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Antagonization of monoamine reuptake transporters by agmatine improves anxiolytic and locomotive behaviors commensurate with fluoxetine and methylphenidate

Hira Rafi*, Hamna Rafiq and Muhammad Farhan

Abstract

Background: Agmatine (AGM) is known for its protective effects including neuroprotection, nephroprotection, gastroprotection, cardioprotection, and glucoprotection. Studies have validated the neuroprotective role of AGM as antidepressant, anxiolytic, locomotive, and antipsychotic agent in psychopathologies. Fluoxetine (FLX) is the most extensively prescribed antidepressant while methylphenidate (MPD) is the most frequently prescribed psychoactive stimulant for ADHD (attention deficit hyperactivity disorder) treatment worldwide. The mechanism of action of FLX and MPD involves reuptake inhibition of serotonin and dopamine and norepinephrine at presynaptic transporters. Present study was designed to determine the safety and efficacy of AGM administration along with conventional antidepressant and psychostimulative drugs. The study also aimed to establish underlying mechanism of action of AGM at monoamine reuptake transporters.

Results: AGM significantly ameliorated locomotion in activity box and open field while anxiolytic behaviors in light/dark transition box and EPM were also improved ($p<0.01$). The growth and appetite of animals were enhanced along with antidepressive behavior in FST ($p<0.01$). Moreover, co-administration of AGM with FLX or MPD improved rats' behaviors as compared to single AGM administration.

Conclusion: Present study determined the significant anxiolytic, locomotor, and antidepressive effects of AGM compared with FLX and MPD. The study also showed improved behaviors of rats treated with combined doses of AGM with FLX or MPD along with food intake and body weights. This study has also proposed the potential mechanism of action of AGM at monoamine receptors that may lead to inhibition of monoamine reuptake transporters that may lead to increase in 5-HT, D, and NE concentrations at synaptic level.

Keywords: Agmatine, Fluoxetine, Methylphenidate, Behaviors, Monoamines

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1 Background

Agmatine (AGM) is an ubiquitary compound synthesized by the action of arginine decarboxylase (ADC) enzyme from the precursor arginine. It is widely known to exert its neuroprotective effects during neurodegeneration processes such as apoptosis, oxidative stress, inflammation, brain edema, and many other neurological diseases [1–3]. Endogenous agmatine inside the brain reveals anxiolytic, antidepressive, anticonvulsive, antinociceptive, and neuroprotective effects [4–6]. Furthermore, AGM levels exhibit potential incorporation in aging progression and memory- and cognition-related structure of the brain [7]. AGM is considered as an eligible candidate that is capable of modulating various target sites simultaneously and appropriate therapeutic substance for numerous disorders [8].

Fluoxetine (FLX) is a selective serotonin inhibitor class of antidepressant possessing high affinity for 5-HT transporter thus tempering synaptic serotonin levels [9]. It is a widely known antidepressant used to treat depression, anxiety, and other personality disorders [10]. The mechanism involves inhibition of serotonin reuptake into presynaptic terminal that leads to increase extracellular serotonin levels [11]. Various preclinical studies have demonstrated the antidepressant effects of FLX in animal models such as reserpine model, chronic mild stress, FST model, restraint model, and olfactory bulbectomy. FLX has also been assessed in anxiety, cognition, and various other psychological models [12]. FLX inhibits serotonin reuptake and affects sexual behavior [13] thus produces antidepressant effects. Long-term FLX treatment induces a constant increase in serotonin levels in various regions of the brain including the striatum, diencephalon, hippocampus, and frontal cortex [14, 15] while no altering difference on noradrenaline and dopamine were observed [16]. Chronic FLX treatment prompted neurogenesis in subgranular zone of hippocampus, increased cell proliferation, and long-lasting survival of new granule neurons [17–19].

