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Neurochemical and behavioral effects of fluoxetine on midazolam induce dependence in an animal model of addiction

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ABSTRACT: In the present study we have monitored effects of repeated coadministration of fluoxetine with 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 serotonin 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 effects of repeated administration of fluoxetine with midazolam. Effects of fluoxetine and midazolam coadministration were monitored on motor activities in familiar and novel environments, hot plate test, forced swim test, conditioned place preference test and levels of dopamine, 5-HT and their metabolites. Both midazolam (2.5mg/kg) and fluoxetine (1mg/kg) were administered orally for 12 days. Conditioned place preference test was performed on day 13. 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 show attenuation of midazolam-induced reinforcement upon repeated co-administration of fluoxetine. These could be implicated to increased therapeutic utility of midazolam and related benzodiazepines.

Keywords: Midazolam, fluoxetine, conditioned place preference, dopamine, serotonin.

INTRODUCTION

Results from the previous experiment showed that midazolam at the dose of 2.5mg/kg can significantly produce therapeutic effects without affecting dopamine metabolism. However repeated administration of midazolam could result in its abuse (Zaporowska-Stachowiak *et al*., 2019). While studying the dependence and drug abuse it was found out that the abuse profile of midazolam is like diazepam. Midazolam also exhibits withdrawal symptoms including insomnia and dysphoria. Therefore it is recommended that slow dose tapering method should be used for midazolam discontinuation, after extended therapy (Owusu *et al*., 2019).

There is a well-established role of serotonin in the addictive- and reinforcing effects of drugs of abuse. Drugs of abuse modulate brain serotonergic neurotransmission, which plays important role in drug addiction and vulnerability to drug relapse. Most important targets of drugs of abuse include serotonergic raphe nuclei and their forebrain projections. Serotonergic system involved mainly in impulsivity; a core behavior that contributes to the vulnerability to addiction and relapse. A dysregulation of serotonergic system is involved in comorbid mood and addictive disorders (Gómez-Coronado *et al*., 2018).

The present experiment was therefore designed to test the role of serotonin in the attenuation of addictive- and reinforcing effects of lorazepam. The drug was injected at a dose of 1.0 mg/kg. Fluoxetine, at this dose, preferentially increases the release of serotonin in synapse, resulting in its functional availability. It was hypothesized that enhanced release of 5-HT, could normalize midazolam-induced addictive- and reinforcing effects.

MATERIALS AND METHODS

Animals

Male Albino Wistar rats (weighing 180-220g) were purchased from HEJ Research Institute of Chemistry, Karachi and 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, 7 days before the start of experiment so that they could become familiar to the environment. Animals were tested in light phase. Before starting the experiment, ratswere accustomed to various handling procedures in order to nullify the psychological affliction of environment. All protocols for experimentation were approved and performed in strict accordance with National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985) and the Institutional Animal Ethics

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Drug

Apomorphine–HCl (Sigma, St. Louis, USA) was dissolved in saline and injected intra-peritoneally at a dose of 1.0 mg/kg. Drug solution was freshly prepared before each experiment. Control animals were injected with saline (0.9% NaCl) at a dose of 1.0 ml/kg.

Experimental protocol

Twenty four male Albino Wistar rats were randomly divided into four groups, each containing six rats: (i) saline-saline (ii) saline-fluoxetine (iii) saline-midazolam and (iv) midazolam-fluoxetine injected rats. On day 0, body weights of rats were recorded and food pallet were provided in the cages. Basal values of Skinner's box-, open field- and conditioned place preference test were monitored. One compartment of the conditioned place preference apparatus was paired with midazolam while other was paired with saline. On day 1,3,5,7,9,11 all rats were injected with saline (1ml/kg) and sequestered in the saline paired compartment of conditioned place preference apparatus for 10 minutes. Cage crossings in saline paired compartment of conditioned place preference apparatus were also recorded. On day 2,4, 6, 8, 10 and 12 rats were injected with midazolam (2.5 mg/kg) or saline respectively and sequestered in midazolam paired compartment of conditioned place preference apparatus for 10 minutes. Cage crossings in midazolam paired compartment of conditioned place preference apparatus were also recorded. Fluoxetine was injected daily at the dose of 1mg/kg. Activities in open field apparatus were monitored on day 2 and 12**.** On Day13, partition between saline- and midazolam paired compartments was removed and number of entries as well as time spent in saline- as well as midazolam paired compartments were recorded. Animals were decapitated and brain samples were collected for neurochemical analysis by HPLC-EC (High Performance Liquid Chromatography with Electrochemical Detection).

Activity in novel environment (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 injected 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 and Haleem, 2019).

Activity in a familiar environment (Skinner's box)

Transparent Perspex cages (26×26×26 cm) with sawdust 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 injected with drug or vehicle. Numbers of cage crossings were counted 10 min post-injection for 20 min (Ikram & Haleem, 2011; Ikram *et al*., 2011).

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−TO)/ (Tmax−TO) × 100]. Where Tx= individual latency time determined at appropriate intervals after administration of the examined analgesics; TO = was the individual latency time determined before analgesic injection and Tmax = 20sec. (Ikram *et al*., 2020).

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 6min-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 and Haleem, 2017).

