



## EVALUATION OF SEDATIVE, HYPNOTIC AND ANTICONVULSANT EFFECTS OF ETHANOLIC EXTRACT OF *GLYCYRRHIZA GLABRA* USING ANIMAL MODELS

Swati Mishra\*<sup>1</sup>, T.R.Swain<sup>2</sup>, M.Mohanty<sup>3</sup>

<sup>1</sup> Department of Pharmacology, I.M.S. & SUM Hospital, SOA University, BBSR-03, India.

<sup>2</sup> Department of Pharmacology, S.C.B. Medical College & Hospital, Cuttack, India.

<sup>3</sup> Department of Pharmacology, KIMS, BBSR, India.

\*Corresponding author's E-mail: [mishraswati017@gmail.com](mailto:mishraswati017@gmail.com)

Accepted on: 31-05-2012; Finalized on: 31-07-2012.

### ABSTRACT

The present study was undertaken to investigate the sedative, hypnotic & anticonvulsant effect of ethanolic extract of *Glycyrrhiza glabra* root (GGRE) in mice using phenobarbitone sleeping time, maximal electroshock induced seizure models. GGRE at doses 75, 150, and 300 mg/kg was administered orally for 7 successive days in separate groups of Swiss young albino mice. Decrease in the onset of sleeping time and increase in the duration of sleeping time was observed in GGRE at doses 150 and 300 mg/kg. The onset of sleep and duration of sleep at the dose 300 mg/kg was comparable to that of Diazepam (1 mg/kg). GGRE at doses 150 & 300 mg/kg showed increase in onset of seizure and decrease in duration of seizure which was comparable to the standard drug Phenytoin (60mg/kg). Thus, it may be concluded that GGRE may possess anticonvulsant & sedative, hypnotic effect.

**Keywords:** Anticonvulsant, Sleeping time, Sedative, Hypnotic.

### INTRODUCTION

Herbs have been the oldest remedies used by all cultures throughout history. India has one of the oldest, richest and diverse cultural living traditions associated with the use of medical plants. In the traditional system of medicine, the roots and rhizomes of *Glycyrrhiza glabra* L. (family: Leguminosae) have been employed clinically for centuries for their anti-inflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities.<sup>1</sup> Several secondary plant metabolites were isolated from rhizomes of *G. glabra* and were investigated for the COX-2 inhibitory activity.<sup>2</sup> Water soluble portion of the aqueous extract of *Glycyrrhiza glabra* exhibited antidepressant activity on forced swim test and tail suspension test<sup>3</sup>. Anti-inflammatory and antioxidant properties of liquorice may be contributing favorably to the memory enhancement & it is possible that the beneficial effect on learning and memory may be because of facilitation of cholinergic transmission in brain<sup>4</sup>. They also possess antipyretic, antimicrobial, antiherpes, and anxiolytic activities<sup>5</sup>. Roots have demulcent, antacid, anti-ulcer, anti-inflammatory, expectorant, tonic, diuretic, laxative, and sedative properties<sup>6</sup>. People all over the world are looking for the alternative system of medicines, which are claimed to be safe, equally effective and provide better answer to chronic diseases. Thus, to have an experimental base, the modern herbal medicines need to adopt a reverse pharmacological approach. Keeping these facts in view, the present study has been designed to throw light & explore central actions like Sedative Hypnotic & anticonvulsant.

### MATERIALS AND METHODS

Evaluation of sedative, hypnotic & anticonvulsant action of *Glycyrrhiza glabra* was carried out over a period of 6 months i.e. from June 2011 to December 2012. The study was undertaken under the headings to probe for possible: Sedative & Hypnotic action, Anticonvulsant action.

#### Selection of Animals

About 70 Swiss young albino mice of either sex weighing between 20-25 grams maintained on 24 hours dark and light cycles were selected randomly for the present study. Animals had free access to food and water and maintained under standard laboratory conditions. The animals were acclimatized for at least ten days before behavioural experiments. Experiments were carried out between 9.00 and 15.00 hrs. After the experiments, the used animals were kept separately from others in the animal house to enable observation for development of any complication. Experimental protocol was approved by the institutional animals' ethics committee before the start of the study.

