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Neurochemical and behavioral effects of midazolam: A dose related study

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Abstract: In the present study we have monitored dose dependent effects of midazolam; a benzodiazepine (CNS depressant). It is the primary drug of choice for procedural sedation, preoperative sedation, and in emergency departments. Repeated administration of this drug is reported to have abuse potential and may cause this by increasing dopaminergic neurotransmission. Since an important role of 5-hydroxy tryptamine (5-HT) is there in the pathophysiology of anxiety and addiction, administration of midazolam may involve altered 5-HT metabolism as well. Present study was designed to monitor dose-dependent effects of midazolam and select the optimum dose for further experiments. Effects of midazolam were monitored on food intake, growth rate, activities in familiar and novel environments, light dark box activity, hot plate test, forced swim test and levels of dopamine, 5-HT and their metabolites. Midazolam was administered orally (0mg/kg, 2.5mg/kg, 5.0mg/kg and 10mg/kg) and behaviors were monitored post single midazolam administrations. 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 midazolam and related benzodiazepines.

Keywords: Midazolam, dormicum, light dark box activity, hot plate test, forced swim test.

INTRODUCTION

Midazolam is a benzodiazepine (CNS depressant) which is primary drug of choice for procedural sedation, preoperative sedation and general anesthesia (Basturk *et al.,* 2017; Ashrafi *et al.,* 2010; Niquet *et al.,* 2017; Ramirez *et al.,* 2016). Midazolam and other benzodiazepines have also been used for the treatment of panic disorders, generalized anxiety disorders, insomnia, seizures, alcohol withdrawal, anxiety as well as other indications (Long *et al.,* 2017; El-Dib and Soul, 2017; Sant'Ana *et al.,* 2016; Monte-Secades *et al.,* 2015). For rapid sequence endotracheal intubation, midazolam is a drug of choice to induce sedation. Administration of midazolam through rectal, nasal, oral, intramuscular or intravenous routes has a rapid onset of action. The first-pass metabolism of the drug is extensive, as a result of which only 50% of the drug administered orally, reaches systemic circulation (Layangool *et al.,* 2008).

In the pre-hospital settings, for children with untreated status epileptic, intramuscular midazolam is a safe and effective option for the management of their condition and could be rapidly administered (Welch *et al.,* 2015). Approximately five times greater steady-state potency of midazolam was found as compared to that of diazepam (Olkkola and Ahonen, 2008). Addition of midazolam can also improve the beneficial effects of morphine in controlling the baseline levels of dyspnea (Navigante *et al.,* 2006).

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However, despite its vast clinical/ therapeutic implications, midazolam produces addictive effects upon repeated administration (Kroon and Carobrez, 2009). positive modulation of $GABA_A$ (gamma-aminobutyric acid type A) receptors in the inter-neurons facilitate the firing of dopaminergic neurons present in the ventral tegmental area as induced by midazolam. Underlying drug reinforcement is due to these excitatory afferents which induce drug-evoked synaptic plasticity of dopamine neurons. This triggering of alpha1-containing $GABA_A$ receptors is the major factor to initiate the process. (Tan *et al.,* 2010). Combining midazolam with fentanyl or other opioids produces a potent drug interaction that places patients at a high risk for hypoxemia and apnea (Roback *et al.,* 2005), while midazolam in combination with ketamine is unlikely to cause cognitive complications (Valentim *et al.,* 2013). Therefore, it is important to monitor drug interactions while giving midazolam in conjugation to some other drug.

Both dopamine and serotonin play an important role in reward and motivation. Repeated midazolam administration effects serotonin metabolism. Serotonin may be a genetic moderator of food reinforcement and distinguishing between serotonin's effect on satiety and impulsivity may provide mechanisms by which serotonin influences food reinforcement (Carr *et al.,* 2013). Midazolam can attenuate the repeated stress-induced increase in craving for sweet food. This attenuation was independent of chronic administration or hunger (Silveira *et al.,* 2000). Repeated drug administration also does not

effect the induced decrease in extra cellular dopamine in the nucleus accumbens despite the fact that it differentially affects meso-accumbens and nigrostriatal dopaminergic neurons (Engin *et al.,* 2014). Self-administration of a rapidly infused lethal drug mixture contains zolpidem, propofol, midazolam, thiopental, and amitriptyline can cause death (Colucci *et al.,* 2013). Long term administration of midazolam was significantly found to inhibit the release of different plasma cytokines in pediatric patients after surgery by inhibiting the interleukin-1β induced release of interleukin-6 in the central nervous system (Lu *et al*, 2015).

