

# Anxiolytic Effect of Herbal medicine, *Khamira Gaozaban Ambri Jadwar Ood Salib Wala* (KGJ) in experimental rat models

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**Abstract:** Anxiety and depression leads to a number of morbid states. Search of new agents which are low-priced and safe alternative is necessary. *Khamira Gaozaban Ambri Jadwar Ood Salib wala* (KGJ), is a product of Hamdard Laboratories (Waqf) Pakistan. They claim that it is anxiolytic, anti-convulsant and nervine tonic. However this claim is not scientifically proven. Some components are proved to be anxiolytic but combination may alter the individual properties of drugs. Therefore we designed present study to prove these properties of KGJ scientifically. Thirty male *Sprague Dawley* rats were divided into five groups of six animals in each group. The groups were saline group, control group (receiving Diazepam 1 mg/kg) and three test groups receiving 86, 170 and 360 mg/kg doses of Khamira orally. Assessments of different doses of KGJ in comparison to diazepam were carried out on anxiety paradigms namely "Elevated Plus Maze", "Light And Dark Activity Box" and "Open Field" paradigms. KGJ produced anxiolytic effects in all the behavioral methods, which were not significantly different from the effects produced by diazepam. Basal levels of corticosterone not altered by diazepam were decreased by 86 mg/kg dose of KGJ. Same dose of KGJ also decreased blood glucose levels. In conclusion, these results suggest anxiolytic potential of KGJ with non-sedative property.

**Keywords:** *Khamira gaozaban ambri jadwar ood salib wala*, anxiolytic activity, anxiety.

## INTRODUCTION

In spite of advancements and comforts in life, stress is increasing day by day. Stress is an essential feature of life and is becoming the major cause of a number of diseases like Diabetes Mellitus (Sledge, 2002), Hypertension (Kulkarni et al., 1998), Cancers (Rosch, 1991), Conversion disorders (Sayeed et al., 2005), Anxiety and Depression (Alfonso et al., 2004).

Physical or Psychological stress activates Hypothalamo-hypophyseal system, whose goal is to release cortisol from the adrenal cortex, to cope up with stressful situations (Ziegler & Herman, 2002).

Stress disorders are treated by the administration of anxiolytic and antidepressant agents. Use of these agents is limited largely because of high cost and a number of side effects. Herbal medicines are therefore, given attention because of their low price and fewer side effects.

*Khamira Gaozaban Ambri Jadwar Ood Salib Wala* (KGJ) is an herbal preparation. As described by Said (1970), it has sixteen constituents from animal, plant and mineral sources namely *Bombyx mori* (1%), *Lavendula stocchas* (0.5%), *Nepeta hindostana* (1%), *Santalum album* (1%), *Onosma bracteatum* leaves (2.25%), *Salvia haematodes* (1%), *Centaurea behen* (1%), *Lallementia royleana* (1.5%), *Cheiranthus cheiri* (2%), *Coriandrum sativum* (1%), *Onosma bracteatum* flowers (1%), *Ambra graesa* (0.06%), Silver foil (0.2%), Gold foil (0.025%), *Delphinium denudatum* (0.75%) and *Orchis mascula*

(1.1%). Individual drugs play a major role in treatment but in combination it may lead to alteration of the individual effects or the effects may be synergistic. Therefore poly-herbal preparations are taken into consideration.

One of the ingredients, *Coriandrum sativum* is reported to be anxiolytic (Imamghorashi et al., 2005). *Lavendula stocchas* is a fragrant plant. Its essential oil is used as perfumery. One type of lavender, the *Lavendula angustifolia* is also reported anxiolytic in open field (Shaw et al., 2007). Active ingredients in the oil of *Lavendula stocchas* are reported anxiolytic in Geller's and Vogel's conflict test by Toyoshi and coworkers in 2004 and 2006.

The present study investigates the effectiveness of KGJ in various animal models of anxiety and compares it with the anxiolytic profile of diazepam.

## MATERIALS AND METHODS

### *Experimental Animals*

Experiments were performed on Male Sprague-Dawley rats weighing between 200-270 grams. They were bought from HEJ Research Institute of Chemistry, International Centre of Chemical and Biological Sciences (ICCBS). Animals were housed randomly in appropriate cages of plexiglass material with 5-6 animals/ cage, maintained at 22±2°C with 12 hrs light and dark cycle at Dr. HMI Institute of Pharmacology and Herbal Sciences, Hamdard University, Karachi, according to standard procedures of animal husbandry. All the experiments were done between 9.00-14.00 hrs.