Methylphenidate (MPD) is the most widely prescribed drug for attention deficit hyperactivity disorder (ADHD) treatment [20]. Studies on animal models have found MPD-mediated effects on neurochemistry [21], behavior [22], development [23], self-administration, and cross sensitization [24]. MPD acts predominantly through dopaminergic pathway and slightly alters norepinephrine receptors. It increases dopamine and norepinephrine levels in synapse by inhibiting reuptake of these neurotransmitters [25, 26]. Study revealed increased activation of the frontal cortex, striatum, and parietal areas and reduced activation of basal cingulate in ADHD [27]. It has also been observed that connectivity reduced between ventral striatum and inferior frontal cortex [27].

The study was designed to determine the safety and efficacy of AGM administration along with FLX and MPD on animal's moods and behaviors. The study also aimed to determine the potential mechanism of action of AGM at monoamine reuptake transporters as described in Fig. 1.

2 Methods

2.1 Animal and drug administration

Six- to 8-week-old male albino Wistar rats (120–180 g) were purchased from Dow University of Health and Sciences, Karachi. Rats were acclimatized for 3 days in separate cages under 12:12 h light/dark cycle, temperature 25 ± 1 °C, and free access to water and food. All experiments were approved by the Institutional Advanced Studies and Research Board (BASR/03367/Sc.) and performed in strict accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publication no. 85–23, revised 2011). Agmatine sulfate salt and fluoxetine hydrochloride were purchased from Merck (Sigma-Aldrich). Methylphenidate (Ritalin-Novartis) along with AGM and FLX were dissolved in distilled water and administered orally by stainless steel oral gavage. Water was given as vehicle to control animals. After 28 days of treatment, animals were humanly decapitated for brain and blood sample collection.

2.2 Experimental design

Thirty-six male rats were divided randomly into 6 groups each that receive the following treatments: (a) water (control), (b) agmatine (100 mg/Kg p.o.), (c) FLX (20 mg/Kg (p.o.), (d) MPD (10 mg/Kg p.o.), (e) AGM+FLX (100+20 mg/Kg), (f) AGM+MPD (100+10 mg/Kg) for 28 days daily. All behaviors were recorded 24 h post drug administration as given in Fig. 2.

2.3 Behavioral estimations

2.3.1 Body weight and food intake

Weighed amount of fresh standard rodent diet cubes were given to each animal separately. Leftover diet in cage hooper was weighed so that effect of drugs on feeding and satiety can be observed. Body weights of each rat were observed separately to determine the effect of AGM and other drugs on growth of animals.

2.3.2 Light dark activity

Light/dark transition test is known for analyzing anxiety in rodents. The apparatus consists of two chambers of equal size made up of transparent and black opaque Plexiglas (20 × 30 × 30 cm). The partition dividing the compartment has a 10 × 10-cm door in the middle of the wall through which rat can move from one chamber to another. Single animal was positioned in the mid of bright chamber fronting towards opposite side from the