Conditioned place preference test

Place conditioning was conducted in a three-compartment apparatus with an unbiased design. The compartments were separated by sliding guillotine doors. The middle (shuttle) compartment (10×26×26 cm3) had a smooth floor. The end (preference) compartments (26×26×26 cm3 each) provided distinct contexts, with one compartment having black horizontal stripes on side walls and grid rod floor. The other compartment had vertical stripes and stainless steel mesh floor.

Pre-conditioning place preference

Rats were tested for CPP using a 13-day procedure. On day 1 all animals were tested, before any treatment, to establish pre-conditioning responses and any possible bias for either compartment. Pre-conditioning place preference testing involved placing individual animals in the central shuttle compartment; after 10 s the guillotine doors were removed and animal allowed exploring all three compartments for a time period of 10 min. The time spent in end (preference) compartments were recorded. The animals exhibited no preference for either compartment.

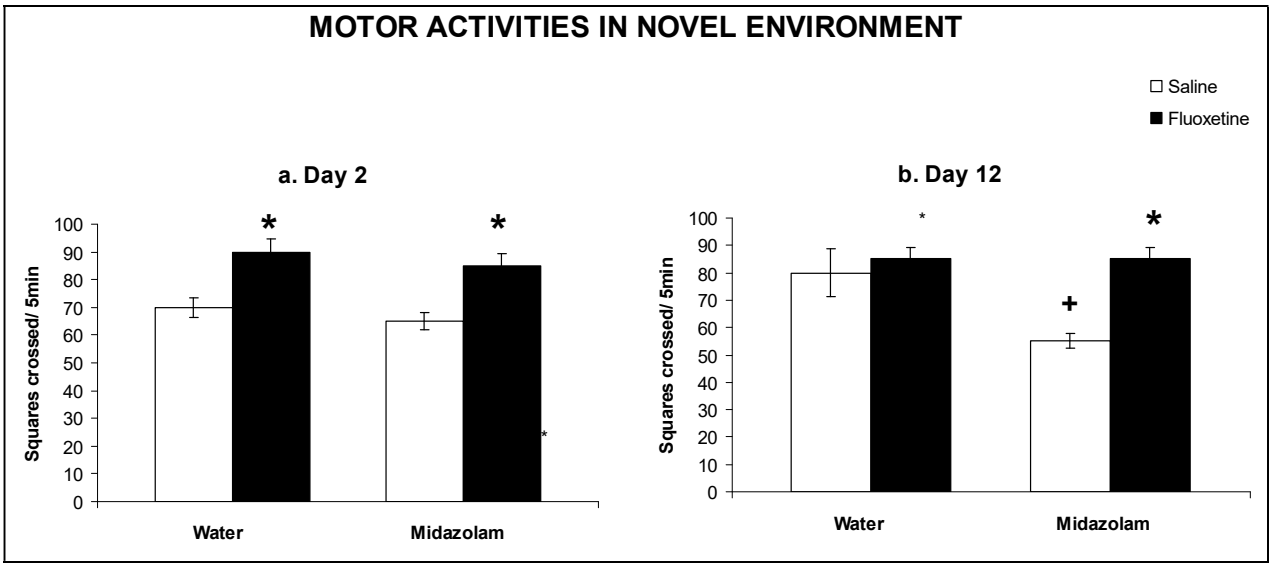

Fig. 1: Effects of midazolam, fluoxetine and their co-administration on activity in novel environment of an open field. Values are means ±SD (n= 6). Significant differences between groups by Tukey's test: *p<0.01 as compared to respective saline injected-; +p<0.01 as compared to respective water treated rats following two-way ANOVA.

