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Neurochemical and behavioral effects of lorazepam: A dose related study

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Abstract: Present study was designed to monitor the dose dependent effects of lorazepam; a benzodiazepine (CNS depressant). It is the primary drug of choice for treatment of anxiety and to produce calming effects. However, repeated administration of this lorazepam causes dependence and this might be caused by increased dopaminergic neurotransmission. Besides dopamine, 5-hydroxy tryptamine (5-HT) has also been reported to have pivotal role in the pathophysiology as well as treatment of anxiety and addiction. Repeated administration of lorazepam might involve altered 5-HT metabolism as well. Present study was therefore designed to monitor dose-dependent effects of lorazepam and to select its optimum dose for further experiments and pharmacological interventions. Effects of lorazepam were monitored on food intake, growth rate, activities in familiar and novel environments, light dark box activity, forced swim test and metabolism of dopamine and 5-HT. oral administration of lorazepam was done at the doses of 0mg/kg, 2mg/kg, 4mg/kg and 6mg/kg. Behaviors parameters were monitored following single administration of lorazepam. Rats were decapitated and whole brain samples were collected and stored at -70°C until neurochemical analysis by HPLC-EC. Findings from the present study could be implicated to increased therapeutic utility of lorazepam and related benzodiazepines.

Keywords: Lorazepam, Ativan, light dark box activity, anxiety, forced swim test.

INTRODUCTION

Benzodiazepines are used for their sedative, anxiety-relieving and muscle-relaxant effects. Lorazepam (Ativan), a benzodiazepine, is used for short-term treatment of severe anxiety or agitation that is restricting individual to control distress (Pomara *et al*., 2015), relieving anxiety, causing sedation before surgery or medical procedures and could be used in epileptic seizures (Prasad *et al*., 2005). GABA receptors are involved in mediating the effects of lorazepam. Lorazepam increases the release of GABA in brain (Olkkola *et al*., 2008) resulting in calming effects, sleepiness, relaxation of the muscles and a decrease in anxiety (Riss *et al*., 2008).

Side effects of lorazepam include: dizziness, drowsiness; weakness; slurred speech, lack of balance or coordination; memory problems; or feeling unsteady. Lorazepam may cause addiction that is, it can be habit-forming and it should only be used with prescription. Overdose of lorazepam induces death. Therapies using lorazepam can cause dependence depending on drug's dosage, period of therapy and potency (Bogenschutz *et al*., 2016). Dependence may be physiological or psychological. Withdrawal symptoms may emerge due to physiological dependence. Psychologically, long-term use of lorazepam may cause over reliance on drug and drug-seeking behavior.

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Present study was therefore designed to monitor the dose-dependent effects of lorazepam on behaviors in order to evaluate the optimum dosage with least side effects. Findings will help in extending therapeutics in epilepsy, anxiety and related disorders.

MATERIALS AND METHODS

Animals

Locally bred male Albino Wistar rats (weighing 180–200 g) were purchased from HEJ Research Institute of Chemistry, Karachi and were housed individually under 12 hr light and dark cycles (lights on at 06:00 hr) and controlled room temperature (24±2 °C) with free access to tap water and cubes of standard rodent diet at least 7 days before starting the experiment so that they could become familiar to the environment. Animals were tested in light phase. Before starting the experiment, rats were accustomed to various handling procedures in order to nullify the psychological affliction of environment. All protocols for experimentation were approved by the Institutional Animal Ethics Committee (Approval No. 3250).

Drugs and doses

Lorazepam (Ativan; Martin Dow Ltd.) was dissolved in water and given orally at the doses of 2.5-, 4.0- and 6.0mg/kg. Drug solutions were freshly prepared before each experiment. Control animals were given tap water (1.0 ml/kg).