Despite a number of clinically important uses, midazolam presents high potential for abuse (Braun *et al.,* 2008). Therefore, chronic use of midazolam may result in the development of tolerance and dependence and person may become addicted to it, even under strict monitoring by the doctor. Therapeutic profile also starts to change when midazolam is abused and tolerance to the beneficial effects is developed. An ultimate increase in the dopaminergic neurotransmission in nucleus accumbens mediates these addictive effects of benzodiazepines (Li *et al.,* 2013). Previous studies from our laboratory show that 5-hydroxytryptamine (5-HT; serotonin) can counter act addictive effects of CNS stimulants (Haleem *et al.,* 2014). However, whether serotonergic agents affect abuse potential and therapeutic profile of benzodiazepines and/or other CNS depressants is yet to be established.

Present study was designed to monitor the dose-dependent effects of midazolam on behaviors and neurochemistry. Findings will help extending therapeutics in epilepsy and anxiety. Associated neurochemical changes will be determined to get an insight in the interaction of serotonin and dopamine in the control of stress, addiction and seizures.

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 12hr 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 (IAEC).

Drugs and doses

Midazolam (Dormicum; Martin Dow Ltd.) was dissolved in water and given orally at the doses of 2.5-, 5.0-, and 10.0mg/kg. Drug was freshly prepared before each experiment. Control animals were given tap water (1.0 ml/kg).

Experimental protocol

Twenty four male Albino Wistar rats were randomly divided into four groups, each containing 6 animals: (i) Control (1ml/kg) (ii) Low (2.5mg/kg) dose of midazolam (iii) Moderate (5.0 mg/kg) dose of midazolam and (iv) High (10mg/kg) dose of midazolam. On day 0, animals and food pallets were weighed and data was recorded. On day 1, animals were administered with tap water (1ml/kg) or respective dose of midazolam. Hot plate test, Skinner's box activity, light dark box activity and open field tests were performed. Animals were then decapitated and brain regions (caudate, hippocampus and nucleus accumbens) were collected and stored 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 9:00-10:00 h (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.

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.

Forced swim test

Each rat was placed individually into the glass cylinders (height 25 cm, diameter 10 cm) 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 4 min 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.

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. 0.1M 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 using SPAA (ver 17) by one-way analysis of variance (ANOVA). 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 dependant effects of midazolam on food intake and growth rates. Analysis of the data on food intake (fig. 1a) by one-way ANOVA showed significant effects of different doses of midazolam on food intake (df= 3,20; F= 31.457; p= 0.0001). Post hoc analysis by Tukey's test showed increased ($p<0.01$) food intake at the dose of 2.5mg/kg as compared to 0mg/kg midazolam administered rats. While food intake was decreased ($p<0.01$) at the dose of 10mg/kg as compared to both 0mg/kg- and 2.5mg/kg midazolam administered rats.

Analysis of the data on growth rates (fig. 1b) by one-way ANOVA showed no significant effects of different doses of midazolam on growth rates (df= 3,20; F= .0427; p= 0.73). Post hoc analysis by Tukey's test showed no significant difference among the groups.

Fig. 2 shows dose dependant effects of midazolam on locomotive activities in familiar as well as novel environment. Analysis of the data on locomotive activities in familiar environment of Skinner's box (fig. 2a) by one-way ANOVA showed significant effects of different doses of midazolam on Skinner's box activity(df= 3,20; F= 14.38; p= 0.0001). Post hoc analysis by Tukey's test showed increased ($p<0.01$) activities at the dose of 2.5mg/kg as compared to 0mg/kg midazolam administered rats. While activities were decreased ($p<0.01$) at the dose of 10mg/kg as compared to 0mg/kg midazolam administered rats. At the dose of 5.0mg/kg and 10mg/kg, activities were decreased as compared to 2.5mg/kg midazolam administered rats.

Analysis of the data on locomotive activities in novel environment of open field (fig. 2b) by one-way ANOVA showed no significant effects of different doses of midazolam on open field activity (df= 3,20; F= 34.25; p= 0.0001). Post hoc analysis by Tukey's test showed increased ($p<0.01$) activities at the dose of 2.5mg/kg as compared to 0mg/kg midazolam administered rats. While at the dose of 5.0mg/kg and 10mg/kg activities were decreased as compared to 2.5mg/kg midazolam administered rats. At 5.0mg/kg, activities were decreased ($p<0.01$) as compared to 0mg/kg midazolam administered rats.

Fig. 3 shows dose dependant effects of midazolam on light dark box activity. Analysis of the data on entries in the light compartment (fig. 3a) by one-way ANOVA showed significant effects of different doses of midazolam on entries in the light compartment (df= 3,20; F= 42.93; p= 0.0001). Post hoc analysis by Tukey's test showed increased ($p<0.01$) at all doses of midazolam as compared to 0mg/kg midazolam treated rats.