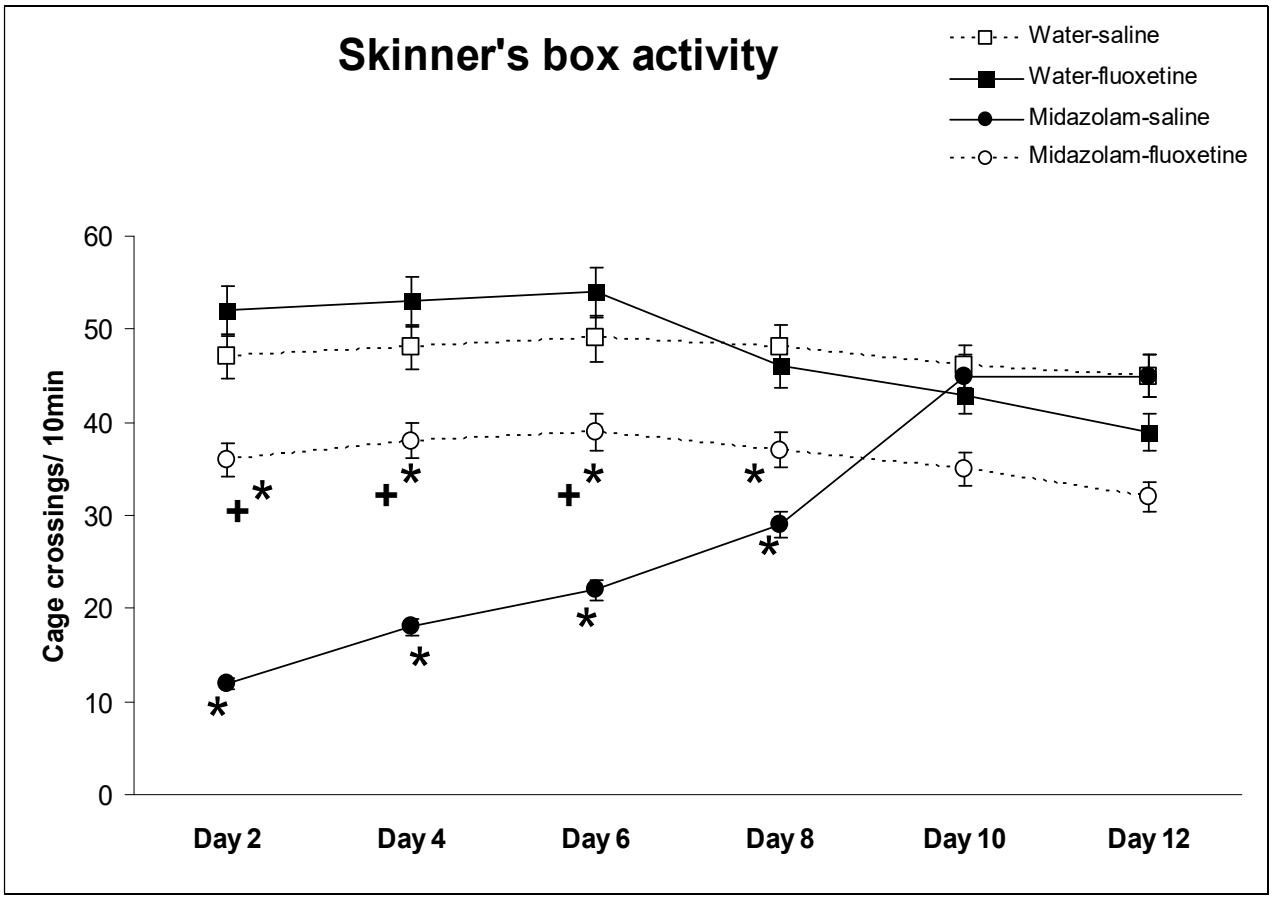

Fig. 2: Effects of midazolam, fluoxetine and their co-administration on motor behavior in a familiar environment. Values are means ±SD (n= 6). Significant differences between groups by Tukey's test: *p<0.01 as compared to respective water injected rats following three-way ANOVA.

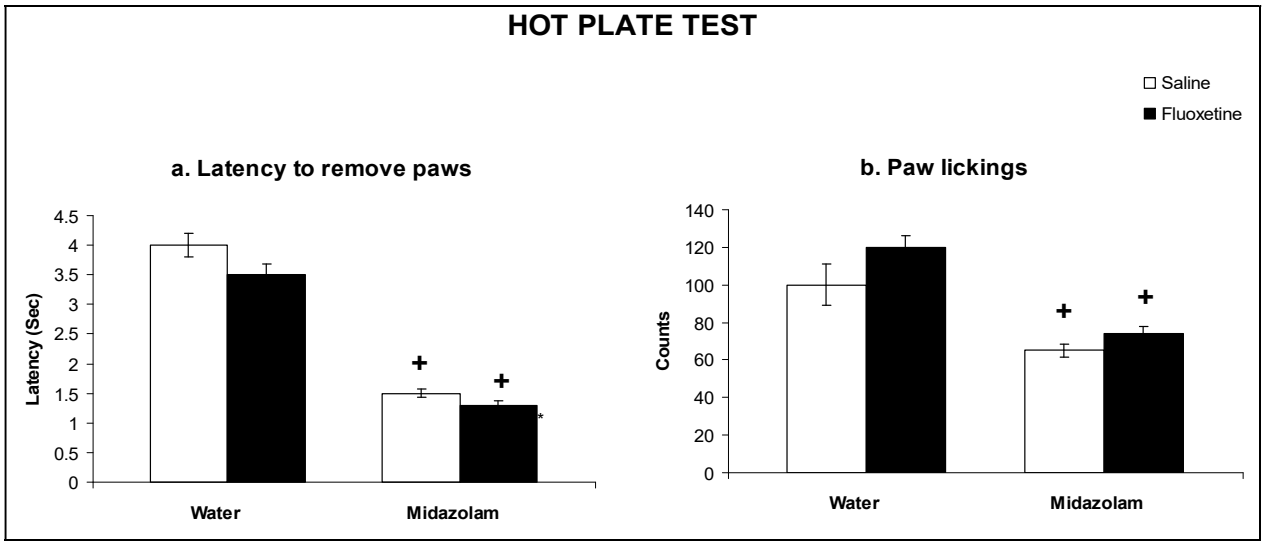

Fig. 3: Effects of midazolam, fluoxetine and their co-administration on hot plate test. Values are means ±SD (n= 6). Significant differences between groups by Tukey's test: +p<0.01 as compared to respective water treated rats following two-way ANOVA.