Experimental Protocol

Twenty-four rats were randomly divided into four groups, each group containing six rats: (i) water (1ml/kg), (ii) lorazepam (2mg/kg), (iii) lorazepam (4mg/kg) and (iv) lorazepam (6mg/kg). Rats all groups were placed individually in the Skinner's box 10min before drug administration to get familiarized with the environment. 10 min post drug administration, animals were orally administered with water or respective doses of midazolam at 9:00 h to 10:00h. Motor activities in familiar environment of Skinner's box were monitored 5min post drug administration for 10min. Pain perception ability was recorded 20 min post drug administration by hot plate test. To assess anxiolytic activity, light/dark box activities were monitored 30min post drug administration. Exploratory activities in novel environment of an open field were monitored 35min post drug administration for 5min. While antidepressant activities were monitored 40 min post drug administration by forced swim test. Animals were decapitated 1hr post drug administration. Plasma and brain samples were collected and stored at -70°C for neurochemical analysis by HPLC-EC.

Behavioral assessment

Food Intake and Growth Rates

Cumulative 24 h food intakes were determined by taking the difference of food given on day 1 at 9:00-10:00h (immediately before injection) and food left at 9:00-10:00h on day 2. Body weights of animals were also monitored at the same time on days 1 and 2. Body weights were reported as %growth rates

Activities in familiar environment of skinner's box

Transparent Perspex cages (26×26×26 cm) with saw dust covered floor were used to monitor activity in familiar environment. Rats were placed individually in these cages to get familiar with the environment. 15 min later the animals were administered with drug or water. Numbers of cage crossings were counted 5 min post-injection for 10 min (Ikram *et al.,* 2007; Ikram and Haleem, 2010; Ikram and Haleem, 2011)

Activities in novel environment of an open field

A square area (76×76 cm) with walls 42 cm high was used to monitor activity in a novel environment. The floor of apparatus was divided by lines into 25 squares of equal size. Animals were administered with drug or vehicle and placed in the central square of the open field immediately after the injection. Numbers of squares crossed with all four paws were counted for 5 min (Ikram *et al.,* 2011).

Light dark box activity

Specifically designed two Perspex compartments of equal dimensions (26x26x26 cm) were used to monitor the activity. One compartment was transparent and other was black walled with an entry between them. Experiment was conducted in a separate room. To determine light and dark field activity, an animal was taken out from home cage and placed for the first time in the light compartment. Number of entries in light compartment and time spent in the light and dark compartments were monitored for 5 minutes (Ikram *et al*., 2018).

Hot Plate Test

Antinociception was assessed using a hot-plate instrument with the plate temperature maintained at 56 ± 0.1°C. Each rat was placed individually with all 4 paws on the plate. Then the response latency to either a hind-paw lick or a jump was recorded. In the absence of a response, the animals were quickly removed from the 56°C hot plate at 20s (cut-off time) to avoid tissue damage. The determined latency time for each animal was converted into the percentage of analgesia according to the formula: % analgesia = $[(Tx-T_O)/ (Tmax-T_O) \times 100]$. Where Tx= individual latency time determined at appropriate intervals after administration of the examined analgesics; T_O = was the individual latency time determined before analgesic injection and Tmax = 20sec (Ikram *et al*., 2019).

Forced Swim Test

Each rat was placed individually into the glass cylinders (height 25 cm, diameter 10cm) containing 10 cm of water at 23-25°C. The animals were left in the cylinder for 6 min. The total duration of immobility was recorded by cumulative stopwatches during the last 4min of the 6-min-long testing period. The rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only the movements necessary to keep its head above the water level (Ikram *et al*., 2018).

Decapitation of rat brain

Dissection procedure was same as described earlier (Bano *et al.,* 2014; Mirza *et al.,* 2013). Animals were killed 1hr post injections, on day 22. The skull plates were cut and membrane covering the brain was removed with the help of fine forceps. Using spatula, brain was taken out and washed with ice-cold saline. The collected brains were immediately stored at -70°C for the estimation of biogenic amines and metabolites using High performance liquid chromatography with electrochemical detection (HPLC-EC).

