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# Comparative Evaluation of Anti-Depressant Activity of Glycyrrhiza Glabra and Piper Nigrum Combination with Individual Drugs Effects and Standard Drug Imipramine in Mice

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Subject: Pharmacology

## Abstract

The main objective of this present study was to compare the anti-depressant property of Glycyrrhizin glabra and Piper nigrum in combination in swiss albino mice with Glycyrrhiza glabra and Piper nigrum individually and standard Imipramine. The doses of Glycyrrhizin glabra and Piper nigrum in suspension form and standard drug were administered into the animals for whole 4 weeks once daily.(Imipramine 20mg/kg, Glycyrrhizin glabra 150mg/kg and Piper nigrum 5mg/kg were individual drug doses and combination dose was Glycyrrhiza glabra 150mg/kg with Piper nigrum 5 mg/kg). The anti-depressant activity was performed using Force swim test and Tail suspension test as laboratory models. The anti-depressant activity was compared to standard drug imipramine. Combination drug therapy has better results than the individual dosage but standard showed optimum results. The results indicate that Glycyrrhiza glabra and Piper nigrum if given in combination is safe.

**Key Words**: *Depression,Glycyrrhizin glabra, Piper nigrum,Force swim test, Tail suspension test.*

## Introduction

Depression is referred to as mood disorder. It can be defined as a pathological complex of psychological, neuro-endocrine and somatic symptoms.[1] It is one of the most common disorders. At any given moment, about 5-6% of the population is depressed[2] and it is estimated about that 10% of the people may become depressed during their lives.[2,3] Depression represents a major public health problem worldwide. The high prevalence of suicide in depressed patients (upto 15%) [4] coupled with complications generally arise from stress and its effects. Over the next 20 years, unipolar depression is projected to be second leading cause of disability worldwide.[5] According to world Health organisation, depression is medical and social problem affecting 340 million people worldwide. The prevalence rate is about 5% annually along with recurrence rates of upto 85% have been reported.[6] Patients with major depression have symptoms that reflect changes in brain moniamine transmitters specifically noradrenaline, serotonin and dopamine.[1]

Loss of neurons in the hippocampus is found in depression and correlates with impaired memory and dysrthymic mood. This is explained on the basis that the drugs increase serotonin level in brain which stimulate neurogenesis and then increase total mass hippocampus and restore mood and memory. Therefore, assisting in fight against mood disorder.[7].Recent evidences showed that individuals with clinical depression showed higher levels of monoamine oxidase A enzyme (MAO-A) in the brain as compared to people without depression. It is an enzyme which decreases the concentrations of monoamines such as serotonin, norepinephrine and dopamine. Lower concentrations of monoamines are well known cause of depression.[7] Licorice belongs to fabaceae family, generally include three species: Glycyrrhizin glabra, uralensis, and inflate.[8] It

consists of several triterpenoid saponins, the most studied of which is glycyrrhizin. Other important are flavonoids, sterols, polysaccharides, coumarins, glabrol, glucose, sucrose, resin and volatile oil.[8]Glycyrrhiza glabra has been experimentally proved as an anti-viral effect [9], anti-hepatoxic effect[10], anti-cancer effect[11], relieve spasmodic effect[12,13], treatment for rheumatoid arthritis[14], anti-ulcer activity[15,16] cognitive function[17], anti-platelet effect[18], anti-microbial effect[19], anti-inflammatory effect[20].

Piper nigrum belongs to family piperaceae and generally its fruit part is used. It consists of a nitrogeneous pungent substance piperine, its alkaloid present in the fruits. [21] Piper nigrum has been experimentally proved as to cure constipation, diahorrea and also for spasmodic effects[22], anti-oxidant effect[23], anti-carcinogenic activity[24], seizure disorders[25,26], anti-inflammatory and analgesic activity[27], cognitive disorders[28]. So, the present study was undertaken to investigate and compare the anti-depressant effects of combination of Glycyrrhiza glabra and Piper nigrum in swiss albino mice using Force swim test and Tail suspension test as animal models. Imipramine, a tricyclic compound is used to standardise the animal models of depression.

## Material and Method

### Study Design

Experimental study was conducted on adult albino mice. The mice were divided into 5 groups with 8 mice in each group. 40 mice were used in each model. The drug doses were administered orally to different group of animals. The dose selection was done on the basis of taking references from previous carried out studies.