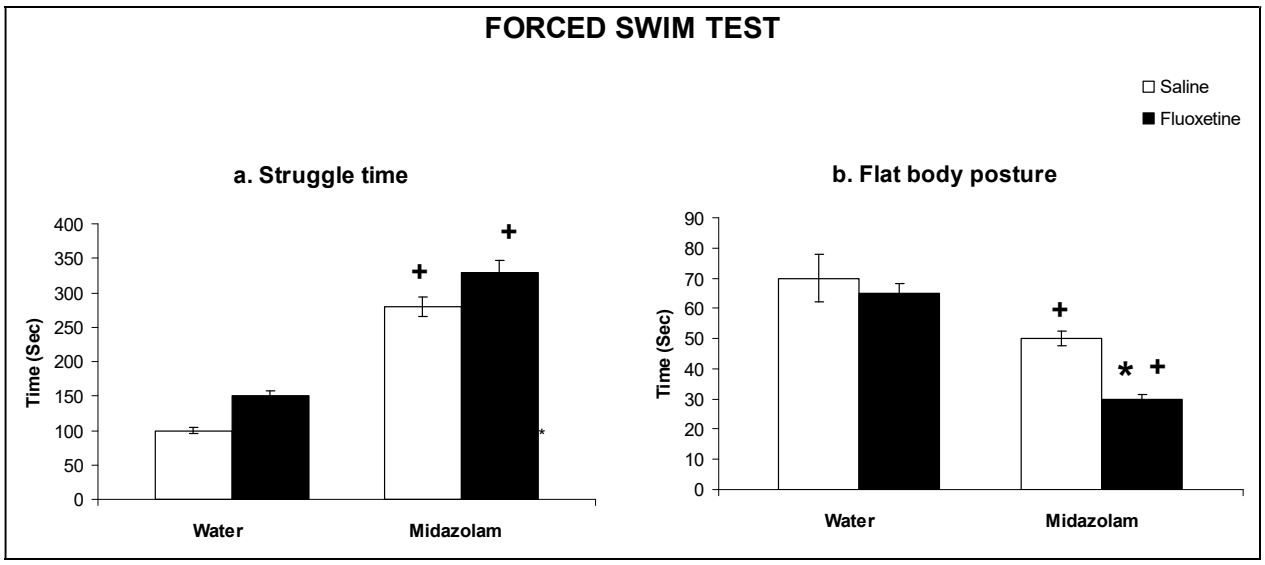

Fig. 4: Effects of midazolam, fluoxetine and their co-administration on forced swim test. Values are means ±SD (n= 6). Significant differences between groups by Tukey's test: *p<0.01 as compared to respective saline injected-; +p<0.01 as compared to respective water treated rats following two-way ANOVA.

Drug conditioning

In 12 days animals went through conditioning (one session per day) in which they were confined to either the horizontal or vertical strip compartment by raising the respective guillotine door. On days 2, 4, 6, 8, 10 and 12 animals were injected with saline (1 ml/kg at 9:00–11:00 h) and placed immediately in the assigned 'Non-Drug' compartment for 30 min. On every other day, i.e. days 3, 5, 7, 9, 11 and 13 animals of each group were injected (as assigned for group) with drug or saline and placed immediately in the 'Drug' compartment for 30 min Video recording was used to determine motor behavior during drug conditioning phase. Animals confined to a compartment were moving across the compartment. Activity scores were counted as number of compartment crossings for 10 min starting 5 min post-injection. The small area (26×26×26 cm3) of the compartment enabled giving a score of 1 for one crossing.

Post-conditioning test

The post-conditioning testing was carried out on day 14. As in the pre-conditioning phase, both guillotine doors were raised and the animals were allowed free access to all compartments for 10 min and time spent in 'Non-Drug' and 'Drug' assigned compartment was monitored to determine place preference.