HPLC-EC Analysis of Biogenic amines and metabolites

Extraction of biogenic amines and metabolites was same as described previously (Ikram *et al.,* 2012). Extraction was performed using 70% perchloric acid. 5 times volume of the extraction medium was added to the brain tissues. Samples were homogenized by using electrical homogenizer and subjected to ultracentrifugation at 6000rpm for 20min at 4°C. Supernatant was separated and injected to HPLC-EC for neurochemical assay. A 5µ Shimpack ODS separation column of 4.0 mm internal diameter and 150mm length was used. 0.1 M phosphate

buffer (PH 2.9) containing EDTA (0.0035%), methanol (14%) and octyl sodium sulfate (0.023%) was used at an operating potential of 2000-3000 psi on Schimadzu HPLC pump. Electrochemical detection (using Schimadzu LEC 6A detector) was done at an operating potential of +0.8V.

STATISTICAL ANALYSIS

Results are represented as means ± SD. Statistical analyses were performed by one-way or two-way analysis of variance (ANOVA) using SPSS software ver 17. Post hoc individual comparisons of groups were performed by Tukey's test. Values of $p<0.05$ were considered significant.

RESULTS

Fig. 1 shows dose dependent effects of lorazepam on food intake. Analysis of the data by one-way ANOVA showed significant effects of different doses of lorazepamon food intake (df= 3, 20; F= 8.23; p= 0.001). Post hoc analysis by Tukey's test showed decreased ($p<0.01$) food intake in rats administered with lorazepam at the dose of 6mg/kg as compared to both 0mg/kg as well as 2mg/kg lorazepamadministered rats.

Fig. 1: Dose dependent effects of lorazepam on food intake. Values are means ± SD (n=6). Significant differences by Tukey's test: *$p<0.01$ as compared to 0mg/kg-; +$p<0.01$ as compared to 2mg/kg lorazepam treated rats, following one-way ANOVA.

Fig. 2 shows dose dependent effects of lorazepam on growth rates. Analysis of the data by one-way ANOVA showed non-significant effects of different doses of lorazepam on growth rates (df= 3,20; F= 0.09; p= 0.964). Post hoc analysis by Tukey's test showed no significant difference among groups.

Fig. 3 shows dose dependent effects of lorazepam on motor activities in familiar environment of Skinner's box. Analysis of the data by two-way ANOVA showed significant effects of post-treatment values (df= 2,40; F= 160.50; p= 0.0001) but not those of lorazepam (df= 3,40; F= 2.27; p= 0.095) and interaction between the two (df= 3,40; F= 0.35; p= 0.783).Post hoc analysis by Tukey's test decreased ($p<0.01$) motor activities in familiar environment in rats as compared to respective pre-treatment values as well as compared to respective 0mg/kg lorazepam treated rats.

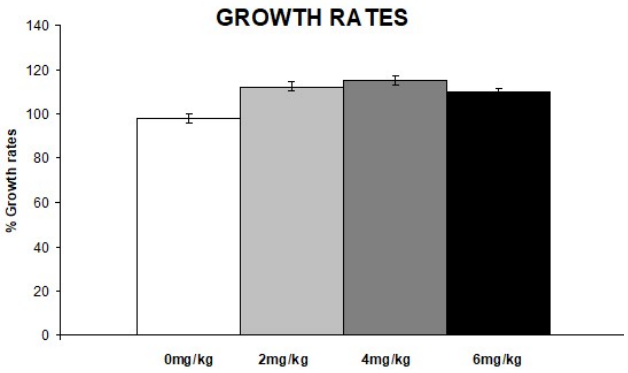

Fig. 2: Dose dependent effects of lorazepam on growth rates. Values are means ± SD (n=6). Differences by Tukey's test were non-significant, following one-way ANOVA.