### Animals

Study was conducted on adult male albino mice of weight 20-30 grams which were free from any diseases. They were kept in polypropylene cages, six mice in each cage, in a controlled environment of 25° C with a 12 hour light and dark cycle. Animals got free access to food and water. Their diet was in the form of pellets which contain protein (20.12%), oil (4.38%), fibre (3.655%) and moisture (8%). Animals were acclimitised to these conditions atleast a week before to the start of the experiment. The Institutional Animal Ethics Committe(IAEC) approved the experimental protocol and care of laboratory animal were taken as per the guidelines of CPCSEA.

### Drugs and Chemicals

Powder of the roots of Glycyrrhizin glabra and fruits of Piper nigrum were obtained from Bharat Ayurvedic Pharmacy.Gum acacia and dist illed water were obtained from the institution. Imipramine was obtained from a registered medical store.

### Study procedure

Mice were assigned to 5 groups of 8 mice each.

**Group 1**- Served as control vehicle only (1 ml p.o) given 60 minutes prior to testing by FST and TST.

**Group 2**- Animals received Glycyrrhizin glabra in suspension form in distilled water and Gum acacia in the dose of 150 mg/kg p.o given 1 hr prior to the induction of depression by Despair Swimming Test and Tail suspension test.[17]

**Group 3**- Received Piper nigrum in suspension form in Distilled water and Gum Acacia (dose 5 mg/kg p.o) 1 hour prior to testing in each animal.[28]

**Group 4**- Animal received combination of Glycyrrhizin glabra and Piper nigrum in suspension form in distilled water and Gum acacia. (dose 150mg/kg and 5mg/kg p.o respectively ) given 1 hour prior to testing.

**Group 5**- Received Imipramine as standard (20mg/kg p.o) 1 hour prior testing.[31]

### Laboratory animals for testing Anti-depressant activity

### Force swimming test

FST is the best viewed as simple tests for anti-depressant activity[16]. In this method, mice were individually forced to swim the cyclinder inside a vertical plexi glass cyclinder (height 50 cm, diameter 20 cm) containing water column of 15 cm of height. Water was changed before the next animal was placed into the tank. Clean water is used each time because the used water has been shown to alter behaviour alarm signals [32]

Rats were gently kept into the water cyclinder for a total six minute session. After an initial 2 minute period of high activity, vigorously swimming in circles, tried to climb the wall or diving to the

bottom, usually each animal assumes a typical immobile posture. A mouse is considered immobile when it remains floating in water without struggling, making only minimum movements of its limbs necessary to keep its head above water. the total duration of immobility will be recorded.[1,33]

**Tail suspension test**

Male swiss albino mice of 20-30 grams were used. This animal model for testing is based on the principle that mice were suspended upside down on a metal rod stand 50-75 cm above the table top by an adhesive tape placed approximately 1 cm from the tip of the tail. This will lead to characteristic behaviour of immobility after initial momentry struggle. Immobility time was recorded during 8 minute period. The immobility during the first two minute due to vigorous activity is not taken into account . Animal is considered to be immobile when it didnot show any movement of body and hanged passively.[1,34]

**Statistical Analysis**

The results were expressed in mean+_ S.E.M. Statistical analysis of the values observed in the experimental methods that are Despair swim test and tail suspension test was done by one way-ANOVA followed by tanhame's test. P<0.01 was considered as statistically significant.

## Results

**Effect on immobility duration in Force swim test**

Effects of oral administration of the test drug on the duration of immobility of mice in force swim test method is shown in table form below. Test drug which is combination of two herbal drugs (Glycyrrhizin glabra-150mg/kg and Piper nigrum-5mg/kg) is found to be safer and there was no mortality rate. The combination showed significant results in chronic studies. There is significant decrease in immobility time of animals treated with combination than the individual drug therapy. The standard Imipramine (20mg/kg) which was administered 1 hour prior to test procedure, reduced the immobility time to the maximum as compared to test drug, individual drugs and placebo. Standard drug showed better results (P>0.05) than combination drug therapy. But combination of Glycyrrhizin glabra and Piper nigrum showed reduction in immobility time and which is significant as compared to individual drug effects and control (P>0.01) in chronic exposure.