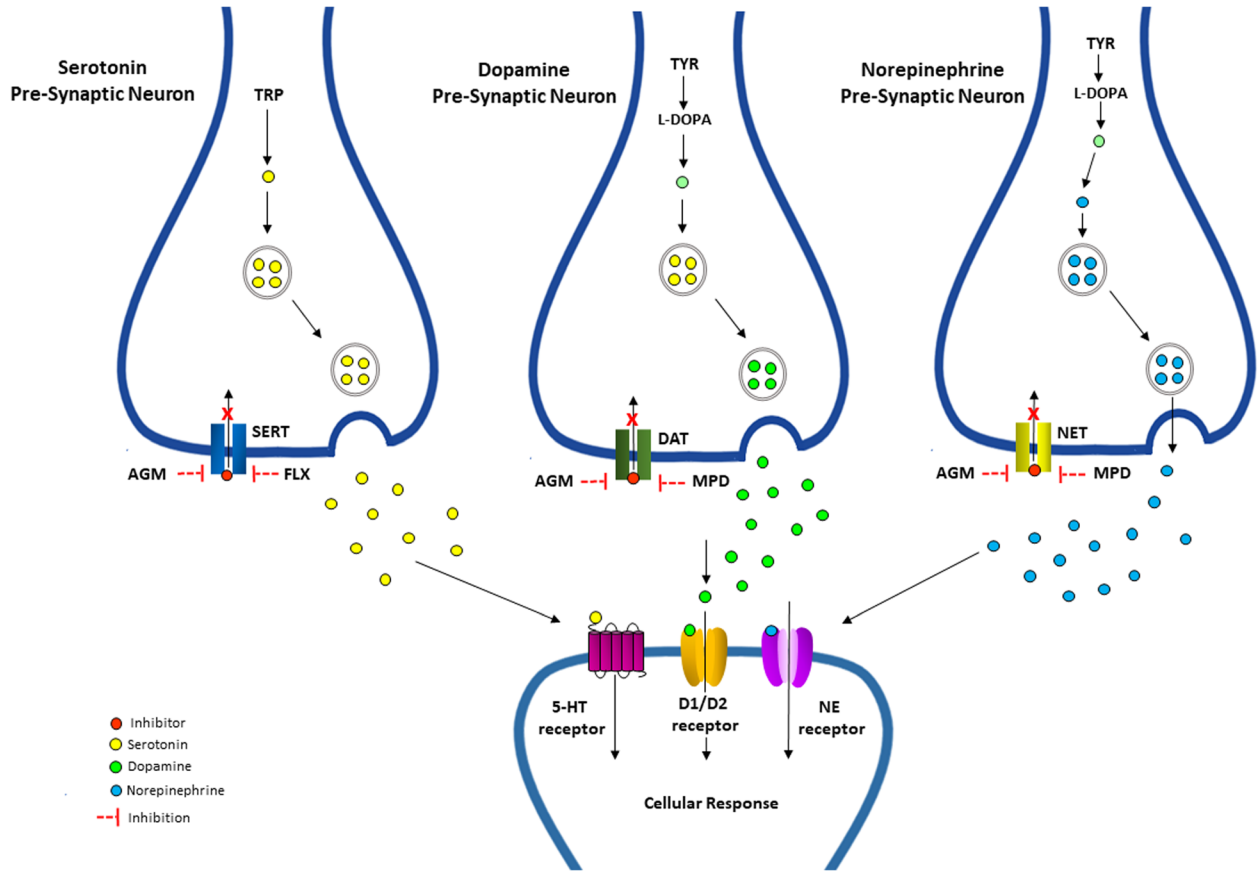

Fig. 1 Potential mechanism of action of agmatine as monoamine reuptake inhibitor. Agmatine (AGM) may block serotonin reuptake transporter (SERT) found on presynaptic neuron. This leads to increase in serotonin (5-HT) levels at synaptic cleft thus induces cellular response at post-synaptic neuron. AGM may also inhibit dopamine reuptake transporter (DAT) and norepinephrine reuptake transporter (NET) at pre-synapse. Similar to serotonin, inhibition of DAT and NET leads to elevated levels of dopamine (D) and norepinephrine (NE) at synaptic cleft. The mechanism of action of AGM at monoamine reuptake transporters is similar to fluoxetine (FLX) and methylphenidate (MPD) at SERT, DAT, and NET, respectively. Consequently, monoamines bind to their specific post-synaptic receptors efficiently and lead to improved mood and anxiolytic and learning behaviors

middle wall opening. Behaviors measured were entries and time spent in light box for 05 min.

2.3.3 Elevated plus maze

Elevated plus maze has been commonly endorsed to observe anxiety in rats. The apparatus consists of plus-shaped four arms in which two arms are open (50 × 10 cm) and two arms are closed (50 × 20 cm) with 15-cm high opaque walls. Open-arm edges were 25-cm high to avoid fall of rat. The maze was elevated 100 cm above the ground. Each rat is positioned at the center of the maze facing enclosed arm. Time spent and entries in open arms were observed in 5-min test period.

2.3.4 Activity box test

The simplest locomotive assessment is to observe activity of animals in home cages. Behavioral observations over a period of 24 h in novel environment can be used to assess anxiety, cardiac rhythm, and exploration [28, 29]. Extent of exploration, anxiety, and locomotion was measured in familiar environment of rats via activity box. The apparatus consists of (26 × 26 × 26 cm) transparent Perspex walls with ground covered with saw chips. Fifteen minutes of habituation of animal is followed by 10 min observation of cage crossings.