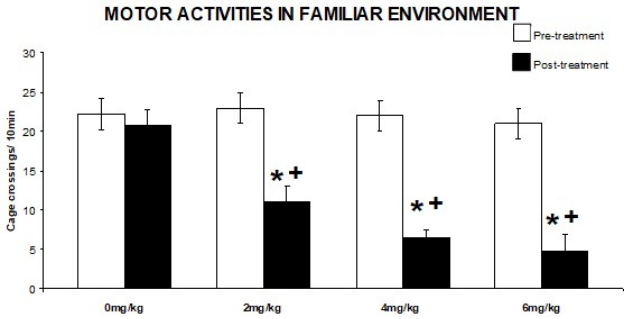

Fig. 3: Dose dependent effects of lorazepam on motor activities in familiar environment of Skinner's box. Values are means ± SD (n=6). Significant differences by Tukey's test: *$p<0.01$ as compared to respective pre-treatment values; +$p<0.01$ as compared to respective 0mg/kg lorazepam treated rats, following two-way ANOVA.

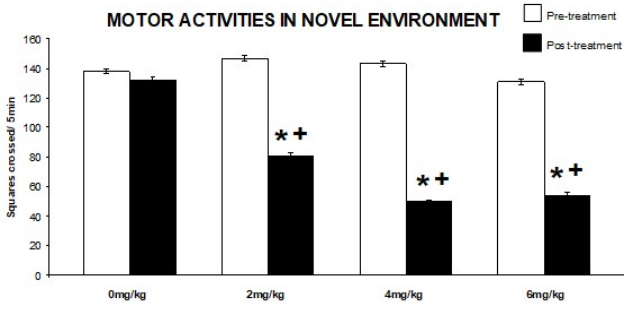

Fig. 4: Dose dependent effects of lorazepam on motor activities in novel environment of open field. Values are means ± SD (n=6). Significant differences by Tukey's test: *$p<0.01$ as compared to respective pre-treatment values; +$p<0.01$ as compared to respective 0mg/kg lorazepam treated rats, following two-way ANOVA.

Fig. 4 shows dose dependent effects of lorazepam on motor activities in novel environment of open field.

Analysis of the data by two-way ANOVA showed significant effects of post-treatment values (df= 1, 40; F= 121.79; p= 0.0001) but not those of lorazepam (df= 3, 40; F= 2.32; p= 0.090) and interaction between the two (df= 3,40; F= 5.09; p= 0.004). Post hoc analysis by Tukey's test decreased (p<0.01) motor activities in novel environment in rats as compared to respective pre-treatment values as well as compared to respective 0mg/kg lorazepam treated rats.

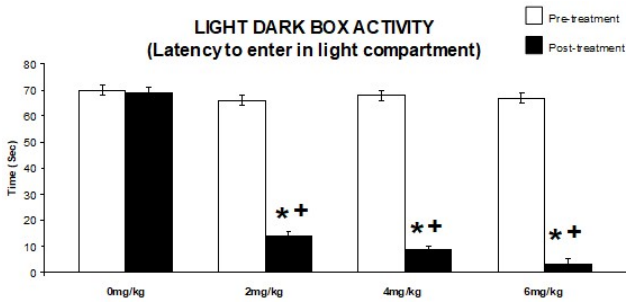

Fig. 5: Dose dependent effects of lorazepam on light dark box activity (latency to enter in light compartment). Values are means ± SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to respective pre-treatment values; +p<0.01 as compared to respective 0mg/kg lorazepam treated rats, following two-way ANOVA.

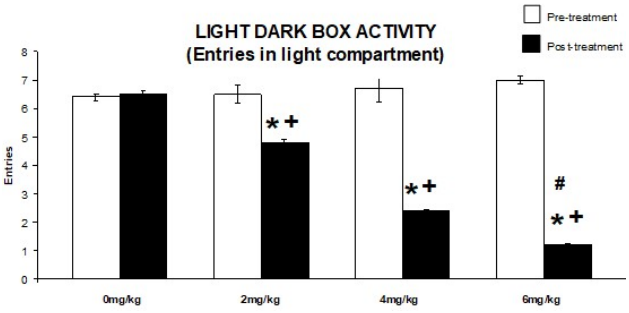

Fig. 6: Dose dependent effects of lorazepam on light dark box activity (entries in light compartment). Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to respective pre-treatment values; +p<0.01 as compared to respective 0mg/kg lorazepam treated rats; #p<0.01 as compared to respective 2mg/kg lorazepam treated rats,, following two-way ANOVA.

