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COMPARATIVE STUDY OF DIAZEPAM AND ASCORBIC ACID IN MICE BY OPEN FIELD AND BALANCE BEAM TEST

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ABSTRACT

Present study was conducted to observe the behavioral effects of cumulative administration of diazepam and ascorbic acid in mice. Ascorbic acid, a water soluble vitamin is a reducing agent and has a role in collagen formation .Whereas diazepam is a benzodiazepine and belongs to sedative hypnotics class. Twenty eight mice were divided into four groups: (1) Control -distilled water (2) Diazepam (3) Ascorbic acid (4) Ascorbic acid plus Diazepam. All drugs were administered orally. After thirty minutes of drug administration open field and balance beam activity was observed in mice. In open field all groups increased number of squares crossed, as compared to control. In balance beam test, balance was more affected by ascorbic acid plus diazepam group. It may be proposed that ascorbic acid co-administration with diazepam can increase the effects of diazepam.

KEYWORDS: Ascorbic acid, Balance beam, Diazepam, Open field.

INTRODUCTION

Anxiety is a common psychological disorder that can affect the daily activities of a person; sometimes it is also associated with depression and can lead to emotional and behavioral despair (Chandra *et al.,* 2013). Anxiety disorders are generally correlated with increased level of oxidative stress in the body (Deoleivera *et al*., 2015). The general symptoms of anxiety are fear, stress and worrying about future events. Anxiety can also affect the autonomic functions of a person that will result in symptoms like increased sweating, increased heart rate, GI discomfort and appetite problems (Nirwane *et al.*, 2015).

Anxiety can usually be treated by the modulation of gamma amino butyric acid i.e. GABA which is an inhibitory neurotransmitter, it works by increasing the influx of chloride ions thereby causing the hyper polarization of neuronal membrane (Gordon, 2002).There are two types of GABA receptors, A and B. The $GABA_A$ receptor is a pentameric receptor consisting of 5 sub units including two α, two β and one γ sub unit, benzodiazepines bind to this receptor and induce conformational change to produce action (Clayton *et al.,* 2015).Benzodiazepines are the major drugs that are used in many psychological disorders due to effect on brain functions like behavior, memory and organization. Benzodiazepines are used in different conditions such as stress, anxiety, convulsions, epilepsy, anesthesia, sleeping and alcohol withdrawal (Pebdani *et al*., 2015).

Diazepam is the most commonly used benzodiazepine, it has rapid onset, high efficacy and very rare cases of life threatening side effects have been reported with this drug (Calcaterra and Barrow, 2014). Along with its action on the chloride ion channel of GABA receptor, diazepam also produce mind relaxing effects due to its action on limbic system, it also has action on sympathetic system and can sometime alleviate systolic blood pressure when used at high doses (Farooq *et al.*, 2008). Despite of its many advantages long term use of Diazepam and other benzodiazepines is also associated with dependency, sedation and drug abuse that leads to the idea towards the development of other anxiolytic drugs with lesser side effects (Rupprecht *et al.,* 2009). To avoid the associated side effects diazepam must not be used for more than two weeks and should always be discontinued after dose tapering and in some cases the antagonist of benzodiazepine i.e. Flumazenil is used to withdraw the drug without any serious side effects (Brett and Murnlon, 2015). Diazepam can cause the generation of free radicals, which necessitates the use of antioxidants like ascorbic acid which can scavenge these free radicals (EL-Sokkary, 2008). Diazepam can increase the oxidative stress by decreasing the level of glutathione and superoxide dismutase that ultimately leads to cell death i.e. apoptosis and administration of Ascorbic acid can prevent this condition by decreasing the level of reactive oxygen species and increasing the level of glutathione (Pavlovic *et al.,* 2012).

Ascorbic acid is water soluble vitamin involved in collagen formation and production of adrenaline, it is also helpful in induction of anesthesia and when used with anesthetics, it decreases the dose of anesthetic agent (Yanmaz *et al*., 2015). The deficiency of ascorbic acid can lead to seizure so it is suggested that ascorbic acid can be used as a preventive and curative therapy for seizure (Warner *et al*., 2015). The deficiency of Ascorbic acid can also lead to scurvy, gum bleeding, immune suppression, and weakness. It can be used at higher doses in several critical conditions without any significant side effects (Berger and Straaten, 2015). Ascorbic acid can also produce memory enhancing effects on brain, delays the aging process and has preventive role in diabetes (Mhaidat *et al*., 2015). Ascorbic acid is also involved in the modulation of different neurotransmitters such as dopamine, acetylcholine, Glutamate and GABA (Esmaeilpour and Abbasnejad, 2013).Ascorbic acid can also be useful in Alzheimer disease and dementia because it can prevent the damage to neurons produced by free radicals. It can also increase the effect of acetylcholine (Raghu *et al.,* 2013). The

deficiency of Ascorbic acid is also associated with behavioral problems and loss of motor co-ordination that may be due to the anxiogenic effects produce by the deficiency of Ascorbic acid and altered metabolism of carbohydrate, protein and lipids that can cause decreased energy production (Pierce *et al.,* 2013).

MATERIAL AND METHODS

Male and female albino mice having weight of 20 to 25 gm were kept for a conditioning period of a week in the animal house of Pharmacology laboratory. Ethics of animal experimentation approved by institutional animal care & use committee of Pharmacy Faculty, University of Karachi was followed. The animals were maintained on standard feed and water adlibitum, at an ambient temperature between 22-25°C, with a 12 hr light and dark cycle. Animals were randomly divided into 4 groups with 7 mice in each group.

All group of mice received drugs orally. Group I served as control and was given distilled water. Other groups were administered drugs as described below:

Group	II	Diazepam	0.14 mg/kg
Group	III	Ascorbic acid	14.28 mg/kg
Group	IV	(Mixed group)	Diazepam &Ascorbic acid both were administered in the doses mentioned above.

