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# SYNERGISTIC POTENTIATING ANTI-ANXIETY ACTIVITIES OF WITHANIA AND ALPRAZOLAM BY LICORICE

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**ABSTRACT:**

Alprazolam is the most commonly used allopathic remedy for the management of anxiety. Several drugs like Withania, Valerian & Centella are the leading herbs for the management of anxiety. Magnesium hydroxide when given alone shows laxative property at all does but if it is given along with Licorice it enhances CNS activity of magnesium salt and produces hypnosis instead of laxation. Hence in the present study attempts have been made to screen possible synergistic potentiating anti-anxiety activity of *Withania* and Alprazolam with Licorice in elevated plus maze and dark light exploration model. In elevated plus maze model time spent in open arm and number of cross entries were increased significantly when Withania extract or Alprazolam were given along with Licorice. To conclude, if Withania or Alprazolam is given in combination with Licorice, there is enhancement of their anti-anxiety activities in mice. The synergism in the anti-anxiety activity may be due to differences in their mechanism of action or there may be increased BBB permeability of Withania and Alprazolam by Licorice because of its triterpenoid nature or may be due to inhibition of certain enzymes responsible for metabolism of Withania and Alprazolam.

**KEYWORDS:** Anxiety, Glycyrrhiza, Alprazolam, Withania.

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## INTRODUCTION

Anxiety is a psychological and physiological state characterized by cognitive, somatic, emotional, and behavioral components[1]. Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions[2]. Anxiety is most the common disorder affecting 15.7 million people in US each year[3]. Number of medicinal plants have been claimed to possess anti-anxiety activity. Withania (*Withania somnifera* L.: Family: Solanaceae) known as Ashwagandha in Sanskrit, has been evaluated by many scientist for its effect on various CNS disorders like anxiety, stress, convulsion, adaptogenic etc.[4] Licorice (*Glycyrrhiza glabra:* Family; Papilionaceae/Fabaceae) contains various triterpenoid saponins like glycyrrhizin and glycyrrhetic acid as well as various flavonoids like liquiritin and isoliquiritin. It has been demonstrated that if magnesium salts are administered along with saponin of Licorice, instead of laxation hypnosis occur[5]. Hence in the present study attempts have been made to study the probable role of Licorice extract in potentiating anti-anxiety activity of Withania and Alprazolam using elevated plus maze test and Dark light Exploration model in mice.

## MATERIALS AND METHODS

**Collection and processing of plant material**: The dried samples of Withania and Licorice were procured from

established local supplier, *Lalubhai Vrajlal Gandhi & Sons*, K-2/51, Gandhi Bazaar, Under Gandhi Road Bridge, Ahmedebad-380001 (India). The materials were authenticated using morphological, microscopical and physicochemical parameters. The voucher specimens were deposited in the Department of Pharmacognosy, K. B. Institute of Pharmaceutical Education and Research. Alprazolam tablet (B. No: 1390, Mfg. Date: Aug.2006, Exp. Date: July 2009), Unison Pharmaceuticals Ltd., (Vatva, Ahmedabad) was purchased from the market. All the chemicals and reagents used for the studies were of analytical grade.

**Preparation of extracts**: The crude materials after authentication were grinded and passed through 40 meshes sieve. The powdered material of Licorice and Withania were macerated with five times quantity of ethanol: water (7:3) for 24 h. The extracts were pooled, dried under vacuum and stored in air tight glass bottles.

Animals: Adult male Swiss albino mice (35–45 g) from our institution's own breeding stock were used. They were housed in groups in polypropylene cages (11 cm×17 cm×28 cm) with wood shavings as bedding, under controlled conditions of light (12 h light–dark cycle, light on at 7 a.m.) and temperature (25±2ºC). The animals had free access to water and food except 1 h before and during the experiments. The animals were allowed to acclimatize to laboratory conditions for 10 days before conducting the study. The experimental protocol was approved by the Institute Animal Ethics Committee (Approval no. KBIPER/08/108) and the experiments were carried out in accordance with the guidelines of CPCSEA on animal experimentation.