#### Extraction Procedure

The roots of *Glycyrrhiza glabra* were collected from the tree in Autumn when 3 to 4 years old from the plants available at Government Botanical Garden, Nrusinghanath, Bolangir district. The plant was certified by a Botanist. The roots were shade dried and coarsely grinded. About 600 grams of powdered material was taken and extracted with 90% ethanol using Soxhlet's apparatus for 24 hours. The extract was filtered and the residue obtained was rejected. The filtrate was evaporated to dry and a dark brown mass was obtained (which is henceforth referred as EXTRACT). The extract



was suspended in polyethylene glycol (PEG) and freshly prepared solution was used for each experiment.

**Solvent**

Polyethylene glycol (PEG) is a non-ionic agent used as a solubilising agent for water insoluble substances like GGRE root extract. The solvent was also used as control in one group of mice.

**Evaluation of sedative and hypnotic action**

The study was undertaken to evaluate any possible sedative, hypnotic effect of ethanol extract of roots of *Glycyrrhiza glabra* by using prolongation of phenobarbitone sleeping time in mice.

- Drugs used:
- a. GGRE
  - b. Phenobarbitone
  - c. Polyethylene Glycol
  - d. Distilled water as vehicle
  - e. Diazepam

35 albino mice were selected randomly by preliminary screening for the experiment and divided into 5 equal groups. Each group of mice were numbered and kept separately in separate cages and the cages were numbered. Standard (Diazepam) administered i.p, GGRE & control (PEG) administered orally. Phenobarbitone sodium (60mg/kg i.p.) was injected 30 minutes after administration of drugs for all the groups. The time elapsed between loss and recovery of the righting reflex was noted and taken as sleeping time. (model by Fujimoto J.M et al, 1960)

**Table 1:** Grouping of animals for assessment of sedative & hypnotic action

Group	Drug	Dose
1	PEG	1 ml / 100 gm.
2	GGRE	75 mg/kg
3	GGRE	150 mg/kg
4	GGRE	300 mg/kg
5	Diazepam	1mg/kg

**Evaluation of anticonvulsant action**

The study was undertaken to evaluate any possible of ethanol extract of roots of *Glycyrrhiza glabra* by using electroshock induced seizure model (MES).

Apparatus used: Electro-convulsimeter (Techno), Ear electrode

- Drugs used:
- a. GGRE
  - b. Phenytoin
  - c. Polyethylene Glycol
  - d. Distilled water as vehicle

35 albino mice were selected randomly by preliminary screening for the experiment and divided into 5 equal groups. Each group of mice were numbered and kept separately in separate cages and the cages were

numbered. Drugs (GGRE, Phenytoin), control (PEG) administered i.p 30 minutes before the starting of the experiment.

**Table 2:** Grouping of animals for assessment of anticonvulsant action

Group	Drug	Dose
1	PEG	1 ml / 100 gm.
2	GGRE	75 mg/kg
3	GGRE	150 mg/kg
4	GGRE	300 mg/kg
5	Phenytoin	60 mg/kg

**Maximal Electroshock Seizure Model (method by Swinyard E.A et al)**

This model of seizure is a standard model for screening anticonvulsant activity of a compound. In this model, electrical current of 150mA was introduced by placing ear electrodes for 0.2 seconds. MES - Convulsion was manifested by tonic flexion, tonic extension, clonic convulsion, stupor and recovery or death of mices (extensor seizure latency). The onset and duration of hind limb extension was recorded and compared with different groups of drugs. The substance having anticonvulsant property reduces or abolishes the extensor phase of MES convulsions.