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### **Drugs and chemicals**

Khamira Gaozaban ambri Jadwar Ood Salib wala, product of Hamdard Laboratories (Waqf) Pakistan, was purchased from the local Hamdard outlet, Matab Hamdard, at Aram Bagh Road, Karachi. Diazepam (10 mg Tablet, Rosch Pakistan) was bought from pharmacy. Normal saline made by NaCl (Extra pure USP-BP from Scharlar S.A. LaJota 86-08016- Barcelona, Spain).

### **Experimental protocol**

Thirty animals were divided into five groups randomly that is saline group, 86 mg/kg, 170 mg/kg, 350 mg/kg doses of KGJ and 1 mg/kg group of diazepam. 86 mg/kg dose is the normally prescribed dose in unani clinical settings whereas 170 mg/kg was the double dose and 360 mg/kg was four times dose.

All the drugs were freshly prepared in the laboratory before experiment, and were administered by oral route with the help of orogastric tube one hour before the behavior testing.

After one hour animals were placed initially in the open field for five minutes and after that in the elevated plus maze for another five minutes as was done by Wijeweera and coworkers (2006). Soon after the test session animals were anesthetized by Sodium Pentothal intraperitoneally and blood was collected by direct cardiac puncture for the analysis of blood sugar which was done at the same time by the glucometer (One touch basic, Roche) and from the remaining blood serum was collected for the purpose of serum corticosterone level detection. The flourimetric method of Mattingley (1962) was used for the determination of corticosterone in serum.

Light and dark box was done on separate group of animals divided similarly as above. The test was done 1 hour after the oral administration of the drug.

### **Behavioral Paradigms**

#### **Open field**

The method was same as described by Khan & Haleem (2007). Open field consist of square arena 80x80cm of white Plexiglas material with transparent floor with opaque 37.5 cm high walls. The arena was divided into 25 equal squares each measuring 16x16cm. A number of behavioral parameters were checked like Grooming, Rearing, Central square movement and locomotor activity for the cut off time of five minutes.

#### **Elevated plus maze**

The apparatus consisted of four identical arms (40x10) radiating from the central platform to form plus sign. Two arms of the maze were open while the other two arms were closed with walls 17 cm in height. Whole of the apparatus was 50 cm elevated from the floor on a wooden stand. Rats were introduced in the center of the apparatus facing the open arm (Peng *et al*, 2000). Percent time spent

in the open arm (Time spent in the open arm/ Total time x 100) and the percent open arm entry (Open arm entry/ Total entries x 100) were calculated for each rat.

#### **Light dark activity box**

Apparatus was almost identical with that described by Khan & Haleem (2008). The apparatus used was a two compartment box, of equal size (26x27x28 cm) with a midway door 10x10 cm. One compartment was dark and the other transparent. Light box was brightly illuminated by a lamp placed 35 cm above the floor of the box as described by Wei *et al* (2007). Drug administered animals were introduced to the light compartment with their back towards the door as by Peng *et al* (2000) and the percent time spent in the light compartment (Time in light compartment/ Total time x 100) and number of transitions between the boxes were monitored for 5 minutes.

### **STATISTICAL ANALYSIS**

All the results analyzed by one way ANOVA followed by Unpaired Student's t-test using computer software "statistical product selective solution" (SPSS-19) version 19. All the results were considered significant when  $p < 0.05$ .

### **RESULTS**

#### **Anxiolytic activity**

Fig. 1 shows the effect of KGJ at doses 86, 170 and 350 mg/kg on different parameters of EPM in rats. Analysis of the data revealed that groups were statistically significant. Unpaired t- test showed a significant rise in Percent of open arm entry by 86 mg/kg, 170 mg/kg and 350 mg/kg ( $p < 0.05$ ) doses (fig. 1A). All the above mentioned doses also showed significant ( $p < 0.05$ ) rise in percent of time spent in open arm (fig. 1B).

Fig. 2 shows effects of KGJ at doses 86, 170 & 350 mg/kg on different parameters of "Light and Dark activity Box. One way ANOVA showed highly significant difference between groups. Un-paired Student's t-test showed marked rise in percent of time spent in the lighted compartment by 86 ( $p < 0.005$ ), 170 ( $p < 0.05$ ) and 360 mg/kg doses ( $p < 0.005$ ).