**Drugs and treatments**: Dose of individual drug was calculated from the literature (Ayurvedic Pharmacopoeia of India) dose for human. That dose was converted to mice dose using standard method using body surface area for conversion of dose. Drugs were administered orally and effects were recorded one hour after drug administration. Licorice and Withania extracts (7.5mg/45gm mice and 5.5 mg/45gm mice) were suspended in distilled water with 1% sodium CMC, or alprazolam (0.7 and 1.4 mg/kg; suspended in distilled water with 1% sodium CMC) were administered orally to mice by gavage. All the doses were prepared immediately before use. Control group was administered with the corresponding vehicle (1% sodium CMC in distilled water).

**Elevated plus-maze test**: The plus-maze for mice consisted of two perpendicular open arms (30cm×5 cm) and two perpendicular closed arms (30cm×5cm×25 cm). The whole apparatus was made of wood, and the maze was 45 cm above the floor[6-8]. The open arms were surrounded by a wooden rim (0.5cm high) to prevent the mice from falling down. One hour after oral treatment, the mouse was placed at the center of the plus-maze facing one closed arm and was observed for 20 min. The parameters observed were number of entries in the open and closed arms and time spent in the open arms. A mouse was considered to have entered an arm when all four legs were on the arm. The percentage of time spent and percentage of entries on the open arms was considered as the anxiety index. All the experimental sessions were video recorded for subsequent analysis by an experimenter blind to drug treatment.

**Light Dark Exploration test**: The testing apparatus was consisting of a light and a dark chambers zone. A polypropylene animal cage, 44 × 21 × 21 cm, was darkened with black paper sticked one-third of its surface[6]. A partition containing a 13 cm long × 5 cm high opening separates the dark one third from the bright two thirds of the cage. Naive male mice were placed into the cage. The animals were treated 60 min before the experiment with the test drugs or the vehicle orally and were observed for 10 min. Groups of 6 animals were used for each dose. The time passed in individual chambers and number of cross entries was recorded.

**Statistics**: Data obtained from elevated plus maze test was statistically analyzed using one way ANOVA, followed by Dunnett test for comparison between various treatment groups. $P < 0.05$ was considered as a significant level. All data are expressed as mean ± S.D. (n=6).

## RESULTS AND DISCUSSION

**Elevated plus maze test**: After oral administration of *Withania somnifera* extract and alprazolam, average number of entries in close arm, open arm, time spent in closed arm and open arm as well as number of cross entries were recorded and the results of the elevated plus maze test are shown in Figure 1 A], B], C], D]. There were increases in average number of entries in open and close arm as well as time spent in open and closed arm after oral administration of *Withania somnifera* extract (5.5mg/45g mice) and alprazolam (0.1mg/45g mice) compared to vehicle. However after oral administration of *Glycyrrhiza glabra* extract (7.5mg/45g mice) there was no significant increase in these values compared to vehicle. After oral administration of *Withania somnifera* extract at 2.75mg/45g mice along with *Glycyrrhiza glabra* extract 3.75mg/45g mice and alprazolam 0.05mg/45g mice along with *Glycyrrhiza glabra* extract 3.75mg/45g mice, there was significant increase in average number of

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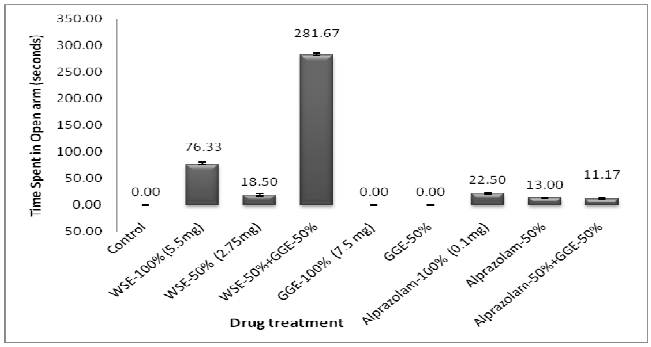