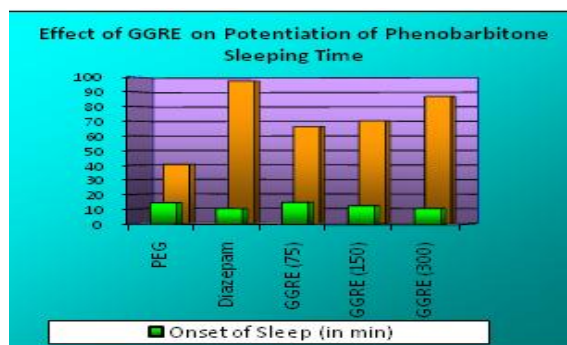
**Statistical analysis**

Effect of GGRE at different doses was compared both with PEG and standard drug by using unpaired and paired t-test. The p value of <0.05 was considered to indicate statistical significance.

**RESULTS AND DISCUSSION**

GGRE at the dose of 75 mg/kg did not alter the onset of Phenobarbitone sleeping time but there was increase in the duration of sleeping time. Significant decrease in the onset of sleeping time and increase in the duration of sleeping time was observed with GGRE at doses starting, 150 and 300 mg/kg compared to the pretreatment value.

At the dose of 75 mg/kg though GGRE could be able to prolong the duration of sleeping time, it did not alter sleep latency significantly. But at all the three doses of GGRE, the onset as well as duration of sleep was significantly less compared to the standard drug Diazepam.



**Graph 1:** Effect of GGRE on potentiation of phenobarbitone sleeping time



**Table 3:** Effect of GGRE on Potentiation of Phenobarbitone Sleeping Time In Mice

Group	Drug	Dose (mg/kg)	Latency/Onset of Sleep (min)		Duration of Sleep (min)	
			Pre Tt.	Post Tt.	Pre Tt.	Post Tt.
1	PEG	1ml/100gm	14.56 ± 0.33	14.42 ± 0.36	40.57 ± 0.59	40.43 ± 0.41
2	Diazepam	1	14 ± 0.24	10.44 ± 0.2 <sup>c</sup>	40.86 ± 0.46	97.58 ± 0.53 <sup>c</sup>
3	GGRE	75	15.53 ± 0.51	14.76 ± 0.71 <sup>a**</sup>	40.85 ± 0.46	66.42 ± 0.37 <sup>c**</sup> ♦
4	GGRE	150	14.33 ± 0.37	12.51 ± 0.2 <sup>b**</sup> ♦	40.53 ± 0.43	70.22 ± 0.41 <sup>c**</sup> ♦
5	GGRE	300	12.84 ± 0.31	10.81 ± 0.23 <sup>b**</sup> ♦	40.55 ± 0.31	86.91 ± 0.35 <sup>c**</sup> ♦

Values as Mean ± SEM

1. a = p < 0.05, b = p < 0.01, c = p < 0.001 as compared to pre treatment value
2. \*\* = p < 0.001 when compared to standard (Diazepam)
3. ♦ = p < 0.001 when compared to control

**Table 4:** Effect of GGRE on Maximal Electroshock Induced Seizure in Mice

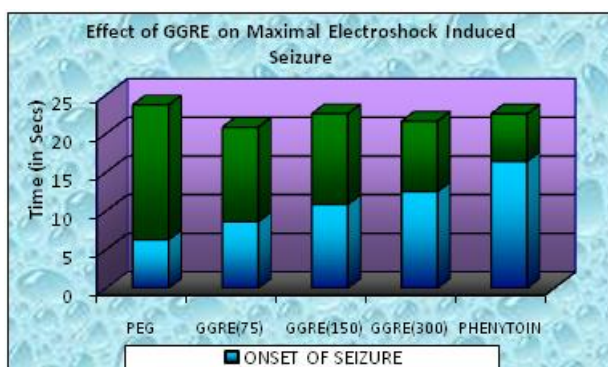
Group	Drug	Dose (mg/kg)	Pre treatment (secs)		Post treatment (secs)	
			Onset of Seizure	Duration of Tonic Seizure	Onset of Seizure	Duration of Tonic Seizure
1	PEG	100	5.99 ± 0.13	17.31 ± 0.33	6.29 ± 0.11	17.43 ± 0.27
2	GGRE	75	6.61 ± 0.10	16.63 ± 0.13	8.62 ± 0.18 <sup>***a</sup>	12.21 ± 0.24 <sup>***a</sup>
3	GGRE	150	6.23 ± 0.13	16.60 ± 0.10	10.86 ± 0.19 <sup>***a</sup>	11.72 ± 0.15 <sup>***a</sup>
4	GGRE	300	6.48 ± 0.04	16.91 ± 0.05	12.53 ± 0.42 <sup>***a</sup>	9.08 ± 0.05 <sup>***a</sup>
5	Phenytoin	60	6.63 ± 0.27	16.75 ± 0.06	16.41 ± 0.05	6.15 ± 0.05

