figure 13 to 15. Diazepam treatment group showed significantly increased time in light area, latency in to enter dark compartment and number of tunnel crossing.

The GG1 has not significant in above paradigms as compare to control group, whereas GG2 showed significantly increase the time in light area latency in to enter dark compartment and number of tunnel crossing

Table No. 5 - Effect of aqueous extract of AEGG from light/dark transition test in mice.

Treatment	Social interaction. time (Sec)	Locomotion
Control	62.3	155
Diazepam (2 mg/kg)	192.45	121.0
AEGG 1 (200 mg/kg)	75.68	143.7
AEGG 2 (400 mg/kg)	170.01	144

Table No. 6 - Effect of Aqueous extract of GG from light/dark transition test in mice.

Treatment	Time in light area(sec)	Latency to enter dark (sec)	Tunnel crossing
Control		25.45	13.17
Diazepam 2 mg/kg		46.33	19.67
AEGG 1 200 mg/kg	116	28.00	13.67
AEGG 2 400 mg/kg	132	42.00	17.00

Effects of aqueous extracts of AEGG from Light/dark transition test after acute treatment with 200 mg/kg and 400 mg/kg extract in mice.

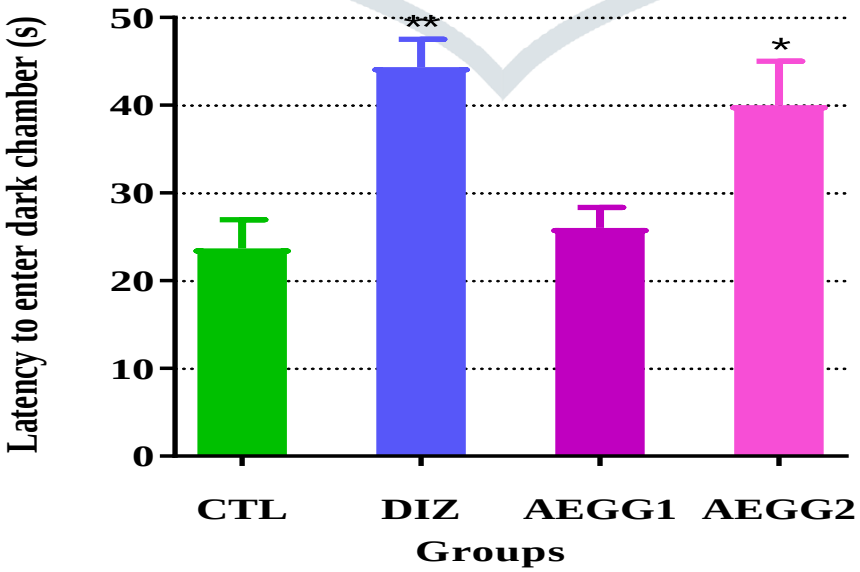


Figure 13- Time spent in the light chamber (sec) in 5-min Light/Dark test.

5.5.2 Ethanolic extract of EEGG on light/dark transition test:

Result of the light/dark transition test shown in table 8 and figure 16 to 18. Diazepam treatment group showed significantly increase time in light area, latency to enter dark compartment and number of tunnel crossing

The EEGG2 was showed significantly increase the time in light area latency to enter the dark chamber and the number of tunnel crossing as compare to control group whereas EEGG2 has not significant in above paradigms.

Treatment	Time in light area(sec)	Latency to enter dark (sec)	Tunnel crossing
Control	72.17	22.00	8.66
Diazepam 2 mg/kg	157.0		18.77
EEGG1 200 mg/kg	106.3		36.83
EEGG2 200 mg/kg	145.8	44.00	16.50

Table No. 7 - Effect of ethanolic extract of EEGG from light/dark transition test in mice.

ects of ethanolic extracts of EEGG from Light/dark transition test after acute treatment with 200 mg/kg and 400 mg/kg extract in mice.

Figure 16- Time spent in the light chamber (sec) in 5-min Light/Dark test

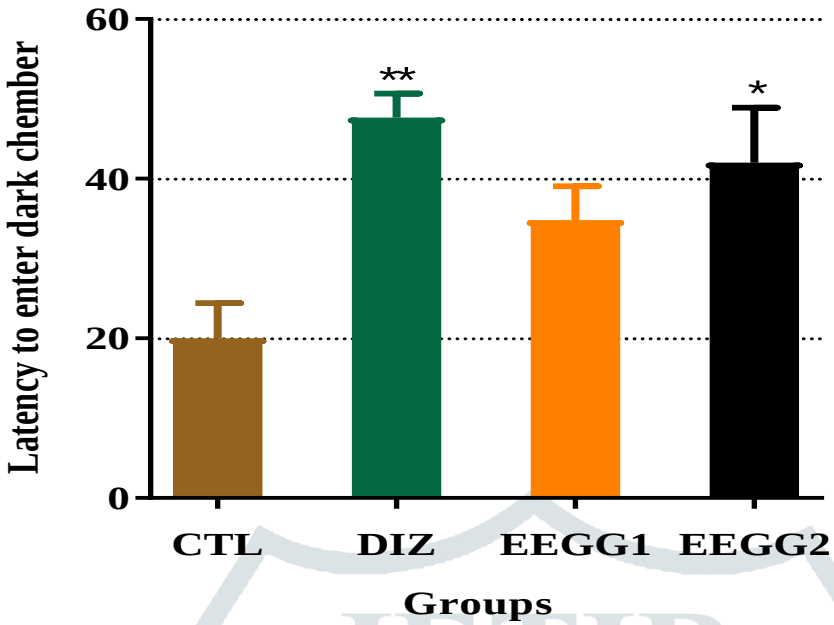


Figure 17- Latency to enter dark chamber (sec) in 5-min Light and Dark test.

