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COMPARATIVE EVALUATION OF THE ANTI-DEPRESSANT EFFECT OF ETHANOLIC AND AQUEOUS EXTRACT OF GLYCYRRHIZA GLABRA IN RATS AND MICE

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ABSTRACT

The main objective of the present study was to compare the antidepressant like activity of ethanolic and aqueous extracts of Glycyrrhiza glabra in albino rats and mice. From acute toxicity study, the dose of ethanolic and aqueous extracts of G.glabra was fixed as 100,200 and 400 mg/kg which was administered orally for 7 days once daily. The antidepressant like activity was performed after single and repetitive treatments of both the extracts for 7 days using forced swimming test(FST) and tail suspension test(TST) as labarotary models. The antidepressant like activity was compared to that of Imipramine(10mg/kg,i.p) administered for seven successive days. *G. glabra(* ethanolic and aqueous) root extracts given in three doses 100 mg/kg, 200 mg/kg & 400 mg/kg decreased the duration of immobility in FST and TST in a dose dependent manner, but significant antidepressant like effect was seen with ethanolic extracts(200 mg/kg & 400 mg/kg) body wt. doses. The efficacy of the extracts was comparable to that of Imipramine (10mg/kg). Thus it can be assumed that the antidepressant like effects of the extracts *G.glabra* may be due to increase in the level of serotonin and monoamine oxidase

inhibitors. Our present comparative study confirmed that the *Glycyrrhiza glabra* ethanolic root extract has higher antidepressant activity as it significantly reduced the immobility behavior as compared to aqueous extracts during depression in animal models and this effect might be due to blockade of hyperactivity of HPA-axis (hypothalamic–pituitary–adrenal).

Key words: *Glycyrrhiza glabra*, antidepressant, forced swim test, tail suspension test

Introduction:

Mental depression is a chronic and heterogeneous disorder affecting the persons mood, thoughts physical health and behavior which may range from mild condition to severe depression which is sometimes called as ‘‘psychotic depression’’ mostly accompanied by hallucinations and delus ions[1]. More than 20% of the adult population suffers from these conditions at some point of time in their life. According to WHO's prediction, depression will be the second most common disease in 2020**[2].**

Evidence has shown that there is involvement of neurogenesis in depression, though the role is not exactly known. Recent research has suggested that there may be a link between depression and neurogenesis of the hippocampus. This horse shoe-shaped structure is a center for both mood and memory. Loss of neurons in the hippocampus is found in depression and correlates with impaired memory and dysrthymic mood. It is explained on the basis that the drugs increase serotonin levels in the brain which in turn stimulate neurogenesis and therefore increase the total mass of the hippocampus and would in theory restore mood and memory, therefore assisting in the fight against the mood disorder [3]**.**

Recent evidences showed that individuals with clinical depression exhibit markedly higher levels of monoamine oxidase A (MAO-A) enzyme in the brain compared to people without depression. MAO-A is an enzyme which reacts with and decreases the concentration of monoamines such as serotonin, norepinephrine and dopamine. Lower concentrations of monoamines are a well known cause of depression [4].

Liquorice, member of Fabaceae family mainly includes three species: *Glycyrrhiza uralensis, Glycyrrhiza glabra*, and *Glycyrrhiza inflata*, is a famous medicinal plant with a long history of pharmaceutical and medicinal use. *Glycyrrhizin glabra* has experimentally proved activities such as

anti-inflammotory activity[5] ,anti microbial activity[6], anti viral activity[7], hepatoprotective activity[8] , anti tumor activity[9] ,memory enhancing activity[10] ,anti platelet activity[11],immuno stimulatory activity[12]. The clinically proved pharmacological activities includes anti ulcer activity[13] ,anti cancer activity[14], anti oxidant activity[15], hepato protective activity[16],eczema and psoriasis[17] and cough relieving effects[18] .