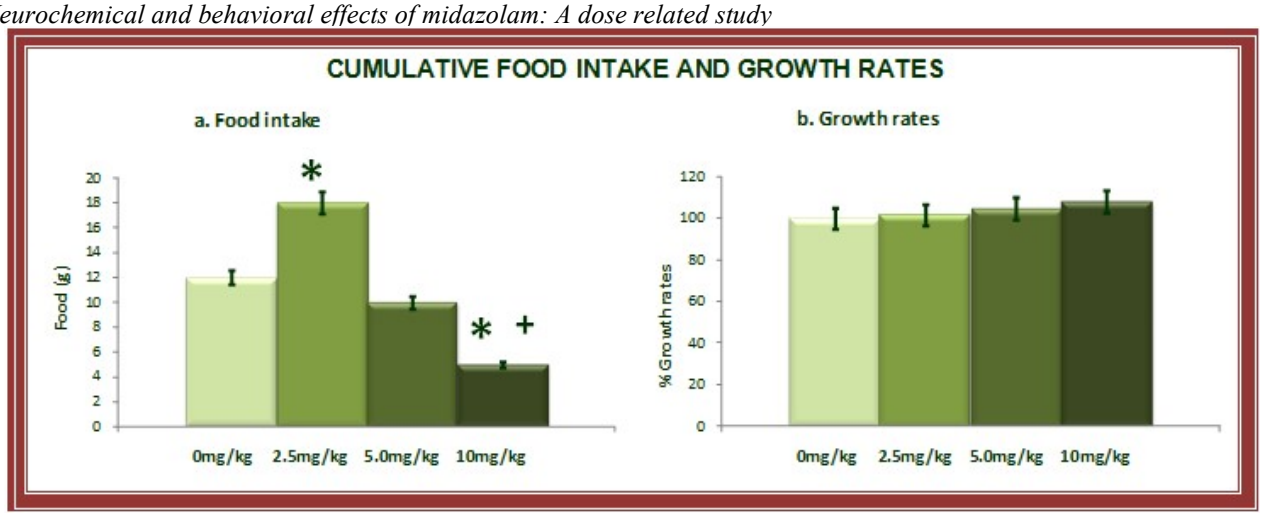

Fig. 1: Dose dependant effects of midazolam on food intake and growth rates. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 from 0mg/kg midazolam administered rats; +p<0.01 from 2.5mg/kg midazolam administered rats following one-way ANOVA.

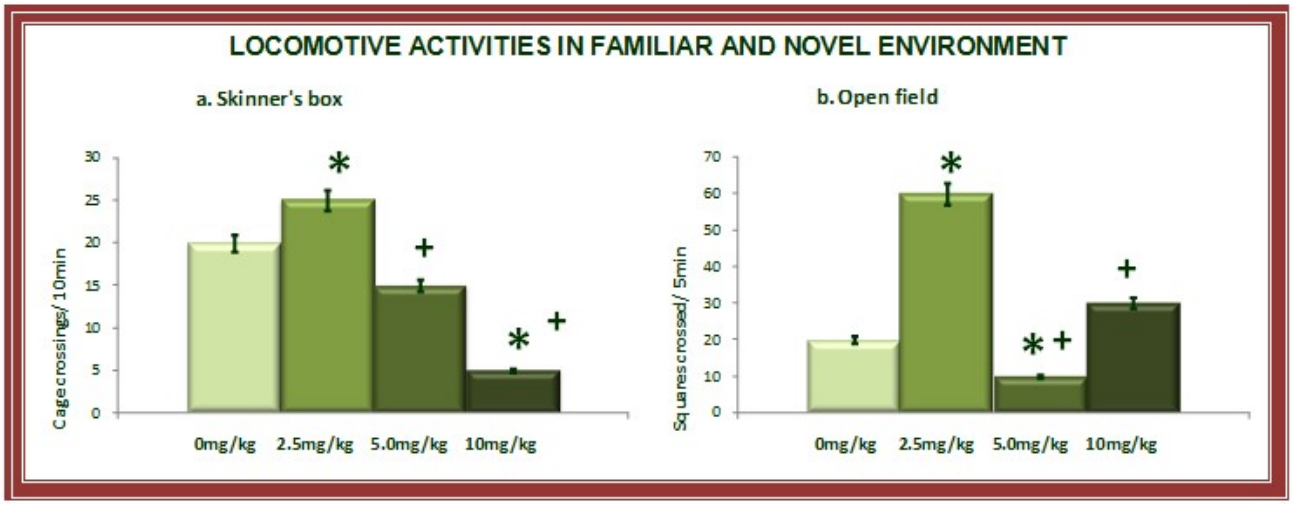

Fig. 2: Dose dependant effects of midazolam on locomotive activities in (a) familiar as well as (b) novel environment. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 from 0mg/kg midazolam administered rats; +p<0.01 from 2.5mg/kg midazolam administered rats following one-way ANOVA.