2.3.5 Forced swim test

Forced swim test is known as a fundamental test to examine depression-like behavior. FST apparatus comprises of a transparent glass chamber (12-cm diameter and 22-cm height). The cylinder was filled up to 10 cm with water (25 °C). Each rat was placed into the apparatus, and struggling of rat was monitored for 5 min. The cylinder was filled with clean water after each test.

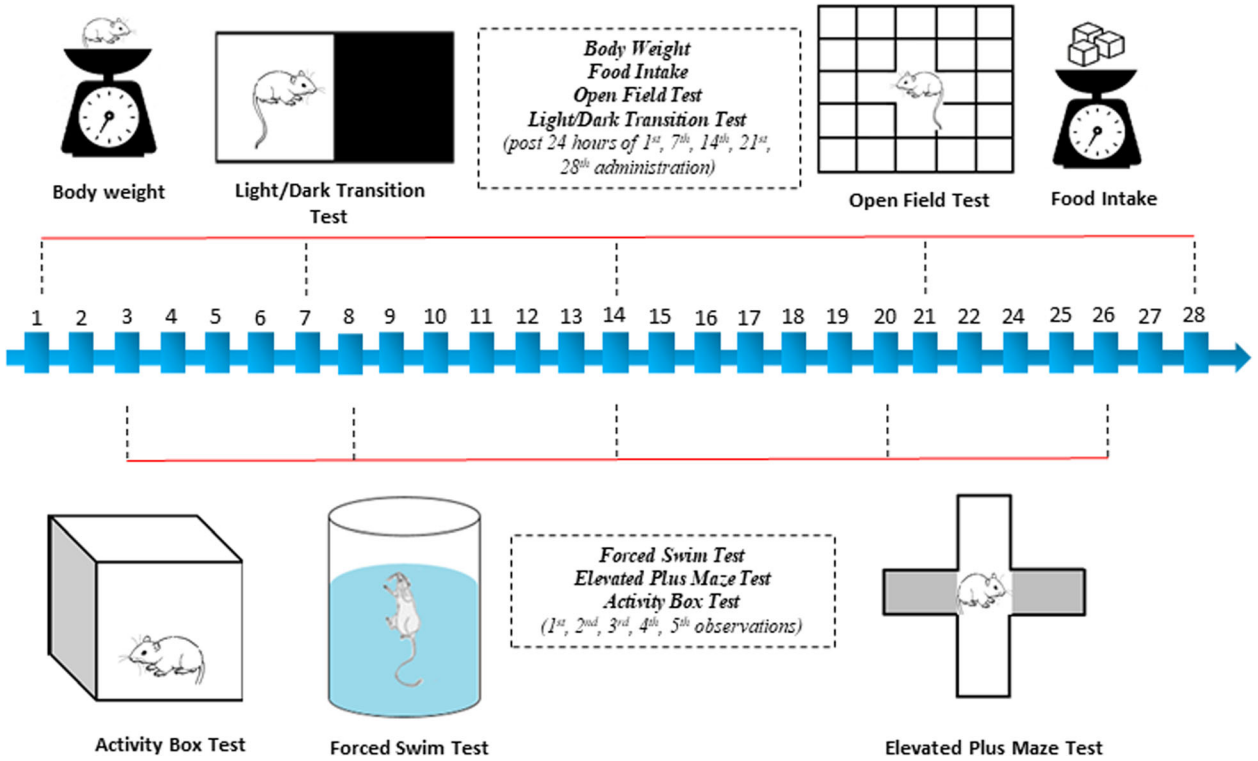

Fig. 2 Schematic representation of experimental protocol and behavioral evaluations

2.3.6 Open field activity

Open field test is an uncomplicated and simple assessment of behaviors that does not require training to animals. The apparatus consisted of 76 × 76 cm square area with 42-cm high opaque plastic walls. The floor was divided into 25 equal squares. Rat was placed in the center box of arena and exploration; anxiety and ambulation were observed in 5 min examination.