Glycyrrhizin, the major constituent of *G. glabra* is a triterpenoid saponin (2 – 9%) which is a mixture of potassium and calcium salts of glycyrrhizic acid. Minor constituents present in *G. glabra* include triterpenoid saponins viz., glabranin A and B, glycyrrhetol, glabrolide, isoglabrolide, isoflavone s viz., formononetin, glabrone, neoliquiritin, hispaglabridin A and B, coumarins viz., herniarin, umbelliferone, triterpene sterols viz., onocerin, hamyrin and stigmasterol [19].

It is evident that MAO inhibitors increase the concentrations of norepinephrine, serotonin and dopamine within the neuronal synapse through the inhibition of MAO enzyme and have antidepressant effects. It was found that glycyrrhizin, major constituent of *Glycyrrhiza glabra* inhibit monoamine oxidase, thereby increasing the levels of monoamines like epinephrine and dopamine in brains of mice [20].

So the present study was undertaken to investigate and compare the antidepressant like effects of the extracts of *Glycyrrhiza glabra* in albino rats and mice using forced swim test and tail suspension test as labarotary models. Imipramine, a tricyclic antidepressant was used to standardize the animal models of depression and to compare the antidepressant activity of both the extracts *G.glabra*. The study was also carried out to explore the underlying mechanism of action of extracts of *G. glabra*.

Materials and Methods:

Study design: The experimental design consist of animals (albino rats and mice) divided into five groups, each containing 6 animals. The ethanolic and aqueous extracts were administered at 3 different doses (200mg/kg, 300mg/kg and 400mg/kg) orally to different groups of animals. The dose selection was set from the acute toxicity study. The Institutional Animal ethical Committee (Regd No. 926/ab/06/CPSCSEA 22.02.2006) approved the experimental protocol (Approval No.19).

Animals:

Swiss albino rats (145-170g) and albino mice (20-30g) were obtained from Ghosh enterprises, Calcutta which were free from any disease used in the present study. The animals had free access to food and water with alternating light and dark cycles of 12 h each and kept under standard labarotary condition. Diets were in the form of pellets consisting of protein (20.12%), total oil(4.38%), dietary fibre(3.655) and moisture(8%) obtained from Rayan's biotechnologies Pvt Ltd, Hyderabad(A.P). The experimental animals were acclimatized for atleast 5 days before starting the experiment.

Drugs and Chemicals:

Coarse powder of the roots of G.glabra was obtained from commercial sources. Imipramine (Sigma Chemicals Co., St.Louis, USA), Ethanol (90%) from Bengal Chemicals was used in the present study.

Vehicle:

Liquorice ethanolic and aqueous extract was diluted in distilled water. Imipramine was dissolved separately in normal saline (0.9% sodium chloride).

Preparation of ethanolic extract of *G.glabra:*

Ethanolic extract of *G. glabra* was prepared by soxhelation process [21] . Dried powder roots of *Glycyrrhiza glabra* (900 grams) was weighed & macerated with 750 ml of 90% ethanol for 24hr. After 24hr the macerated powder was poured into the round-bottomed extractor flask of soxhlet apparatus. 3500 ml of solvent (Ethanol 90%) was added in to the flask and the soxhlet apparatus was placed on the mantle. The flask was fitted with a water-cooled condenser. A desired temperature was set. The extraction was continued for 36 hours, 1-2 cycles per hour. After cooling the plant material was removed by filtration through a cotton plug. The solvent of the extract was evaporated by using rotary evaporater.The yield was observed to be 15.4% which was dried and kept under refrigeration. It was reconstituted in water for injection for further use.

Preparation of Aqueous extract of G.glabra: [22]

Dried powdered roots of *Glycyrrhiza glabra* (250gm) was weighed & macerated with hot distilled water for 48 hour. Preservatives such as sodium benzoate were added. After 48 hour the macerated content was filtered using muslin

cloth. For complete removal of the water the extract was placed in hot air oven & temperature set upto 50˚C. The obtained extract was then freeze-dried and kept at 4˚C. The total yield was found to be 16%.