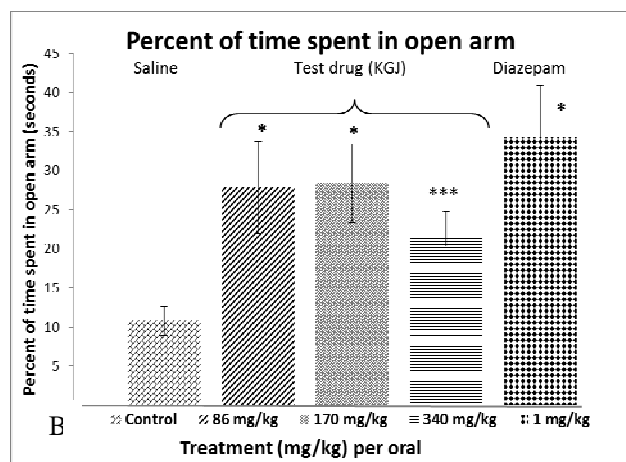
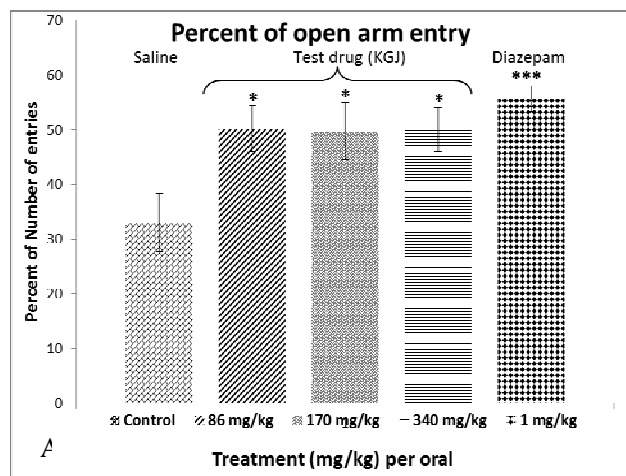
#### **Locomotor activity**

Fig. 3 shows effect of different doses of KGJ on different parameters of Open field. Central square exploration which is a measure of anxiolytic activity was seen by 360 mg/kg dose ( $p < 0.05$ ). However significant rise in total ambulation was observed by 86 and 170 mg/kg doses ( $p < 0.05$ ) and 360 ( $p < 0.005$ ) mg/kg doses.

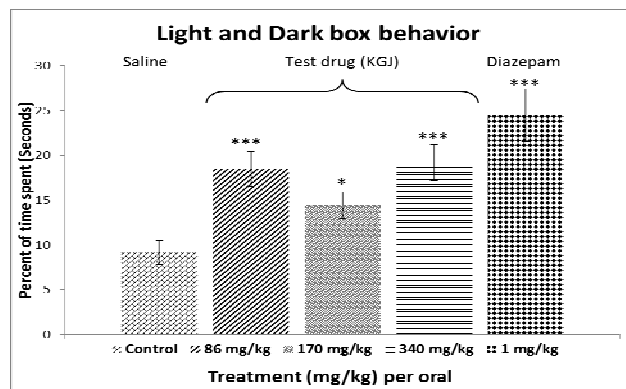
#### **Glucose lowering effect**

Fig. 4 shows the effect of KGJ on blood glucose level, which was decreased significantly by 86 mg/kg ( $p < 0.025$ )

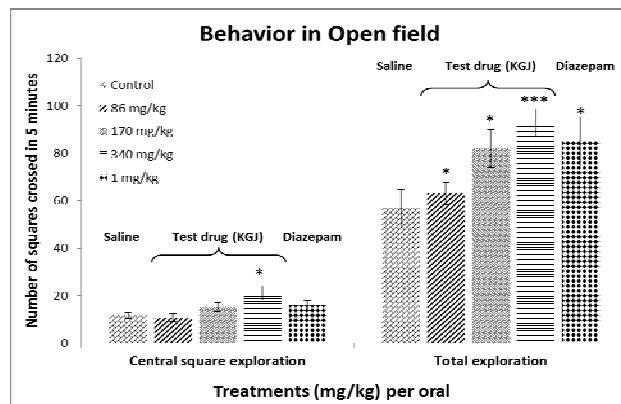
where as remaining doses increased glucose levels non-significantly. ANOVA showed highly significant difference between groups. Diazepam on the other hand non-significantly increased the blood glucose level.