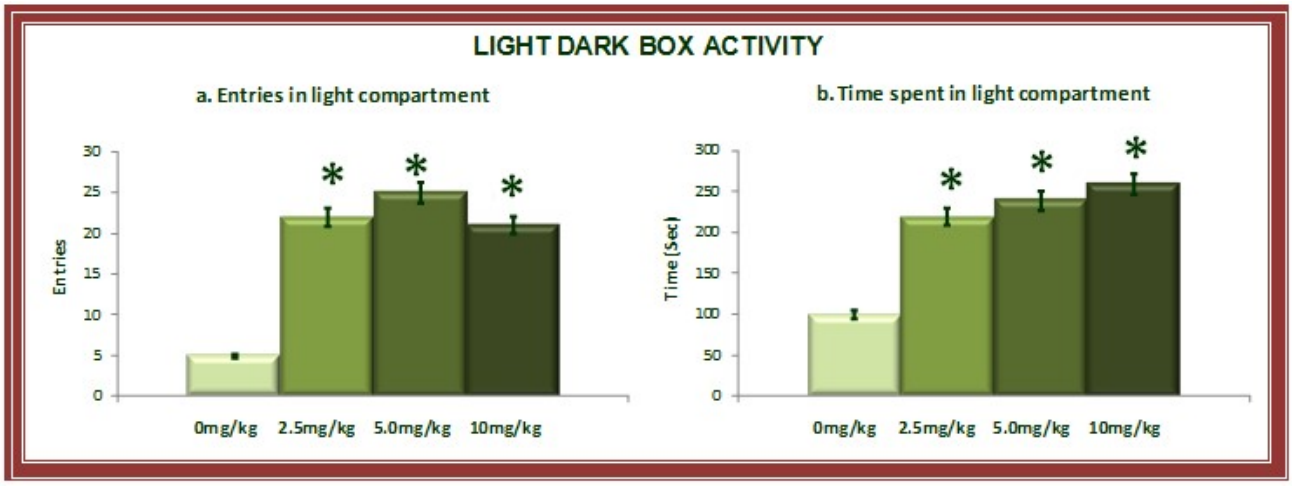

Fig. 3: All the analyses were performed using SPSS software (ver 17). Dose dependant effects of midazolam on light dark box activity. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 from 0mg/kg midazolam administered rats; +p<0.01 from 2.5mg/kg midazolam administered rats following one-way ANOVA.

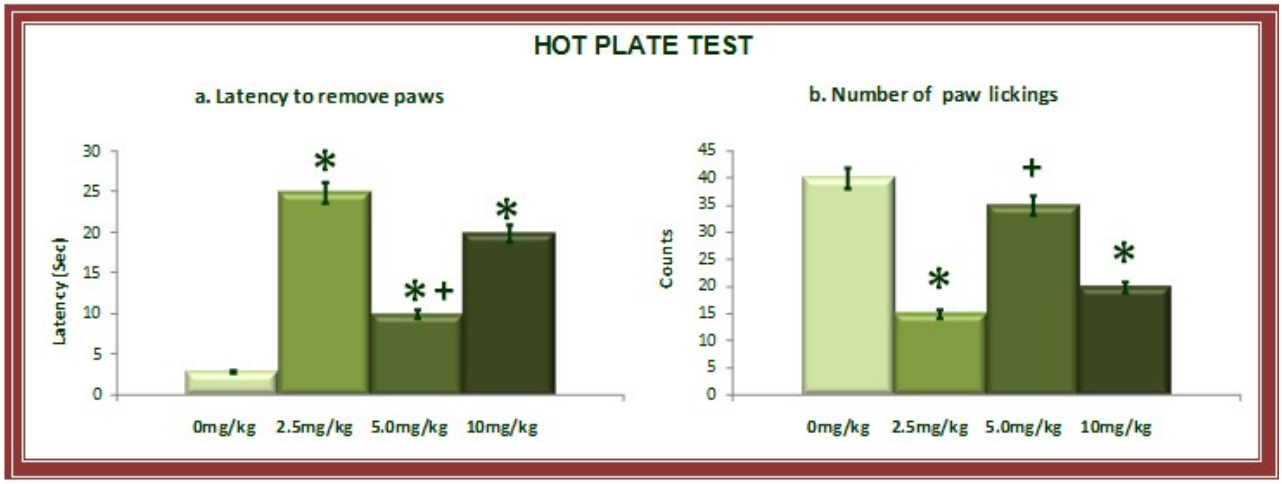

Fig. 4: Dose dependant effects of midazolam on hot plate test. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 from 0mg/kg midazolam administered rats; +p<0.01 from 2.5mg/kg midazolam administered rats following one-way ANOVA.