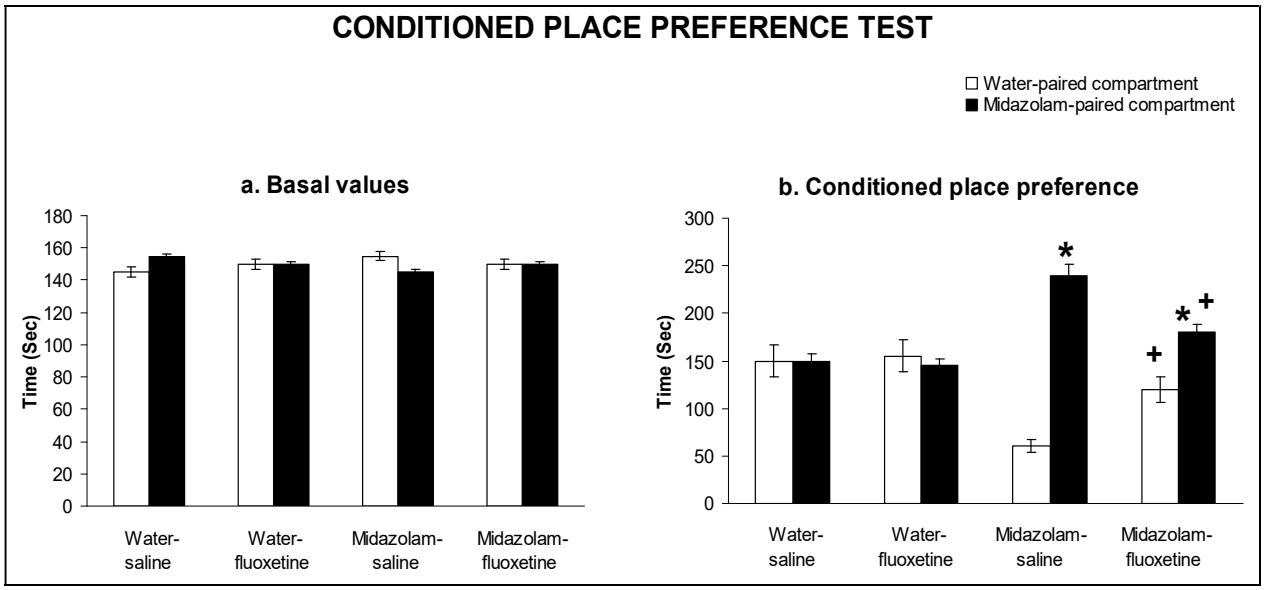

Fig. 5: Effects of midazolam, fluoxetine and their co-administration on conditioned place preference (time spent in compartments). Values are means ±SD (n= 6). Significant differences between groups by Tukey's test: *p<0.01 as compared to respective time spent in water paired compartment-; +p<0.01 as compared to respective saline injected rats following two-way ANOVA.

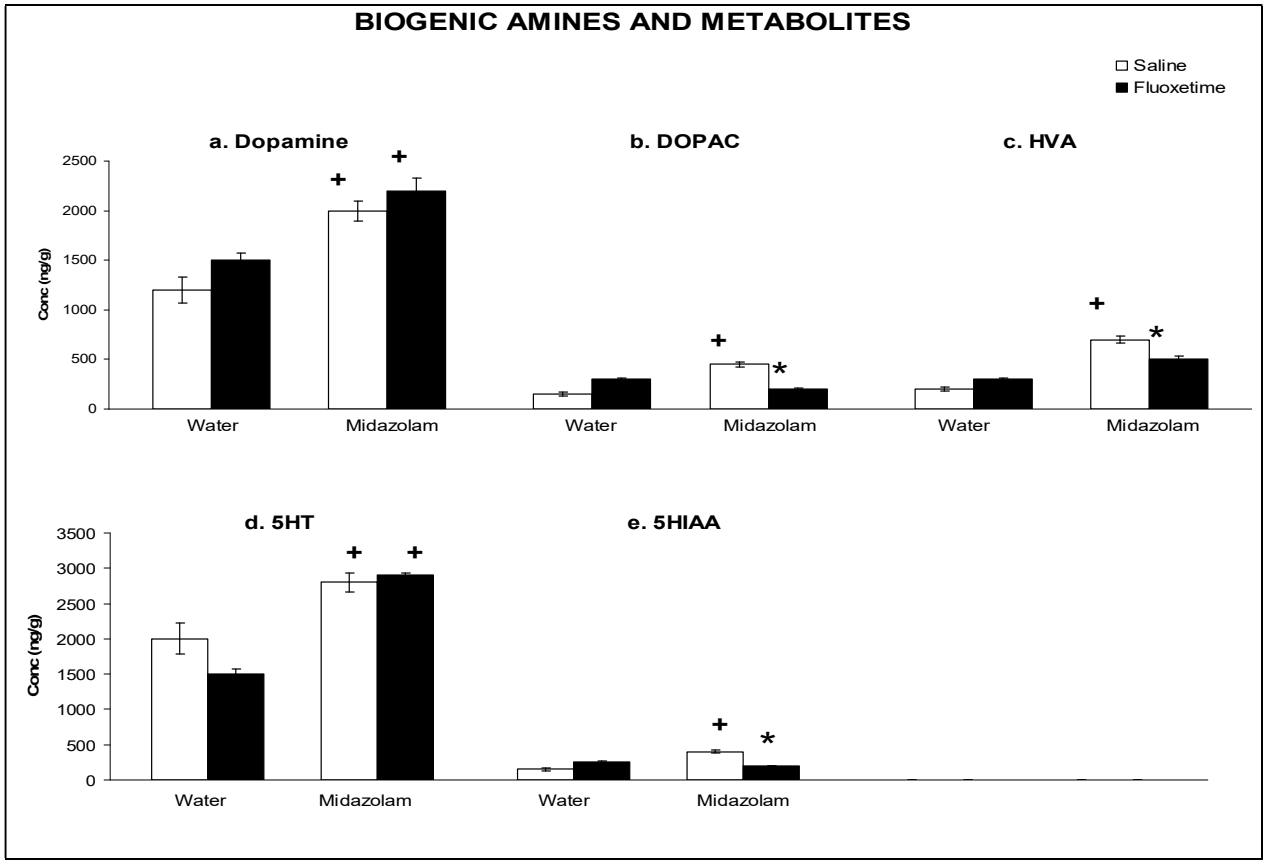

Fig. 6: Effects of midazolam, fluoxetine and their co-administration on biogenic amines and metabolites. Values are means ±SD (n=6). Significant differences between groups by Tukey's test: *p<0.01 as compared to respective saline injected-; +p<0.01 as compared to respective water treated rats following two-way ANOVA.

Dissection of rat whole brain

Dissection procedure was essentially same as described earlier (Ikram *et al*., 2007). After decapitation, fresh brain was washed with ice-cold saline and stored at -70ºC until neurochemical analysis by HPLC-EC (Ikram *et al*., 2018).