Determination of maximum tolerable dose by acute toxicity study:

For acute oral toxicity and determination of maximum tolerable dose, the Organization for Economic Co-operation and Development (OECD) guideline 423 was followed and the behavioral study was performed. Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method).

Albino mice (n = 6), healthy and young adult mice having body weight 25-30 gm of either sex were selected by random sampling technique for the study. The animals were kept fasting for overnight providing only water, after which the extract were administered orally at the dose level of 100, 250, 500, 750, 1000 & 2000mg/kg body weight using cannula and behavior was observed for 48 hours. From the toxicity study, extract of *G.glabra* (ethanolic and aqueous) did not produce any toxicity upto 750mg/kg.Therefore dose between 100 to 500 mg/kg can be used. Hence 100,200 and 400 mg /kg were selected for screening of antidepressant activity.

Laboratory models for testing anti depressant activity:

Forced swimming test:

Behavior despair (Forced swimming test) was most proposed model to test the antidepressant activity [23]. In this method male Albino rats weighing 145-170 g were used. They were brought to the laboratory one day before the experiment and were housed separately in polypropylene cages with free access to food and water. The glass cylinder (diameter=18 cm; height=40 cm) filled with water at 24±1^{0}C to a depth of 15 cm. The water level was 25 cm from the top. Water was changed before the next animal was placed into the water tank. Clean water was used for each behavioral trial because "used water" has been shown to alter behavior alarm signals [24]. The experimental session consisted of two trials. During the conditioning trial, rats were gently placed into the cylinders for 15 min. After the trial, rats were dried and placed into a warm cage. Twenty-four hours later (test trial), the animals were placed again into the cylinders for a 5-min test session. After placing, the rats in the cylinder, the rats were initially showing high activity, vigorously swimming in circles, tried to climb the wall or diving to the bottom. After 2-3 min, the activity of rats gradually

reduced and to be interspersed with phases of immobility. After 5-6 min, immobility reached a plateau where the rats became immobile for approximately 80% of the time. The animal was said to be immobile when the animal remained in water with all four limbs motionless, except for occasional alternate movements of paws and tail necessary to prevent sinking and to keep head/nose above water.

Tail suspension test: [25]

Male mice weighing 20–25 g were used preferentially. They were housed in plastic cages for at least10 days prior to testing in a 12 h light cycle with food and water freely available. Here the mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. Mice were considered immobile when they hang passively and completely motionless for at least 1 min. The duration of immobility was recorded for a period of 5 min.

Study groups: For each test animals were divided into 5 groups each containing 6 animals.

For forced Swim test and tail suspension test:

GROUP-1: Served as control-received vehicle only (0.5ml/100g) 60 min prior to the induction of depression by despair swimming and tail suspension test.

GROUP -2: Received *G.glabra* ethanolic& aqueous extract (100mg/kg) 1 h prior to the induction of depression by despair swimming and tail suspension test.

GROUP-3: Received *G. glabra* ethanolic & aqueous extract (200mg/kg) 1 h prior to the induction of depression by despair swimming and tail suspension test.

GROUP-4: Received *G. glabra* ethanolic & aqueous extract (400mg/kg) 1 h prior to the induction of depression by despair swimming and tail suspension test.

GROUP -5: Received Imipramine (10mg/kg) 30 min prior to induction of the depression by despair swimming and tail suspension test.

Statistical analysis: The results were expressed as Mean± S.E.M. Statistical analysis of the values observed in all the experimental methods viz., Despair swim test, Tail suspension test was performed by one way-ANOVA followed by Dennett's multiple comparison test. $p<0.01$ were considered as statistically significant.