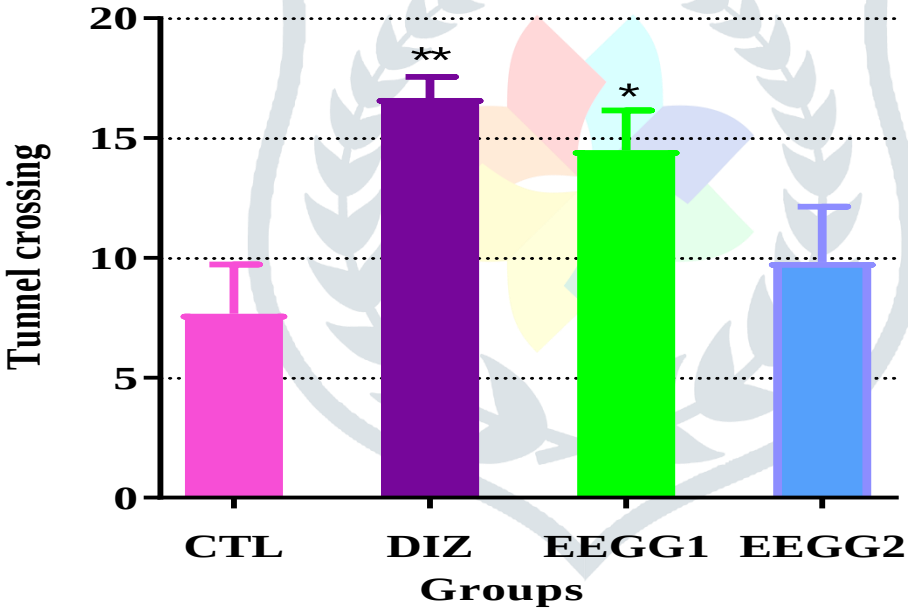


Figure18- Tunnel crossing in 5-min Light and Dark test.

1. DISCUSSION

The study was to evaluate the anxiolytic effect of ethanolic and aqueous extracts of ***glycyrrhiza glabra*** leaves by using behavioural animal models of anxiety. The major finding of present investigation propose the anxiolytic activity on elevated plus maze test, light/dark transition test in mice EPM test is based on a premise where the exposure to an EPM evoked an approach-avoidance conflict that was considerably stronger than evoked by the exposure to an enclosed arm [3]. The decrease in aversion to the

open arm is the result of an anxiolytic effect, expressed by the increased time spent and entries in to the open arm are sensitive to agents thought to act via the $GABA_A$ receptor complex, extract dose AEGG1 shows increase percentage of time spent in open arm but not entry whereas EEGG1 ethanolic extract is insignificant as compare to control. The AEGG2 (400 mg/kg) of aqueous and ethanolic extracts had increased the percentage in time spent and entry in to open arm with decreased in closed arm. It can be suggested that GG (400 mg/kg) of aqueous and ethanolic extracts may have the anxiolytic effects similar to the standard drug as result animal spent more time in open arm and less time in closed arm. EEGG1 (200 mg/kg) of ethanolic extract did not alter the above parameter significantly therefore it does not exhibit anxiolytic effect. There for behavioural alteration induced by higher dose GG (400 mg/kg) of aqueous (AEGG2) and ethanolic extract(EEGG2) and lower dose of EEGG1 (200 mg/kg) of aqueous extract were consistent with dose dependant anxiolytic profile.

Light/dark box is another widely used rodent anxiety model for screening anxiolytic or anxiogenic drugs. It is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors that is novel environment and light [78]. Drugs of anxiolytic activity [79]. In this study, the time spent in light area, latency to enter dark chamber and tunnel crossing is an indices of anxiety. The aqueous extract of AEGG2 (400mg/kg) and ethanolic extract of EEGG2 (400mg/kg) had significantly increased the time spent in light area, latency to enter dark chamber and tunnel crossing, similar to standard drug, suggesting that anxiolytic activity of EEGG leaves extract as compare to control group.. The phytoconstituent like flavonoids were reported for their anxiolytic effect and these constituents were present in aqueous and ethanolic extracts of ***Glycyrrhiza glabra*** leaves, so this active principle might be responsible for anxiolytic effect. The mechanism of anxiolytic activity of ***Glycyrrhiza glabra*** leaves extracts may be due to flavones specifically recognise the central BDZ Receptors and it has been for the anxiolytic effect of aqueous and ethanolic extract of ***Glycyrrhiza glabra*** could be related atleast in part to flavanoids or specifically

7. CONCLUSION

The results obtained from these experimental models clearly confirmed that the anxiolytic activity of aqueous and

(400mg/kg) of ethanolic extract had significant anxiolytic activity comparable to standard drug diazepam (2 mg/kg; i.p) in all models.

The phytoconstituent like flavonoids were reported for their anxiolytic effect and these constituents were present in aqueous and ethanolic extracts of ***Glycyrrhiza glabra*** leaves, so this active principle might be responsible for anxiolytic effect.

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