Values as Mean ± SEM

1. \*\*\* = p < 0.001 when compared to Phenytoin (Onset and Duration of Tonic seizure)
2. a = p < 0.001 as compared to pre treatment value

GGRE at the dose of 75 mg/kg did not show any significant reduction in onset and duration of tonic seizure, but at doses 150 & 300 mg/kg, there was increase in onset of seizure (seizure latency) and decrease in duration of seizure in terms of hind limb extension.

This beneficial effect was comparable to that of standard drug Phenytoin at the dose of 60mg/kg. Water soluble portion of the aqueous extract of *Glycyrrhiza glabra* exhibited antidepressant activity on forced swim test and tail suspension test<sup>3</sup>. The ethanolic extract of root and rhizomes of *Glycyrrhiza glabra* inhibited Pentylene tetrazole and Lithium pilocarpine induced convulsions in mice.<sup>5</sup> The ethanol extract of roots of *Glycyrrhiza Glabra* (GGRE) was used to explore possible anticonvulsant properties using Maximal Electroshock Seizure Model<sup>6</sup>.

**Graph 2:** Effect of GGRE on maximal electroshock induced seizure

## CONCLUSION

At the doses 150 & 300 mg/kg, GGRE produced significant anticonvulsant activities which are comparable to Phenytoin. At the same doses, GGRE also produced significant sedative hypnotic activity which is comparable to standard drug Diazepam. Effect of GGRE in MES induced seizure suggests its possible role in reducing seizure by prolonging the inactivated state of voltage gated sodium channels or by potentiating the GABA mediated synaptic inhibition. The sedative hypnotic effect of GGRE on prolongation of Phenobarbitone sleeping time suggests that it probably acts by potentiating GABA mediated chloride channel opening. Further studies are required to reveal the exact mechanism of action responsible for the anticonvulsant and sedative hypnotic action of GGRE.

## REFERENCES

1. Dhingra D, Parle M, Kulkarni S K; Journal of Ethnopharmacology, Memory enhancing activity of *Glycyrrhiza glabra* in mice. Publisher: Elsevier, 91(2-3),2004, 361-365.
2. Kaura Prabhjit, Kaura Satwinderjeet, Kumarb Subodh & Singhb Palwinder *Rubia cordifolia* L. and *Glycyrrhiza glabra* L. Medicinal Plants as Potential Source of COX-2 Inhibitors, American Journal of Biomedical Sciences, 2(2), 2010, 108-120.
3. Dhingra D, Sharma A. Alt & Complementary therapies, Feb2005, 51-52.



4. Parle M, Dhingra D, Kulkarni SK. Memory strengthening activity of *glycyrrhiza glabra* in exteroceptive and interoceptive behavioral models (2004). *J Med Food*. 7(4): 2004 Winter;462-6,
5. Ambawade Shirish d, Kasture veena s., Kasture sanjay b. anticonvulsant activity of roots and rhizomes of *glycyrrhizaglabra*: *Indian Journal of Pharmacology*, 34: 2002; 251-255.
6. H. Hikino Recent Research on Oriental Medicinal Plants. In: Wagner H, Hikino H, and Farnsworth NR, editors. *Economic and Medicinal Plant Research*. London: Academic Press; 1985. 53.
7. Swinyard EA, Brown WC, Goodman LS. Comparative assay of antiepileptic drugs in mice and rats. *J Pharmacol Exp Ther*, 106: 1952;319-30.
8. Chopra's Indigenous Drugs of India, R.N. Chopra, C. Chopra, K.L. Handa, L. D. Kapur, 1958, 183-187.

\*\*\*\*\*