RESULTS:

Effect on immobility duration in forced swim test:

Effects of oral administration of ethanolic root extract *Glycyrrhiza glabra* on the duration of immobility of rat in Forced swim test (FST) method were shown in **Table-1** and **Figure-1, 2&3**. Low dose 100mg/kg, intermediate dose 200mg/kg of the liquorice extract administered orally for 7 days showed no significant change in immobility whereas the same dose of ethanolic extract of liquorice showed a higher antidepressant effect as evident from the decrease in immobility period.

The highest dose 400mg/kg of the ethanolic extract revealed a maximum antidepressant effect with % change of 48.25 as compared to the control comparatively the aqueous extract showed a % change of 42.5 **(Table-2 and Figure-4,5&6).** Imipramine (10mg/kg i.p.) administered 60 min prior to experiment reduced the immobility periods as compared to control in FST.

Effect on immobility duration in tail suspension test:

Effects of oral administration of ethanolic root extract of *Glycyrrhiza glabra* on the duration of immobility of mice in tail suspension test (TST) were shown in **Table-3 and Figure-7**. Tail suspension stress indudced behavior of aqueous of *Glycyrrhiza glabra* (100, 200, and 400 mg/kg) orally administered for 7 days induces comparative dose related decrease in total period of immobility. The decrease of immobility in ethanolic extract was more as compared to aqueous extract **(Table-4 & Figure-8)** which was qualitatively comparable to that induced by imipramine (10mg/kg, i.p) administered 60 min prior to experiment.

Table 1:Effects of ethanolic extract of Glycyrrhiza glabra & Imipramine on Forced swimming test in Rats.

Gps	Treatment	Dose (mg/k)	Time (hr)	Duration of immobility(Sec)						Mean±SEM	% Change
				Number of rats							
				1	2	3	4	5	6		
I	Control + Depression	D.W.	1	178	164	167	184	168	163	169±3.81	---
			5	188	178	168	176	160	179	172±3.61	---
			12	178	164	167	184	168	163	169 ±5.39	---
II	G.glabra extract +Depression	100	1	151	143	137	147	142	128	** 139±3.26	18.60↓
			5	122	134	132	138	147	148	**139± 3.29	18.85↓
			12	152	157	142	168	142	157	**153±4.99	13.79↓
III	G. glabra extract +Depression	200	1	113	121	111	108	113	99	**110±3.57	34.30↓
			5	116	112	126	127	116	112	** 118±3.31	32.00↓
			12	129	134	139	118	128	137	** 130±3.59	25.28↓
IV	G.glabra extract +Depression	400	1	104	92	94	81	87	83	** 87±2.50	48.25↓
			5	112	79	74	89	92	87	** 84±3.33	50.28↓
			12	112	132	119	98	109	125	** 116±5.98	32.18↓
V	Imipramine +Depression	10	1	58	56	56	64	61	67	** 60±2.17	63.95↓
			5	52	54	43	51	56	58	**52.4±2.6	68.00↓
			12	58	56	56	64	61	67	** 57 ±3.59	65.50↓

Effect of G.glabra(Ethanolic extract)-100200, 400mg/kg &Imipramine10mg/kg on immobility (Forced swim test) by rats.Values are presented as Mean±SEM (n=6), *: indicates($p<0.05$), **: indicates ($p<0.01$) & ***:indicates ($p<0.001$)compared to the control group by one way ANOVA followed by Dunnet multiple comparison test.

Table 2: Effects of aqueous extract of Glycyrrhiza glabra & Imipramine on Forced swimming test in Rats.