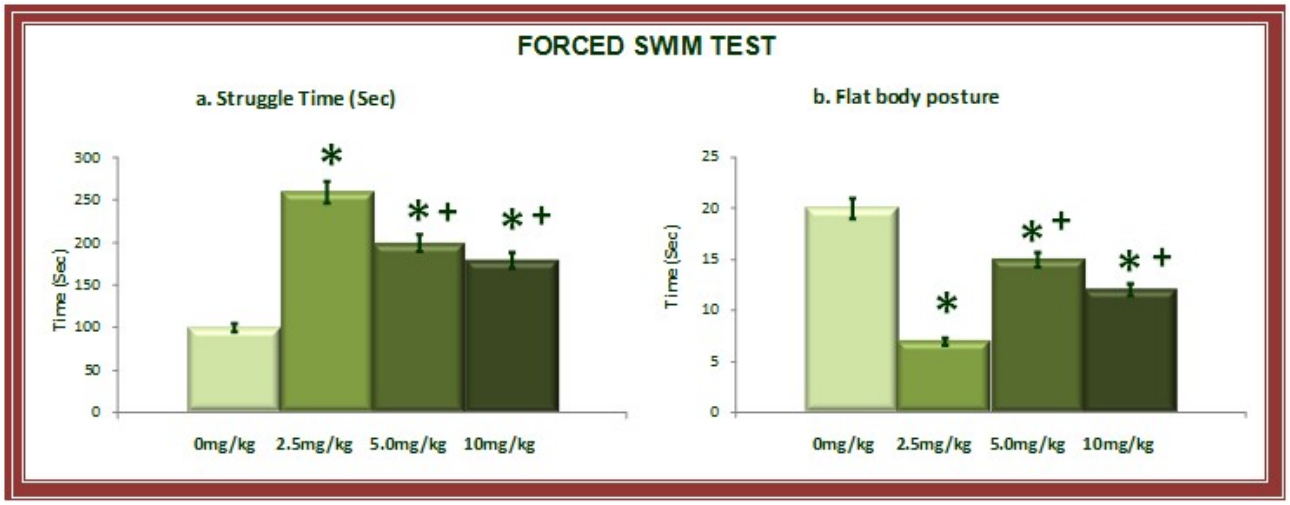

Fig. 5: Dose dependant effects of midazolam on forced swim test. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 from 0mg/kg midazolam administered rats; +p<0.01 from 2.5mg/kg midazolam administered rats following one-way ANOVA.

Analysis of the data on time spent in the light compartment (fig. 3b) by one-way ANOVA showed no significant effects of different doses of midazolam on time spent in the light compartment (df= 3,20; F= 4.98; p= 0.017). Post hoc analysis by Tukey's test showed increased (p<0.01) time spent in light compartment at all three doses of midazolam as compared to 0mg/kg midazolam administered rats. While increased (p<0.01) time spent in light compartment was also observed at all three doses as compared to 0mg/kg midazolam administered rats.

Fig. 4 shows dose dependant effects of midazolam on hot plate test. Analysis of the data on latency to remove paws (fig. 4a) by one-way ANOVA showed significant effects of different doses of midazolam on latency to remove paws (df= 3,20; F= 39.90; p= 0.0001). Post hoc analysis by Tukey's test showed increased (p<0.01) latency to remove paws at all three doses of midazolam as compared to 0mg/kg midazolam administered rats. While latency to remove paws at the dose of 5.0mg/kg midazolam was also decreased (p<0.01) as compared to 2.5mg/kg midazolam administered rats.

Analysis of the data on number of paw lickings (fig 4b) by one-way ANOVA showed no significant effects of different doses of midazolam on number of paw lickings (df= 3,20; F= 42.98; p= 0.017). Post hoc analysis by Tukey's test showed decreased (p<0.01) number of paw lickings at 2.5mg/kg- as well as 10mg/kg midazolam as compared to 0mg/kg midazolam administered rats. Number of paw lickings were greater (p<0.01) in 5.0mg/kg midazolam administered rats as compared to 2.5mg/kg midazolam administered rats.