Fig. 5 shows dose dependent effects of lorazepam on light dark box activity (latency to enter in light compartment). Analysis of the data by two-way ANOVA showed significant effects of post-treatment values (df= 1, 40; F= 34.09; p= 0.0001) but not those of lorazepam (df= 3, 40; F= 2.25; p= 0.096), and interaction between the two (df= 3, 40; F= .448; p= 0.720). Post hoc analysis by Tukey's test decreased (p<0.01) light dark box activity (latency to enter in light compartment) in rats as compared to respective pre-treatment values as well as compared to respective 0mg/kg lorazepam treated rats.

Fig. 6 shows dose dependent effects of lorazepam on light dark box activity (entries in light compartment). Analysis of the data by two-way ANOVA showed significant effects of post-treatment values (df= 1,40; F= 14.53; p= 0.0001) but not those of lorazepam (df= 3,40; F= 4.78; p= 0.06), and interaction between the two (df= 3,40; F= 1.088; p=0.365). Post hoc analysis by Tukey's test decreased (p<0.01) light dark box activity (entries in light compartment) in rats as compared to respective pre-treatment values as well as compared to respective 0mg/kg lorazepam treated rats and as compared to 2mg/kg lorazepam treated rats.

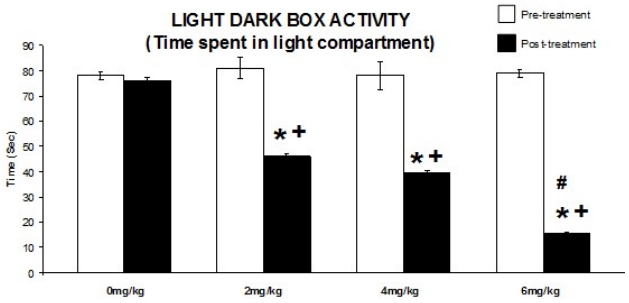

Fig. 7: Dose dependent effects of lorazepam on light dark box activity (time spent in light compartment). Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to respective pre-treatment values; +p<0.01 as compared to respective 0mg/kg lorazepam treated rats; #p<0.01 as compared to respective 2mg/kg lorazepam treated rats, following two-way ANOVA.

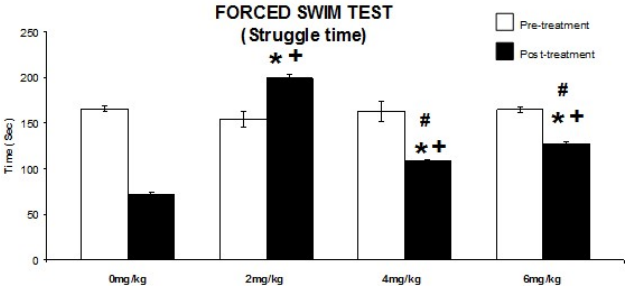

Fig. 8: Dose dependent effects of lorazepam on forced swim test (struggle time). Values are means ± SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to respective pre-treatment values; +p<0.01 as compared to respective 0mg/kg lorazepam treated rats, following two-way ANOVA.