Gps	Treatment	Dose (mg/kg)	Time (hr)	Duration of immobility(Sec)						Mean±SEM	% Change
				Number of rats							
				1	2	3	4	5	6		
I	Control+ Depression	D.W.	1	181	172	158	189	162	167	169±3.81	---
			5	188.	178	168	176	160	179	172±3.61	---- --
			12	178	164	167	184	168	163	169 ±5.39	---
II	G.glabra extract +Depression	100	1	152	157	142	148	142	147	** 148±2.29	11.9↓
			5	142	151	146	148	151	148	** 147 ±3.91	17.14↓
			12	161	152	147	157	148	158	** 153 ±2.51	13.11↓
III	G.glabra extract +Depression	200	1	119	109	127	131	118	137	** 123±4.1	26.39↓
			5	126	122	124	124	126	114	** 122 ±1.8	26.14↓
			12	123	137	136	138	131	134	** 133 ±2.77	21.75↓
IV	G.glabra extract +Depression	400	1	97	94	99	97	102	97	** 97±1.80	42.51↓
			5	94	98	81	107	97	106	** 97 ±3.85	43.85↓
			12	114	127	112	126	118	121	** 119±2.85	32.92↓
V	Imipramine +Depression	10	1	57	59	69	54	56	47	** 60±2.17	65.11↓
			5	52	54	43	51	56	58	** 52.4±2.61	70.28↓
			12	58	56	56	64	61	67	** 57 ±3.59	67.24↓

Effect of G.glabra(Aqueous extract)-100200, 400mg/kg &Imipramine10mg/kg on immobility (Forced swim test) by rats.Values are presented as Mean±SEM (n=6), *: indicates($p<0.05$), **: indicates ($p<0.01$) & ***:indicates ($p<0.001$)compared to the control group by one way ANOVA followed by Dunnet multiple comparison test.

Table 3:

Effects of ethanolic extract of Glycyrrhiza glabra & Imipramine on Tail suspension test in Mice.

Gps	Treatment	Dose (mg/kg)	Duration of immobility(Sec)						Mean±SEM	% Change
			Number of rats							
			1	2	3	4	5	6		
I	Control	D.W.	189	194	163	191	188	193	185±5.79	---
II	G.glabra extract +Depression	100	132	174	121	131	136	127	**136±7.71	28.42↓
III	G.glabra extract +Depression	200	113	115	102	105	125	121	**113±3.63	33.68↓
IV	G.glabra extract +Depression	400	91	84	81	81	86	88	**85±1.62	56.52↓
V	Imipramin+Depression	10	84	57	75	65	72	73	** 68±3.31	64.21↓

Effect of G.glabra(Ethanolic extract)-100200, 400mg/kg &Imipramine10mg/kg on immobility (Tail suspension test) by mice.Values are presented as Mean±SEM (n=6), *: indicates($p<0.05$), **: indicates ($p<0.01$) & ***:indicates ($p<0.001$)compared to the control group by one way ANOVA followed by Dunnet multiple comparison test.

Table 4: Effects of aqueous extract of Glycyrrhiza glabra & Imipramine on Tail suspension test in Mice.

Gps	Treatment	Dose (mg/kg)	Duration of immobility(Sec)						Mean±SEM	% Change
			Number of rats							
			1	2	3	4	5	6		
I	Control	D.W.	189	194	163	191	188	194	185±5.79	---
II	G.glabra extract +Depression	100	139	168	137	178	145	148	**152±6.80	17.89↓
III	G.glabra extract +Depression	200	118	132	148	172	145	151	** 144±7.40	20.63↓
IV	G.glabra extract +Depression	400	108	122	134	114	127	128	** 122±4.59	38.00↓
V	Imipramine +Depression	10	84	57	75	65	72	73	**71±3.75	64.21↓

Effect of G.glabra(Aqueous extract)-100200, 400mg/kg &Imipramine10mg/kg on immobility (Tail suspension test) by mice.Values are presented as Mean±SEM (n=6), *: indicates($p<0.05$), **: indicates ($p<0.01$) & ***:indicates ($p<0.001$)compared to the control group by one way ANOVA followed by Dunnet multiple comparison test.