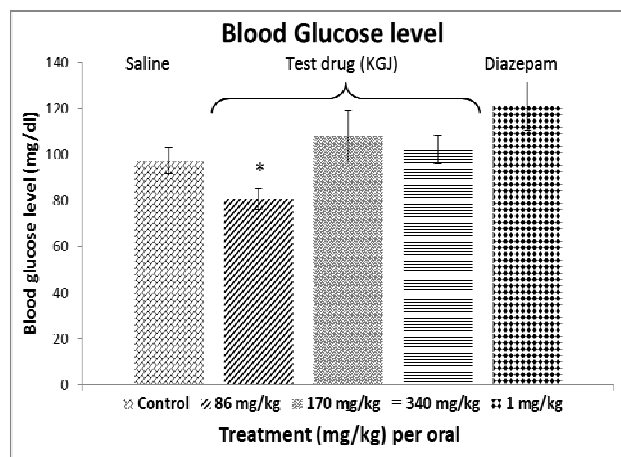
**Fig. 1:** Parameters of Elevated plus maze showing anxiolytic potential of KGJ. A: Percent of open arm entry; B: Percent of time spent in open arm. Value are Average  $\pm$  SEM (n=6). \* = P<0.05; \*\* = P<0.01; \*\*\* = P<0.005



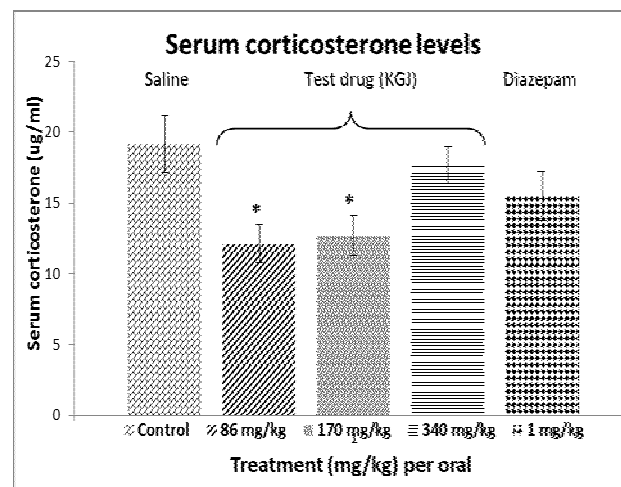
**Fig. 2:** Parameters of light and dark box showing anxiolytic potential. Value are Average  $\pm$  SEM. (n = 6). \* = P<0.05; \*\*\* = P<0.005.



**Fig. 3:** Parameters of open field showing locomotor activity. Value are Average  $\pm$  SEM. (n = 6). \* = P<0.05; \*\*\* = P<0.005.



**Fig. 4:** Blood glucose level after single dose of drug and single exposure to stress produced by novel environment of open field and elevated plus maze. Value are Average  $\pm$  SEM. (n = 6). \* = P<0.05



**Fig. 5:** Serum corticosterone level after single dose of drug and single exposure to stress produced by novel environment of open field and elevated plus maze. Value are Average  $\pm$  SEM. (n = 6). \* = P<0.05.

### Corticosterone lowering effect

Fig. 5 shows the effect of KGJ on serum corticosterone level. One way ANOVA showed significant difference between groups ( $p < 0.05$ ). Unpaired Student's t-test shows that corticosterone level was decreased significantly by 86 ( $p < 0.01$ ) and 170 ( $p < 0.025$ ). Diazepam did not show fall in the corticosterone levels

## DISCUSSION

There is very high incidence of anxiety and associated morbidity in the community. Incidence of mental health problems in the general population was found to be around one in six people, and about 40% of people with mental health problems will have symptoms of both anxiety and depression, as discussed by Rauniar and coworkers (2007). Therefore it is very important problem to address nowadays on priority basis. Several conventional therapies are available like benzodiazepines, etc but these are associated with side effects like sedation, loss of concentration and dependence as discussed by Ashton *et al* (1984). Newer antianxiety agents like buspirone are very effective and with fewer side effects but still cause tachycardia, palpitation and gastric discomfort (Rang & Dale, 2003).

Resistant cases of anxiety that needs long term treatment need newer and safer agents. Therefore attention is diverted towards natural products and herbal drugs.

*Khamira Gaozaban Ambri Jadwar Ood Salib Wala* is very useful drug for anxiety and mental illnesses. It is used from older times. It is said to be anti-anxiety and anticonvulsant.