2.4 Statistical analysis

All obtained data were considered as two-way ANOVA repeated measure designs SPSS version 20, followed by Newman Keuls post hoc test. The results are described as mean ± SD. Significance was considered as p value < 0.05.

3 Results

3.1 Body weight

Data in Fig. 3 was analyzed by two-way ANOVA that explained the significant effects of days (F (4, 30) = 386.462, p<0.01), treatment (F (5, 30) = 212.348, p<0.01) and days × treatment (F (20, 30) = 26.212, p<0.01). Post hoc analysis determined that body weight increased significantly after the 14th (p<0.05), 21st, and 28th (p<0.01) day administration of AGM and decreased after the 1st administration of FLX (p<0.05) and MPD (p<0.01). FLX co-administered with AGM increased significantly body weight after the 7th (p<0.05), 14th, 21st, and 28th (p< 0.01) days of administration, while MPD + AGM after the 1st, 7th, 14th, 21st, and 28th (p<0.01) administration increased body weight significantly when compared with water-treated controls. When treatments were compared with their first administration, water after the 28th (p< 0.05) day; AGM after the 21st and 28th (p<0.01) days; FLX after the 14th, 21st, and 28th (p<0.01) days; and MPD after the 1st, 14th, 21st, and 28th (p<0.01) days of administration increased significantly body weight. However, co-administration of FLX and AGM after the 14th, 21st, and 28th (p<0.01) administration increased body weight significantly when compared with their first administration. However, body weight was significantly decreased after the 1st, 7th, 14th, 21st, and 28th (p<0.01) administration of FLX and MPD when compared with AGM-treated animals.

3.2 Food intake

Data in Fig. 4 was analyzed by two-way ANOVA that explained the significant effects of days (F (4, 30) = 250.104, p<0.01), treatment (F (5, 30) = 124.216, p<0.01), and days × treatment (F (20, 30) = 85.593, p<0.01). Post hoc analysis determined that food intake increased after the 7th and 21st (p<0.01) administration of AGM. However, decreased food intake was observed after the 14th (p<0.01) day of AGM treatment, 21st (p<0.01) day of