Fig. 7 shows dose dependent effects of lorazepam on light dark box activity (time spent in light compartment). Analysis of the data by two-way ANOVA showed significant effects of post-treatment values (df= 1, 40; F= 19.82; p= 0.0001) but not those of lorazepam (df= 3, 40; F= 1.95; p= 0.136), and interaction between the two (df= 3, 40; F= .740; p= 0.534). Post hoc analysis by Tukey's test decreased (p<0.01) light dark box activity (time spent in light compartment) in rats as compared to respective pre-treatment values as well as compared to respective

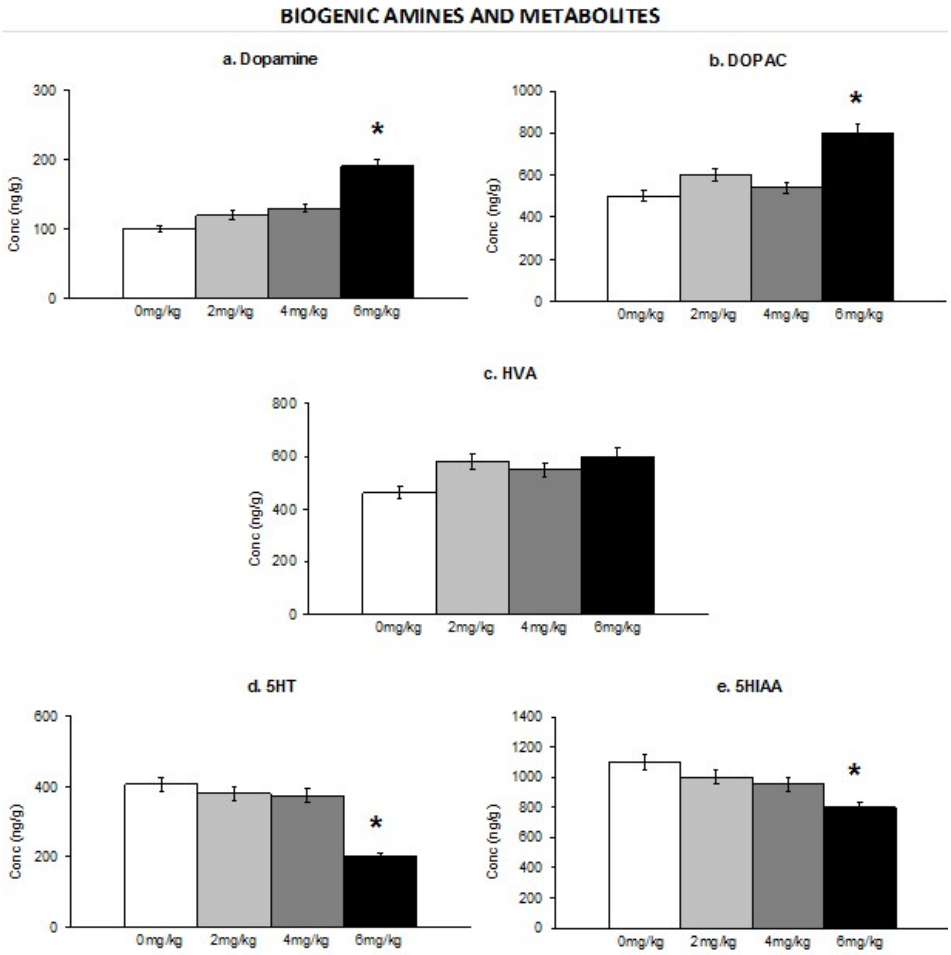

Fig. 9: Dose dependent effects of lorazepam on biogenic amines and metabolites in whole brain of rats. Values are means ± SD (n=6). Significant differences by Tukey's test: *p<0.01 as compared to 0mg/kg lorazepam treated rats, following one-way ANOVA.

0mg/kg lorazepam treated rats and as compared to 2mg/kg lorazepam treated rats.

Fig. 8 shows dose dependent effects of lorazepam on forced swim test (struggle time). Analysis of the data by two-way ANOVA showed significant effects of post-treatment values (df= 1, 40; F= 5.12; p= 0.029), lorazepam (df= 3, 40; F= 12.44; p= 0.008), and interaction between the two (df= 3,40; F= 4.51; p= 0.024). Post hoc analysis by Tukey's test increased (p<0.01) forced swim test (struggle time) in rats with 2mg/kg as compared to respective pre-treatment value as well as compared to respective 0mg/kg lorazepam treated rats. Decreased (p<0.01) forced swim test (struggle time) in rats with 4mg/kg and 6mg/kg as compared to respective pre-treatment values as well as compared to respective 0mg/kg lorazepam treated rats and as compared to 2mg/kg lorazepam treated rats.