**Table 1: Effect of different treatment groups on immobility time of mice in FST on chronic exposure**

| Treatment Groups | N | Mean ± SEM |
|---|---|---|
| Control | 8 | 16.60± 0.56 |
| Glycyrrhizin glabra | 8 | 10.02 ± 0.45 |
| Piper nigrum | 8 | 9.45 ±0.38 |
| Combination of Glycyrrhizin glabra and Piper nigrum. (Test Drug) | 8 | 7.28 ± 0.28 |
| Standard | 8 | 4.97 ± 0.16 |

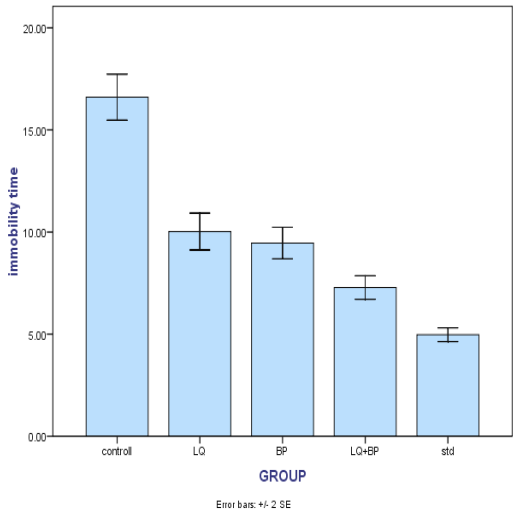


**Fig. 1: Effect of drugs in mice, Results are expressed as mean ±SEM, number of animals in each group (n=8)**

Values are Mean SEM, number of animals(n=8) in each group, Standard denotes p<0.001 as compare to control group of adult mice, Test denotes p<0.01 as compared to control group of adult mice. Test denotes p>0.05 as compares to standard group of adult mice, Data analysed using one-way ANOVA, followed by Tanhame's T2.

**Effect on immobility duration in Tail suspension test**

Animals in control group were immobile for maximum period of time in 8 minutes. There was significant decrease in immobility time of animals after the treatment with test drug. The decrease in immobility time on treatment with test drug was more than in individual drug therapy. Standard

drug still showed more better better results than the test drug on the duration of immobility of mice in tail suspension test are shown in below tables.

**Table 1: Effect of different treatment groups on immobility time of mice in TST on chronic exposure**

| Treatment Groups | n | Mean ± SEM |
|---|---|---|
| Control | 8 | 32.75 ± 0.65 |
| Glycyrrhizin glabra | 8 | 17.12 ± 0.35 |
| Piper nigrum | 8 | 17.6 ± 0.47 |
| Combination of Glycyrrhizin glabra and Piper nigrum. (Test Drug) | 8 | 14.6 ± 0.29 |
| Standard | 8 | 11.72± 0.24 |

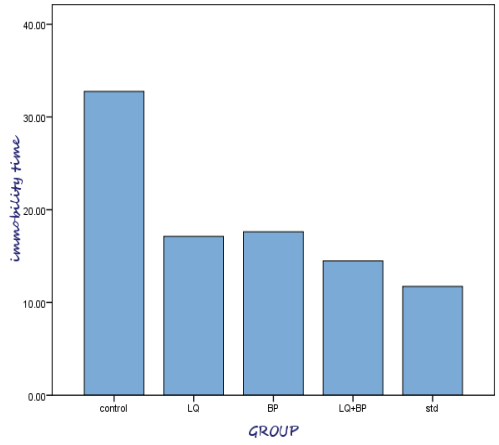


**Fig.2: Effect of drugs in mice, Results are expressed as mean SEM, number of animals in each group (n=8)**

Values are Mean SEM, number of animals (n=8) in each group, Standard denotes $p<0.001$ as compare to control group of adult mice, Test denotes $p<0.01$ as compared to control group of adult mice. Test denotes $p>0.05$ as compares to standard group of adult mice, Data analysed using one-way ANOVA, followed by Tanhame's T2

## Discussion

The present study of the comparision of the Anti-depressant effects of test drug and individual drugs on mice revealed that there was significant reduction in the immobility time when Glycyrrhizin glabra and Piper nigrum were given in combination. The anti-depressant effect of combination of Glycyrrhizin glabra and Piper nigrum was more as compared to individual drug effects. Force swim test and tail suspension test are the most common and the accepted paradigms for the depression study. In FST, when the mice are forced to swim in a restricted space from from which they can not escape are escape are induced to characteristic behaviour of immobility. This behaviour of immobility shows a state of despair which can be reduced by several anti-depressants which are also therapeutically effective in humans. In TST, the immobility behaviour shown by rodents, when they are put into an unavoidable and inescapable stress reflect behavioural despair which is reduced by some clinically effective anti-depressants. These anti-depressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by tail.

## Conclusion

The results conclude that the preclinical study on mice of test drug after oral administration using two paradigms possess significant effects of anti-depressant activity. Combination of Glycyrrhizin glabra and Piper nigrum showed more anti-depressant effects than individual drug therapy. Thus, the combination of Glycyrrhizin glabra and Piper nigrum can be explored for the management of depressive disorders.

## "Cite this article"

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