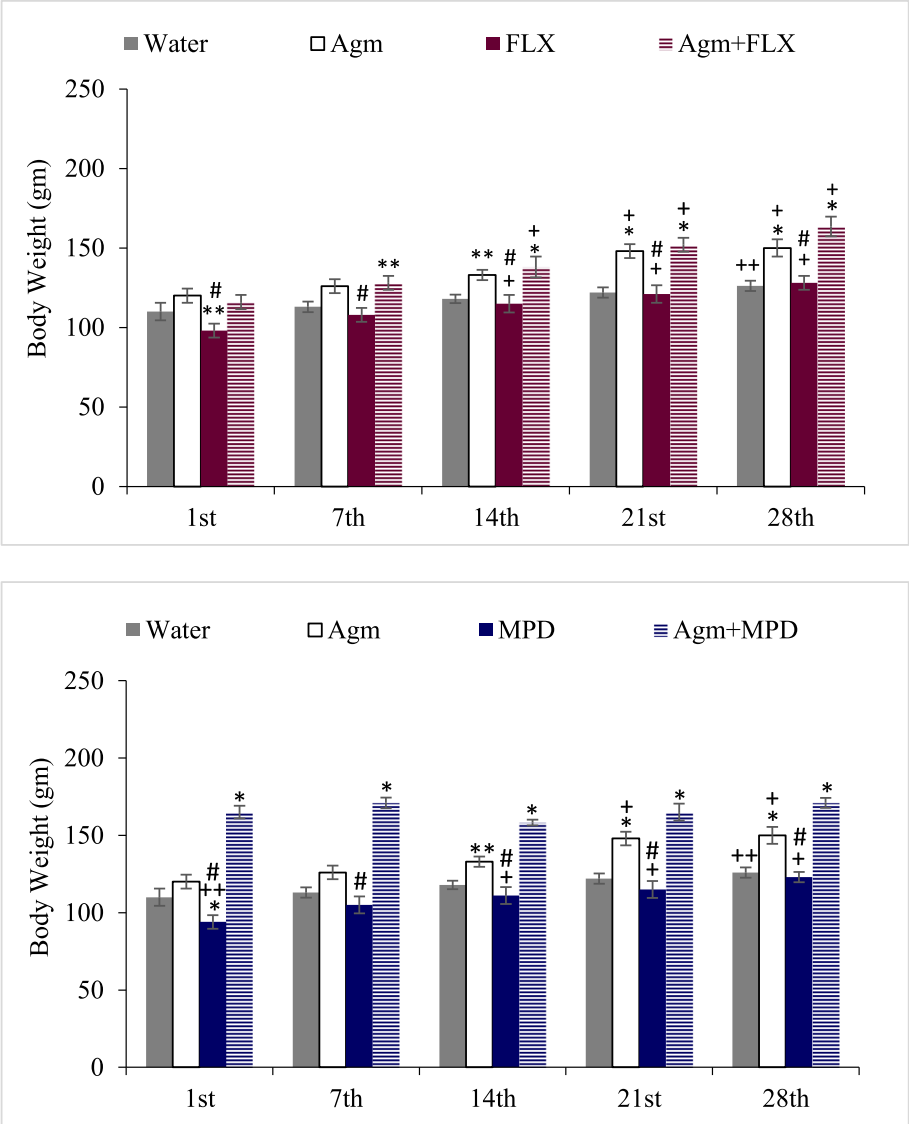

Fig. 3 Body weight: values are expressed as means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.01, **p<0.05 from water-treated control group to drug-treated groups. +p<0.01, ++p<0.05 from drug's 1st administration. #p<0.01, ##p<0.05 from AGM-treated animals to FLX- and MPD-treated animals following two-way ANOVA

FLX, and after the 1st, 7th, 14th, 21st, and 28th (p<0.01) administration of MPD while co-administration of AGM with MPD after the 1st (p<0.05), 14th, and 28th (p<0.01) administration decreased food intake significantly.

When compared with the first administration, significantly increased food intake was observed after the 14th (p<0.01) and 28th (p<0.05) days in water control and in AGM after the 7th (p<0.05) and 21st (p<0.01) days of administration. Similar results were observed in MPD-treated animals after the 7th and 28th (p<0.01) and 21st (p<0.05) administration and after the 14th, 21st, and 28th (p<0.01) of FLX and AGM co-administration. MPD and AGM co-treated animals have shown increased food intake after the 7th (p<0.05) and 21st (p<0.01) days. Food intake was observed significantly decreased after the 7th, 14th, and 21st (p<0.01) administration of FLX and the 1st, 7th, 14th, 21st (p<0.01), and 28th (p<0.05) administration of MPD when compared with AGM-treated animals.

3.3 Light/dark transition test

3.3.1 Light/dark transition test (time spent)

Obtained data from Fig. 5a was analyzed by two-way ANOVA that explained the effects of days (F (4, 30) = 3796.738, p<0.01), treatment (F (5, 30) = 316.151, p< 0.01), and days × treatment (F (20, 30) =154.937, p<0.01)