“Elevated Plus Maze” is an etiologically proper animal model of anxiety, because according to Grundmann and coworkers (2007) it uses fear of novel open space and fear of balancing on a relatively raised narrow platform as anxiety producing stimulus. Indices of anxiety like open arm entry and time spent in the open arm are sensitive drugs which act through GABA receptors; therefore diazepam is taken as positive control (Emamghoreishi *et al*, 2005). It is used as standard anxiolytic and reference compound for inducing anxiolytic-like effects as discussed by Malaviya *et al* (2009).

As can be seen from the result that human dose caused significant rise in Open arm entry and time spent in the open arm. The results were comparable with diazepam whose effect was in accordance with previously published reports.

Light and dark box is a widely used behavioral paradigm for screening anxiolytic agents in rodents as discussed by Wei *et al* (2007) that was described by Crawley & Goodwin in 1980. According to Peng *et al* (2000) this test

is based on natural dislike of rodents to brightly lit, novel and aversive environment. Mild stressor usually decreases exploratory behavior in rodents as discussed by Wei and coworkers (2007). It has been postulated by Ruiz *et al* (2006) that the time spent by animal in the brightly lit compartment is the most consistent behavior.

In the present work anxiolytic activity of KGJ is demonstrated with increased time spent in the light compartment and increase in the number of crossings between two boxes. The effects were comparable with the standard drug diazepam whose effects are proved by a number of studies namely studies conducted by Peng *et al*, (2000) and Ruiz *et al*, (2006).

Most popular animal model of anxiety is elevated plus maze, as stated by Bradley *et al*, (2007) but open field is emerging recently as a very important behavioral model of anxiety and has been validated in the detection of a number of anti-anxiety agents by Shaw *et al* (2007), such as benzodiazepines. Previously it was used as behavioral model for sedative/ hypnotic activity and locomotor activity.

In the “Open Field Model”, the anxiety behavior is triggered by two factors, i.e., individual testing by separation from its social group and fear of large, novel and isolated arena. In such situations rodents show spontaneous preference to the periphery of the apparatus and reduced movement. Anxiolytic treatment increases exploratory behavior as observed by Wijeweera *et al*, (2006), Malaviya *et al* (2009) as well as by the author. Grooming usually increases with fear and anxiety in rodents. Placement in the novel environment increases grooming. According to Shaw *et al*, (2007) this parameter is reversed by anxiolytic agents. It is stated by Rauniar *et al*, (2007), that rearing is a parameter of exploration and when a rodent is placed in novel environment this behavior is decreased due to stress and the reversal of it takes place in case of administration of anxiolytic agents. Novel environment increases spontaneous freezing, corner sitting and decreases locomotor activity. Anxiolytic agents without sedative property, increases ambulation and conversely decreases freezing behavior.

In the light of above discussion, it can be clearly seen from the results, that all the parameters in the four times dose of KGJ show anxiolytic behavior. Here again anxiolytic potential of the drug is proved.

It can also be seen from the results that human dose significantly decreased the blood sugar level but double and four times dose did not have the same effect, which may be due to increased amount of sugar because of increased drug dose. May be increased amount of sugar has decreased the hypoglycemic effect of certain ingredient herbs of the product. Interestingly diazepam

showed significant rise in the blood sugar level. This may be due to the fact that diazepam, as concluded by Yamada and coworkers (2000) increases the adrenaline levels. Most important effect of adrenalin is that it induces hyperglycemia (Rang & Dale, 2003). This hyperglycemic potential of diazepam is also mentioned by Syvälahti and coworkers in 1975.

It is also shown in the results that serum corticosterone levels are significantly decreased by human and double dose of KGJ. Petreglia and coworkers (1986) reported a decrease in the level of corticosterone following the administration of 0.3 mg/kg dose of diazepam in the stressful situation whereas basal levels were unaffected, following the treatment of drug. Similarly we found no change in the basal corticosterone levels at the dose of 1 mg/ kg dose of diazepam.

Three doses that were studied in the experiment showed powerful anxiolytic potential of KGJ. Diazepam produces antianxiety effects by increasing GABAergic function as reported by Chen *et al* (2006) and Ruiz *et al* (2006). It may be interesting to investigate the mechanism by which KGJ produces antianxiety effects.

## CONCLUSION

In conclusion the present study shows that KGJ has strong anxiolytic profile at the doses that are commonly prescribed for human and the higher doses. In view of fewer side effects, this inexpensive medicine may have advantage over conventional anxiolytic compounds. However, it is important to explore the mechanism by which KGJ may elicit antianxiety effects. Further extensive study on different doses with acute and chronic administration of the agent is necessary.

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