Following tests were performed after 30 minutes of dosing.

Open field: Mice were placed in the centre of square arena for 5 minutes that consist of 25 small squares. The floor of arena was transparent and the side walls were made up of white Plexiglas. Number of boxes crossed (i.e. horizontal activity) was counted. The field was cleansed in between the readings with an ethanol swab (Walsh and Cummins, 1976; Brown *et al.*, 1999).

Balance beam: The balance beam test was performed using beams of diameter 8 and 15 mm. The mice were placed towards the end of beam and allowed to cover a distance of 60cm over it, the time of crossing the distance on beam was noted in seconds (Deacon, 2013).

Statistical analysis: Data was analyzed by one way Anova followed by post hoc test (tukey HSD). $p \leq 0.05$ was considered significant and $p \leq 0.01$ was considered highly significant.

RESULTS

Open field: Table 1 and figure 1 represent open field results, where the mean value of the number of boxes crossed was increased by Diazepam ($p<0.01$), ascorbic acid ($p<0.05$) and Mixed group ($p<0.01$) as compared to control.

Table 1. Number of boxes crossed in open field.

No. of boxes crossed			
Control	**Diazepam**	**Ascorbic**	**Mixed group**
136.5 ± 4.67	168^{**} ± 2.05	187.5^{*} ± 13.79	177^{**} ± 10.28

N = 7, values are expressed as mean ± SEM, *p≤0.05, **p≤0.01

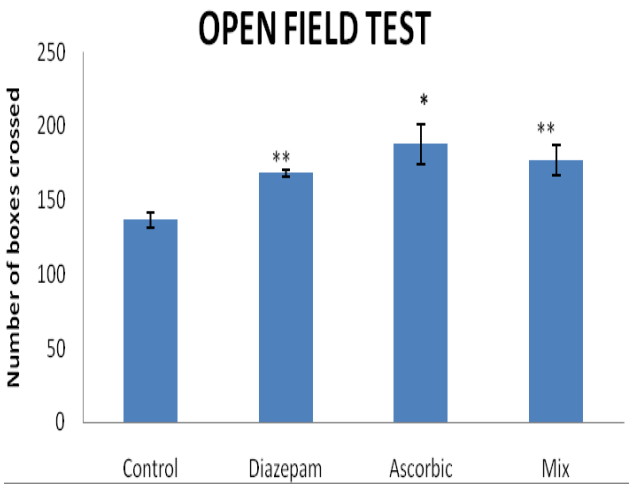

Fig. 1. Effect of diazepam, ascorbic acid and mixed group in open field.

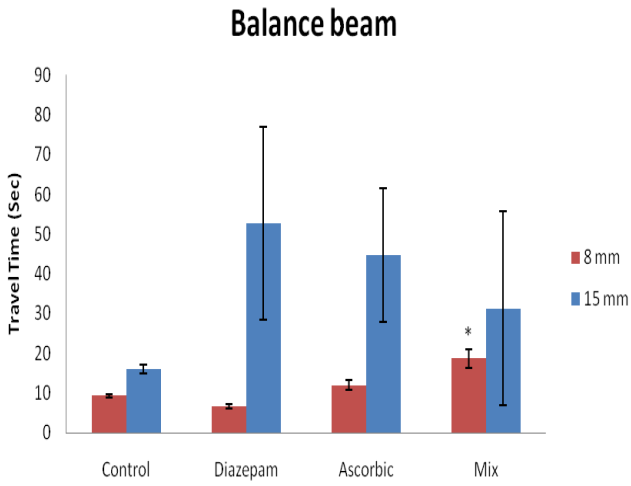

Fig. 2. Effect of diazepam, ascorbic acid and mixed group on travelling time of mice in 8mm and 15 mm beams.

Balance beam test: Table 2 and Figure 2 present balance beam results**.** The mixed group (diazepam and ascorbic acid) showed significant increase in travelling time on 8mm beam.

Table 2. Travel time on balance beam.

Travel Time (sec)							
8 mm				15 mm			
Control	**Diazepam**	**Ascorbic**	**Mix**	**Control**	**Diazepam**	**Ascorbic**	**Mix**
9.33 ± 0.33	6.66 ± 0.66	12 ± 1.15	18.66* ± 2.4	16 ± 1.15	52.66 ± 24.28	44.66 ± 16.82	31.33 ± 24.34

N= 7, values are expressed as mean ± SEM, *p≤0.05, **p≤0.01

DISCUSSION

Anxiety can occur with disorders like diabetes, hypertension, Parkinson and Alzheimer's disease. Increased oxidative stress can be the reason for many such diseases. Antioxidants can be used in combination with anxiolytic drugs in the treatment of anxiety and other stress induced conditions (Gautam *et al*., 2012). In a previous study performed on zebra fish, the effect of anxiogenic agent was decreased by co administration of ascorbic acid due to its ability to prevent neuronal alteration produced by anxiogenic agent (Puty *et al*., 2014).

The focus of our article is to check the effect of ascorbic acid in open field and balance beam test and to compare it with diazepam. The significant increase in number of boxes crossed in open field is indicating that ascorbic acid is producing anxiolytic action as that of diazepam. The concurrent administration of diazepam and ascorbic acid in mice increased exploration and hence anxiolytic effect. This may be justified by the role of ascorbic acid in the formation of different neurotransmitters; it also has an antioxidant action linked with anxiolytic properties (EL-Sokkary, 2008).

The mixed group increased travelling time on balance beam of 8 mm diameter, which needs further research to ascertain that the combination somehow affects balance. However on the basis of current study we propose that ascorbic acid may prove a novel anxiolytic agent.

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