Neurochemical analysis by HPLC-EC

HPLC-EC determination was carried out as described before (Mirza *et al*., 2013; Ikram *et al*., 2012). A 5µ Shim-pack ODS separation column of 4.0 mm internal diameter and 150mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer of PH 2.9 at an operating potential of 2000-3000 psi on Schimadzu HPLC pump. Electrochemical detection was achieved on Schimadzu LEC 6A detector at an operating potential of +0.8V (Ikram *et al*., 2012). Analysis of biogenic amines and metabolites (DA: dopamine; DOPAC: 3,4-dihydroxyphenylacetic acid; HVA: homovanillic acid.; 5HT: 5-hydroxytryptamine; 5HIAA: 5-hydroxyindoleacetic acid).

STATISTICAL ANALYSIS

Results are given as means±SD. Analysis of the data was performed by two-way- or three-way ANOVA, wherever applicable; using SPSS ver 19. Post hoc comparisons were done by Tukey's test. Values of $p<0.05$ were considered statistically significant.

RESULTS

Fig. 1 shows effects of midazolam, fluoxetine and their coadministration on motor activity in novel environment of an open field. Analysis of the data on day 2 (fig. 1a) by two-way ANOVA showed significant effects of midazolam (df= 1,20 ; F= 34.76; p= 0.0001), fluoxetine (df= 1,20 ; F= 74.81; p= 0.0001) as well as interaction between the two (df= 1,20 ; F= 96.11; p= 0. 0001). Post-hoc analysis by Tukey's test showed increased ($p<0.01$) squares crossed in water-fluoxetine- as well as midazolam-fluoxetine injected rats as compared to respective saline injected rats. Analysis of the data on day 12 (fig. 1b) by two-way ANOVA showed significant effects of fluoxetine (df= 1,20 ; F= 54.76; p= 0.0001). Effects of midazolam (df= 1,20 ; F= 83.72; p= 0.0001) as well as interaction between the two (df= 1,20 ; F= 93.56; p= 0.0001) were significant. Post-hoc analysis by Tukey's test showed decreased ($p<0.01$) squares crossed by midazolam-saline injected rats as compared to respective water treated rats. While squares crossed by midazolam-fluoxetine injected rats were increased ($p<0.01$) as compared to to respective saline injected rats.

Fig. 2 shows effects of midazolam, fluoxetine and their coadministration on motor behavior in a familiar environment. Analysis of the data by three-way ANOVA showed significant effects of midazolam (df=1,120; F= 82.36; p=0.0001) but not that of fluoxetine (df=1,120; F= 54.87; p=0.0001) as well as interaction between the two (df=1,120; F=76.29; p=0.0001). Effect of repeated measures (days) (df=5,120; F=102.5; p=0.0001) was significant. Interactions of midazolam*repeated monitoring (df=5,120; F=54.76; p=0.0001), repeated monitoring* fluoxetine (df=5,120; F=92.76; p=0.0001) and midazolam*fluoxetine*repeated monitoring (df= 5,120; F= 65.82; p=0.0001) were all significant. Post-hoc analysis by Tukey's test showed decreased ($p<0.01$) activity in midazolam-saline injected rats on day 2 till day 8 but not later on. This decreased activity was attenuated in by the co-administration of fluoxetine ($p<0.01$).

Fig. 3 shows effects of midazolam, fluoxetine and their coadministration on hot plate test. Analysis of the data on latency to remove paw (fig. 3a) by two-way ANOVA showed non-significant effects of fluoxetine (df=1,20; F= 1.94; p=0.065), midazolam (df=1,20; F=3.29; p=0.023) as well as interaction between the two (df= 1,20; F=4.98; p=0.054). Post-hoc analysis by Tukey's test showed no significant differences among groups. Analysis of the data on paw lickings (fig. 3b) by two-way ANOVA showed significant effects of fluoxetine (df=1,20; F=258.14; p= 0.0001), midazolam (df=1,20; F=139.58; p=0.0001) as well as interaction between the two (df= 1,20; F= 71.69; p=0.0001). Post-hoc analysis by Tukey's test showed decreased ($p<0.01$) number of paw lickings in midazolam-saline as well as midazolam-fluoxetine injected rats as compared to their respective water treated rats.

Fig. 4 shows effects of midazolam, fluoxetine and their coadministration on forced swim test. Analysis of the data on struggle time (fig. 4a) by two-way ANOVA showed significant effects of fluoxetine (df= 1,20; F= 67.54; p= 0.0001), midazolam (df= 1,20; F= 49.76; p= 0.0001) as well as interaction between the two (df= 1,20; F= 196.43; p= 0.0001). Post-hoc analysis by Tukey's test showed increased ($p<0.01$) struggle time in midazolam-saline as well as midazolam-fluoxetine injected rats as compared to their respective water treated rats. Analysis of the data on flat body posture (fig. 4b) by two-way ANOVA showed significant effects of fluoxetine (df= 1,20; F= 87.54; p= 0.0001), midazolam (df= 1,20; F= 87.45; p= 0.0001) as well as interaction between the two (df= 1,20; F= 102.54; p= 0.0001). Post-hoc analysis by Tukey's test showed decreased ($p<0.01$) time to maintain flat body posture in midazolam-saline as well as midazolam-fluoxetine injected rats as compared to their respective water treated rats. This was more decreased ($p<0.01$) in midazolam-fluoxetine injected rats.