Fig. 9 shows dose dependent effects of lorazepam on biogenic amines and metabolites in whole brain samples of rats. Analysis of the data by one-way ANOVA showed significant effects of various doses of lorazepam on dopamine (df= 1,20; F= 24.52; p= 0.001), DOPAC (df= 1,20; F= 65.21; p= 0.001), HVA (df= 1,20; F= 38.95; p= 0.001), 5HT (df= 1,20; F= 91.74; p= 0.001) and 5HIAA (df= 1,20; F= 68.25; p= 0.001). Post hoc analysis by Tukey's test showed increased (p<0.01) levels of dopamine and DOPAC in rats treated with 6mg/kg lorazepam as compared to rats treated with lorazepam at the dose of 0mg/kg. While levels of 5HT and 5HIAA were decreased (p<0.01) in these rats as compared to rats treated with lorazepam at the dose of 0mg/kg.

DISCUSSION

Previously it has been reported that increased food intake in humans and non-human subjects is caused by benzodiazepine agonists. Studies have also shown that diazepam significantly increased sucrose pellet consumption and proposes that increase in consumption of palatable food as caused by benzodiazepine

administration, is mediated through α1GABAA receptors; a GABA receptor subtype (Angela *et al*., 2006). Parabrachial nucleus located in the caudal brainstem is the probable site of action for benzodiazepines (Cooper, 2005). Results from our experiment show non-significant increase in food intake by lorazepam at the dose of 2mg/kg. While significant decrease in food intake was observed at the dose 6mg/kg. Present study shows no significant changes on growth rates at different doses of lorazepam.

A progressive increase in locomotor activity from first till last administration of diazepam, has been reported earlier (Djeridane *et al*., 2005). Others have reported that the effects of benzodiazepines on locomotive activity require α1-GABAA receptor subunit expression in amygdala (Scott A *et al*., 2010). This experiment represented dose related effects of lorazepam on locomotive activity in familiar as well as novel environment. Results of present study show that motor activities in familiar environment were decreased as compared to their control group and respective pretreatment groups. Moreover, motor activities in novel environment were also decreased as compared to their control group and respective pretreatment groups.

Findings from this study show decreased light dark box activity (latency to enter in light compartment) in rats as compared to respective pre-treatment values as well as compared to respective 0mg/kg lorazepam treated rats. This study also shows decrease in light dark box activity (entries in light compartment) in rats. Present study also shows that decrease in light dark box activity (time spent in light compartment) in rats. Studies have shown that anxiolytic effects of benzodiazepines are believed to be mediated through BZ2 receptors located in the limbic system. (Charles E *et al*., 2013). Studies have showed that benzodiazepines produce anxiolytic effect, through GABAA receptor α2 and α3 subunits are involved in anxiolytic effects (Vinkers *et al*., 2009). Previous studies illustrate that benzodiazepines-induced anxiolysis is mediated via α2-containing GABAA receptors ass tested in the in conditioned fear models and unconditioned tests of anxiety (Smith, 2012).

Previously it has been reported that depression coexist with anxiety. There are suggestions that benzodiazepines chronic use carries risks of dependence and long-term administration causes low efficacy (Watanabe *et al*., 2007). Present study showed that struggling time was increased 2mg/kg and decreased at 4 mg/kg and 6 mg/kg of lorazepam, which shows that overdose may cause depression like side effects.

CONCLUSION

Results from this experiment illustrate that lorazepam at the dose of 2mg/kg can produce therapeutic effects with least side effects. It is therefore suggested lorazepam at the dose of 2mg/kg could be used for studying its behavioral effects while keeping its therapeutic profile unaffected and therapeutic interventions to attenuate lorazepam-induced addictive effects.

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