**Fig. 1 A]** Time spent in open arm of Eleveted Plus Maze by mice and drug treatment

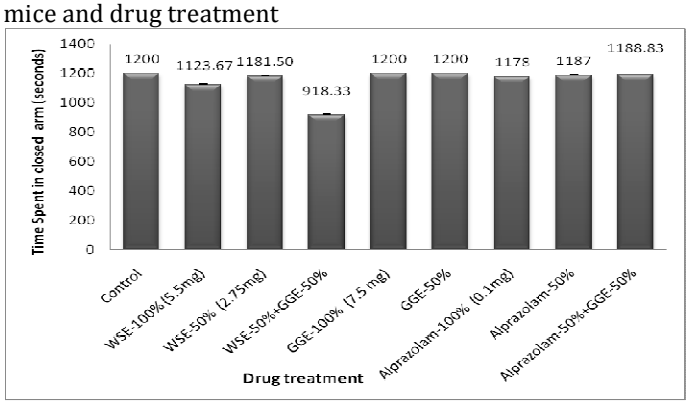


**Fig. 1 B]** Time spent in Closed arm of Eleveted Plus Maze by mice and drug treatment

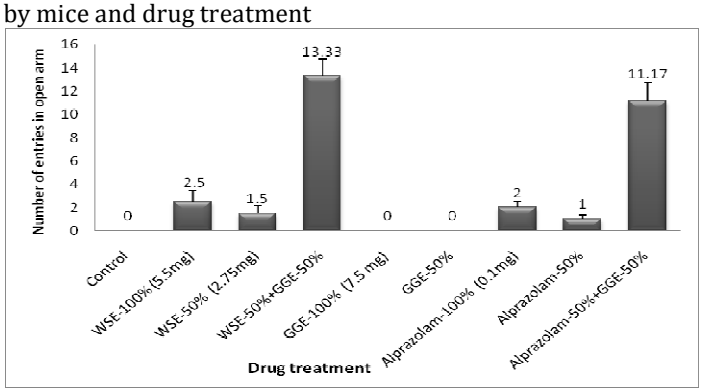


**Fig. 1 C]** Number of entries in open arm of Eleveted Plus Maze by mice and drug treatment

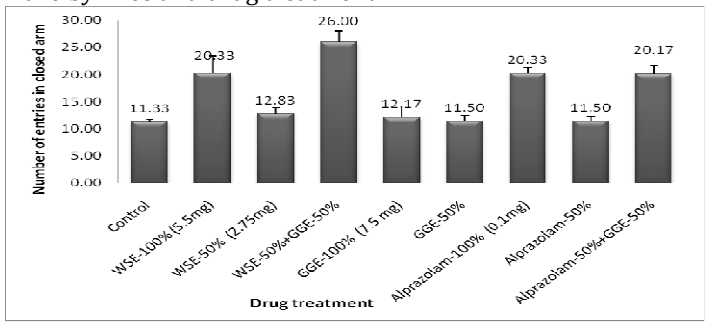


**Fig.1 D]** Number of entries in closed arm of Eleveted Plus Maze by mice and drug treatment

**WSE=*Withania somnifera* ext, GGE=*Glycyrrhiza glabra* ext**

entries in open and close arm as well as time spent in open and closed arm compared to vehicle and full dose of individual extract and alprazolam. The results of the same are presented in the individual graph. The administration of Withania extract and alprazolam individually to mice resulted in a significant reduction in the anxiety and fear as evidenced by increase in the percentage number of entries in the open arms and time spent in the open arms. The results indicate synergistic potentiating activity of *Withania somnifera* and alprazolam with *Glycyrrhiza glabra*.

**Light Dark Exploration test**: In light dark exploration model, if the drug is having anti-anxiety activity, it should increase the average number of cross entries between light and dark chamber[7]. After oral administration of *Withania somnifera* extract at 5.5mg/45g mice and alprazolam at 0.1mg/45g mice, the average number of cross entries between light and dark chamber and time spent in light chamber were increased compared to vehicle while there was significant decrease in time spent in dark chamber compared to vehicle (Fig. 2). However after oral administration of *Glycyrrhiza glabra* extract there was no significant change in any activity in light and dark chamber. After giving oral dose of only *Withania somnifera* extract at 5.5mg/45g mice, *Glycyrrhiza glabra* at 7.5mg/45g mice and alprazolam at 0.1mg/45g mice individually, the average number of cross entries between light and dark chamber were increased significantly to 30.5, 27.5 and 32.17 respectively from 24.33 of vehicle. After oral administration of *Withania somnifera* extract at 2.75mg/45g mice along with *Glycyrrhiza glabra* extract 3.75mg/45g mice and alprazolam 0.05mg/45g mice along with *Glycyrrhiza glabra* extract 3.75mg/45g mice, average number of cross entries between light and dark chamber were increased to 36.67 and 42.83 from 30.5 and 32.17 of *Glycyrrhiza glabra* and alprazolam at their full dose i.e. 5.5mg/45g mice and 0.1mg/45g mice respectively. If the drug is having anti-anxiety activity it should increase time spent in light chamber and decrease in time spent in dark chamber. The results of the same presented in graph indicates that if *Withania somnifera* or alprazolam is given individually, they shows anti-anxirty activity but when they are given along with *Glycyrrhiza glabra* there is significant increase in anti-anxiety activity and even 50% of the recommended dose gives activity more than 100% of the recommended dose.