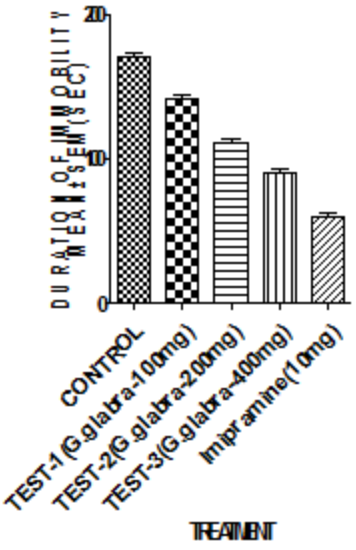

Figure 1 After 1 hour

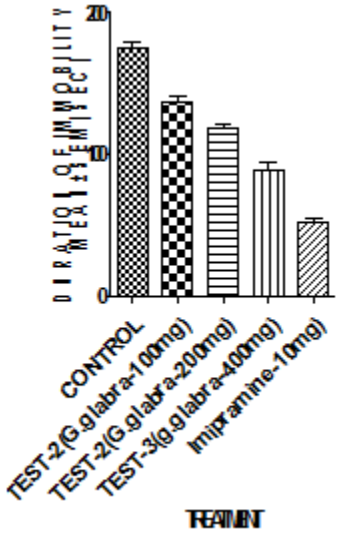

Figure 2 After 5 hour

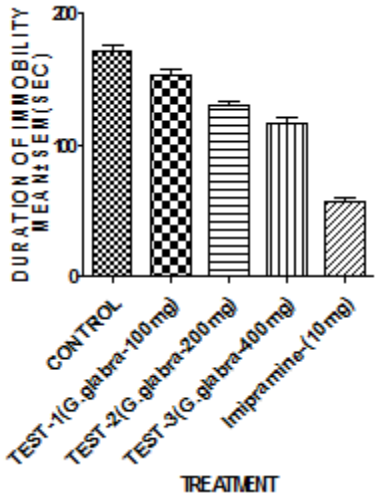

Figure 3 After 12 hour

Fig 1-3: Effects of Ethanolic extract of Glycyrrhiza glabra & Imipramine on Forced swimming test in Rats. Each column represents mean±SEM(n=6)