Fig. 5 shows effects of midazolam, fluoxetine and their co-administration on conditioned place preference. Analysis of the data on basal values of time spent in compartments (fig. 5a) by two-way ANOVA showed non-significant effects of fluoxetine (df= 1,20 ; F= 0.387; p= 0.064), midazolam (df= 1,20 ; F= 1.765; p= 0.06) as well as interaction between the two (df= 1,20 ; F= 0.549; p= 0.932). Post-hoc analysis by Tukey's test showed no differences among the groups. Analysis of the data on conditioned place preference (fig. 5b) by two-way ANOVA showed significant effects of fluoxetine (df= 1,20; F= 89.31; p= 0.0001). Effects of midazolam (df= 1,20; F= 83.17; p= 0.0001) as well as interaction between the two (df= 1,20; F= 80.57; p= 0.0001) were all significant. Post-hoc analysis by Tukey's test showed increased ($p<0.01$) time spent in midazolam-paired compartment by midazolam-saline- and midazolam-fluoxetine treated rats as compared to time spent in the water-paired compartment respectively. While this was attenuated ($p<0.01$) in midazolam-fluoxetine injected rats.

Fig. 6 shows effects of midazolam, fluoxetine and their coadministration on biogenic amines and metabolites. Analysis of the data on dopamine levels (fig. 6a) by two-way ANOVA showed significant effects of fluoxetine (df= 1,20; F= 75.51; p= 0.0001), midazolam (df= 1,20; F= 76.69; p= 0.0001) as well as interaction between the two (df= 1,20; F= 78.56; p= 0.0001). Post-hoc analysis by Tukey's test showed increased ($p<0.01$) dopamine levels in midazolam-saline as well as midazolam-fluoxetine injected rats. Analysis of the data on DOPAC levels (fig. 6b) by two-way ANOVA showed significant effects of fluoxetine (df= 1,20; F= 92.54; p= 0.0001), midazolam (df= 1,20; F= 66.85; p= 0.0001) as well as interaction between the two (df= 1,20; F= 67.73; p= 0.0001). Post-hoc analysis by Tukey's test showed increased ($p<0.01$) DOPAC levels in midazolam-saline injected rats. While levels of DOPAC were decreased in midazolam-fluoxetine injected rats. Analysis of the data on HVA levels (Fig. 6c) by two-way ANOVA showed significant effects of fluoxetine (df= 1,20; F= 67.54; p= 0.0001), midazolam (df= 1,20; F= 45.22; p= 0.0001) as well as interaction between the two (df= 1,20; F= 45.67; p= 0.0001). Post-hoc analysis by Tukey's test showed increased ($p<0.01$) HVA levels in midazolam-saline injected rats. While levels of DOPAC were decreased in midazolam-fluoxetine injected rats.

Analysis of the data on 5-HT levels (Fig. 6d) by two-way ANOVA showed significant effects of fluoxetine (df= 1,20; F= 58.54; p= 0.0001), midazolam (df= 1,20; F= 45.33; p= 0.0001) as well as interaction between the two (df= 1,20; F= 54.65; p= 0.0001). Post-hoc analysis by Tukey's test showed increased ($p<0.01$) 5-HT levels in midazolam-saline as well as midazolam-fluoxetine injected rats. Analysis of the data on 5-HIAA levels (Fig. 6e) by two-way ANOVA showed significant effects of fluoxetine (df= 1,20; F= 45.22; p= 0.0001), midazolam (df= 1,20; F= 21.33; p= 0.0001) as well as interaction between the two (df= 1,20; F= 56.42; p= 0.0001). Post-hoc analysis by Tukey's test showed increased ($p<0.01$) 5-HIAA levels in midazolam-saline injected rats. While levels of 5-HIAA were decreased in midazolam-fluoxetine injected rats.

DISCUSSION

Present study shows the effect of midazolam, fluoxetine and their coadministration on food intake and growth rate in an animal modal of addiction. There is no significant difference in cumulative food intake and on growth rate. It has been reported that neonatal exposure to fluoxetine promotes reduction in body weight, disturbs the serotonin hypophagic response, and increases the serotonin and serotonin transporters in young animals. This may result in disturbing the inhibitory action of serotonin on food intake (Pinheiro *et al*., 2019).

Previous studies have shown that administration of fluoxetine decreased latency to move in open field and increased number of squares crossed (Gray and Hughes, 2015). Activities in open field were monitored after first and sixth injection of midazolam to maintain the novel effects of environment as daily monitoring in the open field would result familiarization to environment. It was found that midazolam decreased locomotor activity in novel environment while locomotor activity in midazolam-fluoxetine injected rats were increased. In the open-field test, coadministration of fluoxetine with benzodiazepines such as diazepam, increases the exploration of the novel area of open field (Zhao *et al*., 2018).

Present study showed significant decrease of motor activity in midazolam treated rats which was attenuated by fluoxetine. Midazolam reduced the locomotor activity and hence the exploratory behavior of the rat. Motor activity in midazolam treated rats was decreased from day 2 to day 8 but not later-on. This is in accordance with previous studies suggesting that the mean activity is decreased at low and moderate doses of midazolam (Charalambous *et al*., 2017).