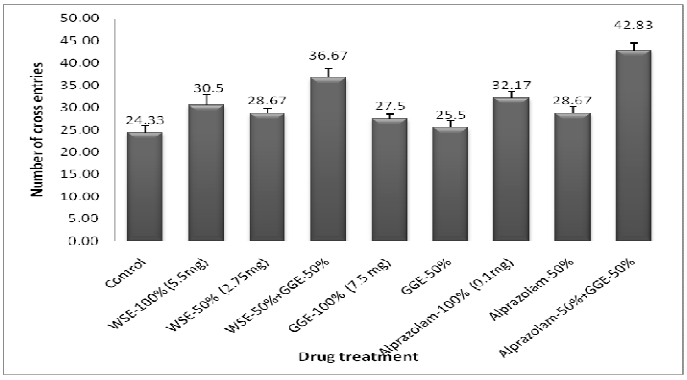


**Fig. 2 A]** Number of cross entries after drug treatment in Light Dark Exploration test

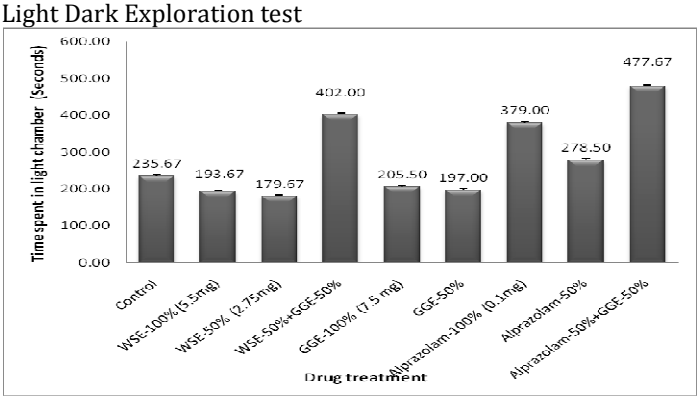


**Fig. 2 B]** Time spent in light chamber after drug treatment in Light Dark Exploration test

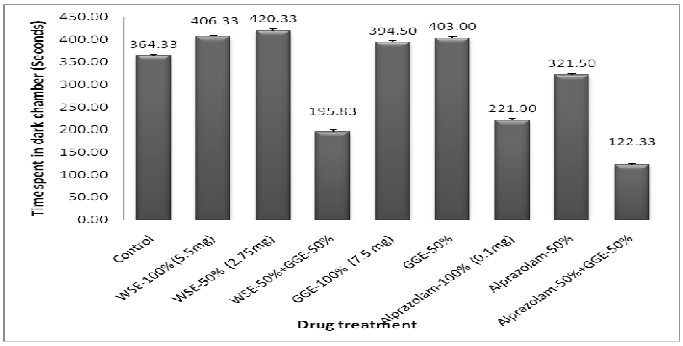


**Fig.2 C]** Time spent in dark chamber after drug treatment in Light Dark Exploration test

## CONCLUSION

The results obtained in the present studies support the hypothesis that Licorice (*Glycyrrhiza glabra*) potentiates the anti-anxiety activity of selected drugs. The mechanism of potentiation of anti-anxiety activity by Licorice needs to be elucidated. The possible mechanism of potentiation of activity could be an increase in blood-brain barrier permeability of the drug by the constituents of Licorice extract, or an increase in the bioavailability of the drug due to increased absorption through gastro-intestinal tract or competitive inhibition of the metabolizing enzymes by the constituents of Licorice.

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