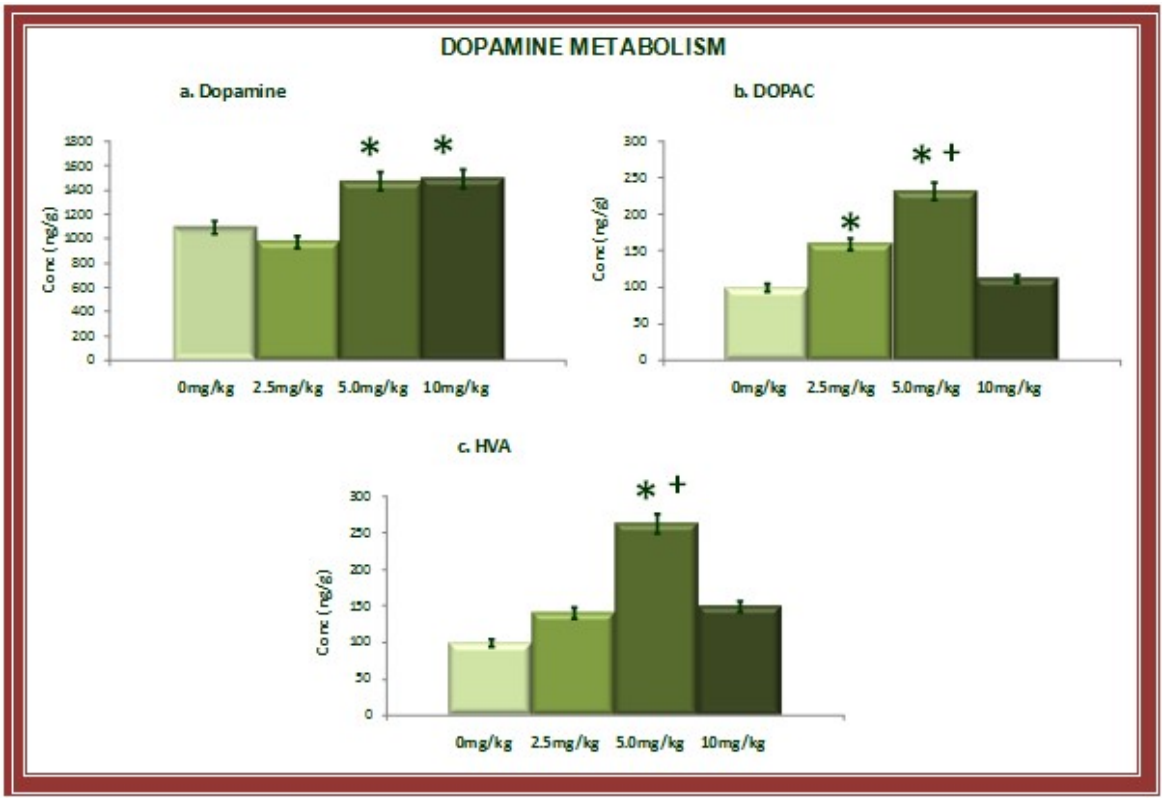

Fig. 6: Dose dependant effects of midazolam on dopamine metabolism. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 from 0mg/kg midazolam administered rats; +p<0.01 from 2.5mg/kg midazolam administered rats following one-way ANOVA.

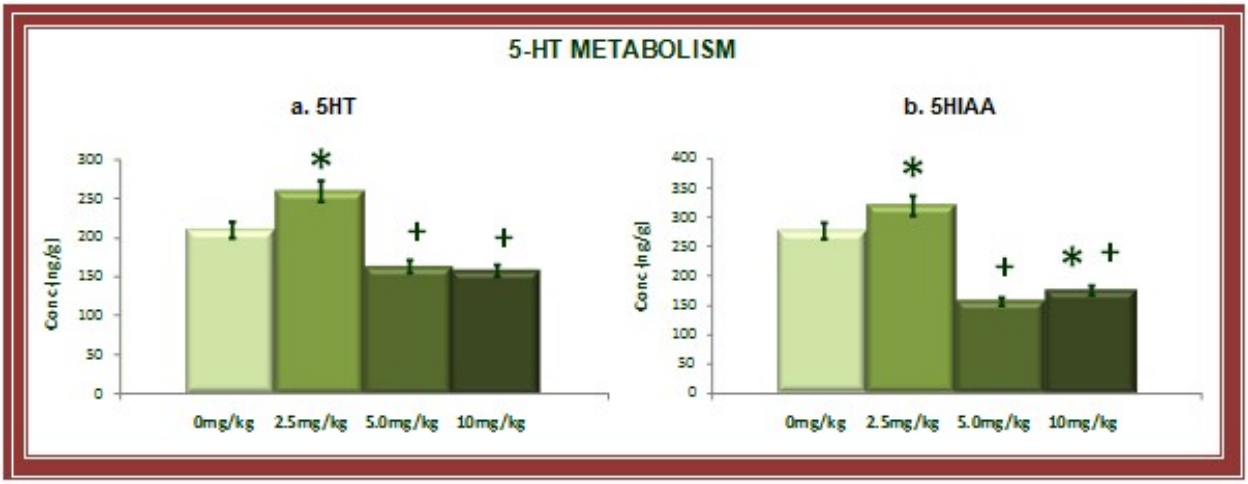

Fig. 7: Dose dependant effects of midazolam on 5-HT metabolism. Values are means±SD (n=6). Significant differences by Tukey's test: *p<0.01 from 0mg/kg midazolam administered rats; +p<0.01 from 2.5mg/kg midazolam administered rats following one-way ANOVA.