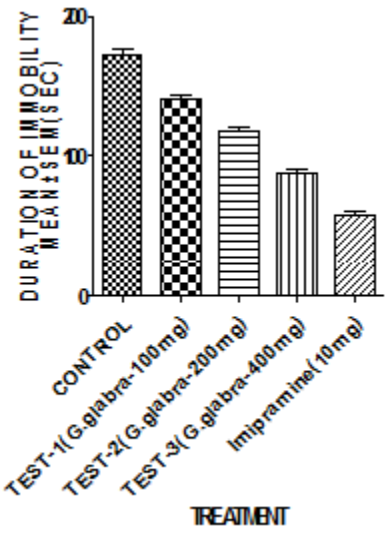

Figure 4 after 1 hour

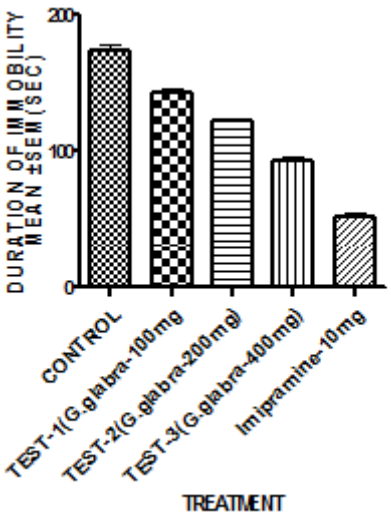

Figure 5 after 5 hour

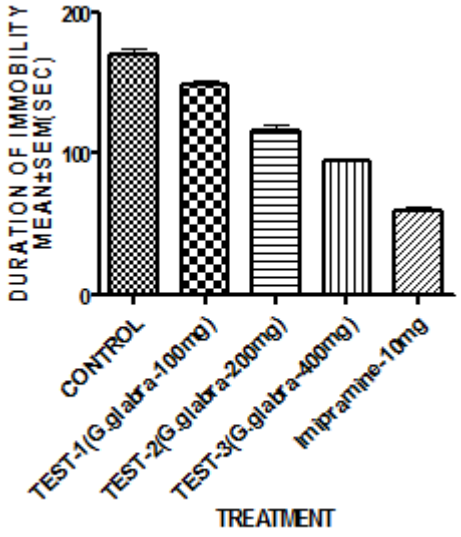

Figure 6 After 12 hour

Figure 4-6: Effects of aqueous extract of Glycyrrhiza glabra & Imipramine on Forced swimming test in Rats. Each column represents mean±SEM(n=6)

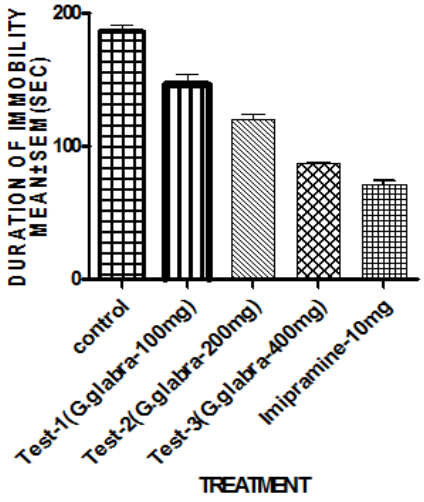

Figure-7: Effects of ethanolic extract of Glycyrrhiza glabra & Imipramine on Tail suspension test in Mice. Each column represents mean±SEM(n=6) .

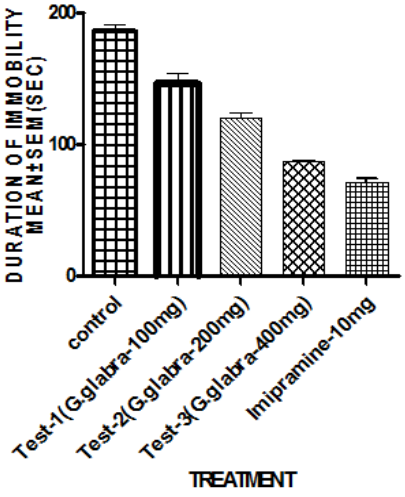

Figure-8: Effects of aqueous extract of Glycyrrhiza glabra & Imipramine on Tail suspension test in Mice.Each column represents mean±SEM(n=6) .

Discussion:

The present study of the comparative assessment of the antidepressant effect of the *G.glabra* (aqueous and ethanolic) extract on animals revealed that there was significant reduction in the immobility time at high dosage (200 & 400 mg/kg). The antidepressant effect of the ethanolic extract was more as compared to aqueous plant extract. Forced swimming test and tail suspension test are the accepted models for the study of antidepressant activity. When mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several antidepressants which are therapeutically effective in human depression. In tail suspension test the immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail [26].

Probable mechanism of the antidepressant effect

It was believed that CNS is the main biochemical cause of depression which is a metabolic disorder of the monoamine neurotransmitters. According to monoamine hypothesis it was proposed that depletion of 5-HT(5-hydroxy tryptamine), NE(norepinephrine) and DA(dopamine) in addition to activation of monoamine oxidase in the CNS causes depression [27]. The standard drug Imipramine is a tricyclic antidepressant which inhibits the cellular uptake of and increases the level of serotonin and monoamine oxidase inhibitors. It showed similar antidepressant activity in terms of immobility as that of the highest dose of plant extract which suggests that it has similar mechanism as of imipramine.

Conclusion: The results conclude that the preclinical study on rodents of the ethanolic and aqueous extracts of after oral administration using two models possess significant antidepressant activity but the ethanolic extract showed higher antidepressant activity comparable to aqueous extract. Thus the ethanolic extract of G.glabra can be explored for the management of depressive disorders.

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