In conditioned place preference basal values of time spent in compartment show no significant difference among groups. ome anesthetic agents, such as benzodiazepines, have the potential to be abused, and they can cause a pathological dependence in animals and humans over time (Collins *et al*., 2020). In present study of conditioned place preference time spent in midazolam paired compartment were increased while this effect was attenuated by fluoxetine. Midazolam motivated the animals to spent more time in a compartment that had been positively reinforced by the midazolam effect.

Latency to remove paws in hot plate test showed no significant difference among mean in present study. Intraperitoneally administered midazolam had precautionary analgesic effects on acute thermal., and inflammatory induced pain in rats (Zhang *et al*., 2020). Present study showed that midazolam decreased the number of paws licking in rats. Other study which was performed to investigate antinociceptive effects of different types of nociception in mice. They concluded that systemically administered midazolam had antinociceptive effects on acute thermal., acute mechanical and acute inflammatory-induced nociception in mice. The antinociceptive potency of midazolam was the same for both acute thermal-induced nociception and mechanical-induced nociception (Chiba *et al*., 2009). Previous studies reported that midazolam did not induce any detectable reduction in motor response in rats (So *et al*., 2014). Preemptive analgesic effect was obvious with NSAIDs due to their mode of action, competing with arachidonic acid for binding to cyclooxygenase and decreasing the formation of prostaglandins (Nkanu *et al*., 2019).

Present study showed increased struggling time in midazolam as well as fluoxetine injected rats. However, there was decreased in time to maintain flat body posture in midazolam as well as midazolam-fluoxetine injected rats. Previous studies showed that benzodiazepine agonist reduced the immobility time and increase in struggling time in forced swim test suggesting an antidepressant-like profile for benzodiazepine (Duan *et al*., 2019). The anti-depressant like effects of benzodiazepine agonist showed that acute stress promotes a marked reduction of the density of peripheral type benzodiazepine receptors. It has also been suggested that peripheral benzodiazepine receptors play a role in physiological adaptation to stress, anxiety and depression (Okazaki and Glass, 2017).

Analysis of the data showed significant increase in dopamine levels in midazolam injected rats, which was attenuated by fluoxetine administration. Benzodiazepines are widely used in clinical setup but will lead to addiction in vulnerable individuals. Addictive drugs increase the levels of dopamine. Benzodiazepines increase firing of dopamine neurons of the ventral tegmental area through the positive modulation of GABAA receptors in nearby interneurons. Such disinhibition, which relies on α1-containing GABA-A receptors expressed in these cells, triggers drug-evoked synaptic plasticity in excitatory afferents onto dopamine neurons and underlies drug reinforcement (Steketee and Liu, 2018). Dopaminergic neurotransmission in prefrontal cortex is also enhanced by aversive stimuli. Whereas the levels of DOPAC and HVA were increased in midazolam-saline injected rats but decreased in midazolam-fluoxetine injected rats. Other studies showed that Chronic variate stress increased levels of DOPAC whereas in the hypothalamus, levels of HVA and DOPAC were decreased, as well as the DOPAC/DA ratio, while no difference was found in amygdala (Gamaro *et al*., 2003). Previous studies were observed increased levels of DOPAC in hippocampus and frontal cortex, suggesting an increased catabolism of DA to DOPAC by intraneuronal monoamine oxidase, which may reflect increased metabolism of dopaminergic neurons in these structures. This finding agrees with other reports in the literature which report that exposure to mild stress increases the dopaminergic activity in several brain regions (Pruessner *et al*., 2004).

Benzodiazepines have been reported to reduce 5-HT turnover after acute and chronic treatment (Benítez *et al*., 2008), suggesting that the decrease in 5-HT turnover reflects reduced activity of 5-HT-containing neurones and thus decreased release of 5-HT. A more recent study using *in vivo* microdialysis showed that both systemic and local administration of benzodiazepine agonists (diazepam and flurazepam) inhibited the release of 5-HT from the ventral hippocampus, and this effect could be reversed by administration of the benzodiazepine antagonist flumazenil (Sakaue *et al*., 2001). However, Vogel type conflict test, significant increase of 5-HT release was monitored in the dorsal hippocampus, while pre-treatment with Midazolam suppressed the dosage-dependently increased 5-HT release and attenuated conflict behaviour. These findings suggest that the activation of serotonergic neuronal activity in the dorsal hippocampus is linked to mediation of anxiety-related behaviour (Rex *et al*., 2005). In present study level of 5-HT were increased in midazolam-saline as well as in the midazolam-fluoxetine injected rats. However, 5-HIAA level were increase in midazolam-saline injected rats but decreased in midazolam-fluoxetine injected rats. This suggests involvement of fluoxetine-induced increased 5-HT in the anxiolytic effects of midazolam as well as fluoxetine.

CONCLUSION

Results from the present study suggest attenuation of midazolam-induced reinforcement upon repeated co-administration of fluoxetine. Since repeated administration of increases serotonergic availability, it is concluded that midazolam-induced increased dopaminergic neurotransmission could be normalized by fluoxetine co-administration. It is therefore suggested that co-administration of fluoxetine with midazolam can attenuate its addictive effects without affecting its therapeutic profile.

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