Fig. 5 shows dose dependant effects of midazolam on forced swim test. Analysis of the data on struggle time (fig. 5a) by one-way ANOVA showed significant effects of different doses of midazolam (df= 3,20; F= 14.65; p= 0.0001). Post hoc analysis by Tukey's test showed increased (p<0.01) struggle time at all three doses of midazolam as compared to 0mg/kg midazolam administered rats. While struggle time was decreased (p<0.01) at the doses of 5.0mg/kg- and 10mg/kg as compared to the midazolam dose of 2.5mg/kg.

Analysis of the data on duration of flat body posture (fig. 5b) by one-way ANOVA showed no significant effects of different doses of midazolam (df= 3,20; F= 13.87; p= 0.017). Post hoc analysis by Tukey's test showed decreased ($p<0.01$) duration of flat body posture at all three doses of midazolam as compared to 0mg/kg midazolam administered rats. While duration of flat body posture at the dose of 5.0mg/kg- and 10mg/kg was greater ($p<0.01$) as compared to 2.5mg/kg midazolam.

Fig. 6 shows dose dependant effects of midazolam on dopamine metabolism. Analysis of the data on dopamine levels (fig. 6a) by one-way ANOVA showed significant effects of different doses of midazolam (df= 3,20; F= 16.79; p= 0.0001). Post hoc analysis by Tukey's test showed increased ($p<0.01$) dopamine levels at the doses of 5.0mg/kg- and 10mg/kg midazolam as compared to 0mg/kg midazolam administered rats.

Analysis of the data on DOPAC levels (fig. 6b) by one-way ANOVA showed significant effects of different doses of midazolam (df= 3,20; F= 21.32; p= 0.0001). Post hoc analysis by Tukey's test showed increased ($p<0.01$) DOPAC levels at the doses of 2.5mg/kg- and 5mg/kg midazolam as compared to 0mg/kg midazolam administered rats. While levels of DOPAC were greater ($p<0.01$) at the doses of 5.0mg/kg as compared to 2.5mg/kg midazolam.

Analysis of the data on HVA levels (fig. 6c) by one-way ANOVA showed significant effects of different doses of midazolam (df= 3,20; F= 22.98; p= 0.0001). Post hoc analysis by Tukey's test showed increased ($p<0.01$) HVA levels at the dose of 5.0mg/kg midazolam as compared to both 0mg/kg and 2.5mg/kg midazolam.

Fig. 7 shows dose dependant effects of midazolam on 5-HT metabolism. Analysis of the data on 5-HT levels (fig. 7a) by one-way ANOVA showed significant effects of different doses of midazolam (df= 3,20; F= 34.95; p= 0.0001). Post hoc analysis by Tukey's test showed increased ($p<0.01$) 5-HT levels at the dose of 2.5mg/kg midazolam as compared to 0mg/kg midazolam administered rats. While levels of 5-HT were decreased ($p<0.01$) at the dose of 5.0mg/kg- as well as 10mg/kg midazolam as compared to 2.5mg/kg midazolam.

Analysis of the data on 5-HIAA levels (fig. 7b) by one-way ANOVA showed significant effects of different doses of midazolam (df= 3,20; F= 27.16; p= 0.0001). Post hoc analysis by Tukey's test showed increased ($p<0.01$) 5-HIAA levels at the dose of 2.5mg/kg midazolam as compared to 0mg/kg midazolam administered rats. While levels of 5-HIAA were decreased ($p<0.01$) at the dose of 10mg/kg midazolam as compared to 0mg/kg midazolam administered rats. While levels of 5-HIAA were decreased ($p<0.01$) at the dose of 5.0mg/kg- as well as 10mg/kg midazolam as compared to 2.5mg/kg midazolam.

DISCUSSION

In the present study, midazolam increased food intake at the dose of 2.5mg/kg. This could be explained in terms of anxiolytic effects of midazolam as well as stimulation of the parabrachial nucleus. Since stress-deficits include reduced food intake and midazolam, being anxiolytic (Qiu *et al.,* 2015), anxiolytic could attenuate stress-induced hypophagia. Others (Söderpalm and Berridge, 2000) have reported enhancement of food 'preference' which is mediated by the parabrachial nucleus at the same doses that increase 'wanting' for food, suggesting that palatability enhancement is one psychological mechanism through which benzodiazepine stimulation of the parabrachial nucleus causes an increase in eating behavior. It has also been suggested that midazolam activates opioidergic transmission and opioid-dependent palatability enhancement of the conditioned stimulus to eliminate conditioned aversion to a sweet taste (Yasoshima and Shimura, 2017). We monitored increased food intake (fig. 1) only at the dose of 5.0mg/kg suggesting that at this dose, midazolam could be used as an anxiolytic to attenuate a decrease in food intake. While at the dose of 5.0mg/kg and 10mg/kg, these effects were not observed. Rather, there was a decrease in food intake which could be explained in terms of reversal of therapeutic effects of midazolam at high doses.

Locomotive activities of the midazolam were also increased at the same dose (i.e., 2.5mg/kg) when monitored in both novel as well as familiar environment (fig. 2). These could be related to anxiolytic effects of midazolam. Acting as an anxiolytic, midazolam can increase amelioration. It has been reported that anxiolytic-like effects of low dose of midazolam are obtained after its administration into the amygdala, as assessed by central open field activity (Heldt and Ressler, 2006). This could be explained in terms of regional dissociation of the anxiety-related and motor-related parameters by the midazolam administration. It has also been suggested that both anxiolytic and stimulant phenomenon of benzodiazepines are regulated by different central mechanisms.

Light dark box activity was performed to access the anxiolytic effects of midazolam. Results show anxiolytic effects of midazolam at all three doses (fig. 3). Although anxiolytic effects of midazolam were prevailing at all three doses, the associated hyperlocomotive effects as observed in novel and familiar environments (fig. 2) by the low dose only, indicate the optimum dose. It has been suggested that open field, elevated plus maze and light dark box activity should be integrated in one experiment to test the wide range of unconditioned exploratory behaviors involved in anxiety (Ramos *et al.,* 2008). Taken together, results from present study suggest that midazolam at the dose of 2.5mg/kg produces optimum

therapeutic effects. In the hot plate test (fig. 4), midazolam produced analgesic effects at the low dose (2.5mg/kg). The same were not observed at high doses of midazolam i.e., 5.0mg/kg- and 10mg/kg. There is evidence that midazolam pretreatment could suppress morphine-induced withdrawal response and this is achieved by inhibiting hypersensitization of spinal cord neurons and increasing met-enkephalin and beta-endorphins levels in both pons and striatum (Jun-Li *et al.,* 2002). Analgesic effects of midazolam could also be mediated via the same mechanism (i.e., increase in met-enkephalin and beta-endorphins levels). Antidepressant effects of midazolam were monitored in forced swim test (fig. 5). Effects were more potent at the low dose (2.5mg/kg). These antidepressant- as well as anxiolytic-like effects of midazolam are reported to be mediated via translocator protein through stimulation of allopregnanolone biosynthesis (Qiu *et al.,* 2015).

An increased dopamine metabolism was observed at moderate and high doses of midazolam. While increase in dopamine metabolism was not observed (fig. 7) at low dose of midazolam (2.5mg/kg). Repetitive administration of midazolam has reinforcing effects (Kerr *et al.,* 2010) which are also associated with binge drug use and polysubstance use. These reinforcing effects of midazolam involve receptors that contain the $GABA_A$ receptors and α1 subunit are important for triggering drug-evoked synaptic plasticity in the ventral tegmental area (Tan *et al.,* 2011). Since other two doses (5.0- and 10mg/kg) of midazolam are causing potent increase in the dopaminergic release, the low dose of midazolam, i.e., 2.5mg/kg should be selected as optimum dose since it gives a good therapeutic profile besides moderately increasing dopaminergic metabolism. While serotonergic metabolism at this dose (2.5mg/kg) was increased as compared to, other two doses (fig. 7). This might be the reason for comparatively low increased in dopamine metabolism at this dose of midazolam as serotonin negatively regulates dopaminergic release.

CONCLUSION

From the results of present study we concluded that midazolam had anxiolytic, antidepressant as well as analgesic effects of midazolam were observed effectively at the dose of 2.5mg/kg. At the same dose, midazolam showed decreased increase in the dopaminergic metabolism as compared to other higher doses of midazolam (i.e., 5.0 and 10mg/kg) with accompanied increase in 5-HT metabolism. This suggests that the midazolam would be least addictive at this dose i.e., 2.5mg/kg, along with a better therapeutic profile. Since midazolam increased locomotive activities in novel as well as familiar environment, this must be further investigated that whether these increased activities are due to anxiolytic effects of midazolam or could also lead to behavioral sensitization/ tolerance upon repetitive administration. Results from hot plate test and forced swim test showed no impairment of therapeutic effects at low dose (2.5mg/kg). There was also no associated increase in dopaminergic metabolism to increase the reinforcing effects of midazolam. This must be further investigated and findings will be beneficial for improving therapeutics in epilepsy, depression and related disorders.

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