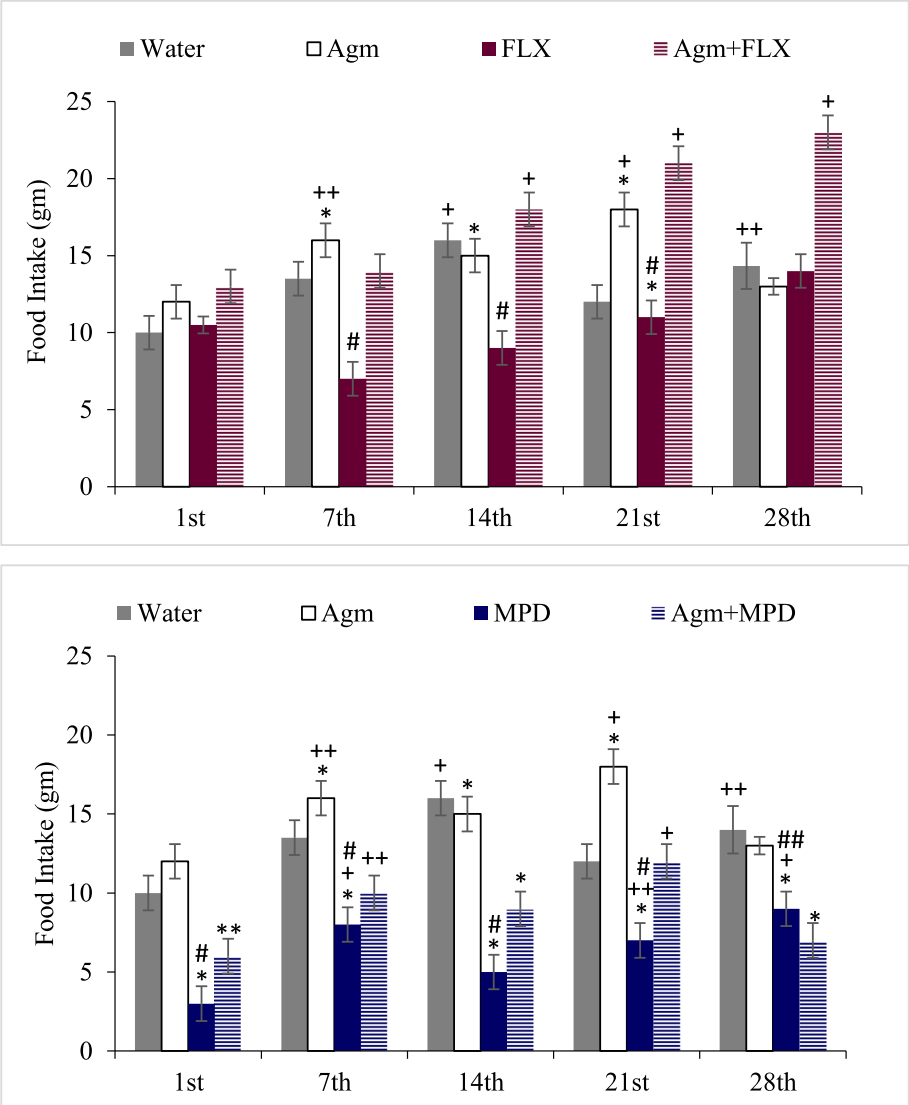

Fig. 4 Food intake: values are expressed as means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.01, **p<0.05 from water-treated control group to drug-treated groups. +p<0.01, ++p<0.05 from drug's 1st administration. #p<0.01, ##p<0.05 from AGM-treated animals to FLX- and MPD-treated animals following two-way ANOVA

to be significant. Post hoc analysis determined that time spent increased after the 1st (p<0.05), 7th, 14th, 21st, and 28th (p<0.01) days of AGM administration and after the 7th, 14th, 21st, and 28th (p<0.01) days of FLX administration while MPD and combined dose of AGM with FLX and MPD after the 1st, 7th, 14th, 21st, and 28th (p<0.01) administration significantly enhanced time spent in light compartment when compared with water-treated controls.

When compared with the first administration, AGM after the 7th (p<0.05), 14th, 21st, and 28th (p<0.01) administration and FLX and combined dose of FLX and AGM after the 7th, 14th, 21st, and 28th (p<0.01) administration increased time spent in light box, whereas MPD and co-treatment of MPD and AGM after the 14th, 21st, and 28th (p<0.01) administration increased time spent significantly in light box. Time spent was observed significantly increased after the 14th, 21st, and 28th (p<0.01) administration of FLX and 1st and 7th (p< 0.01) administration of MPD when compared with AGM-treated animals.

3.3.2 Light/dark transition test (entries)

All obtained results from Fig. 5b were analyzed by two-way ANOVA that explained the significant effects of days (F (4, 30) = 1465.233, p<0.01), treatment (F (5, 30) = 193.362, p<0.01), and days × treatment (F (20, 30) = 97.432, p<0.01). Post hoc analysis determined that AGM

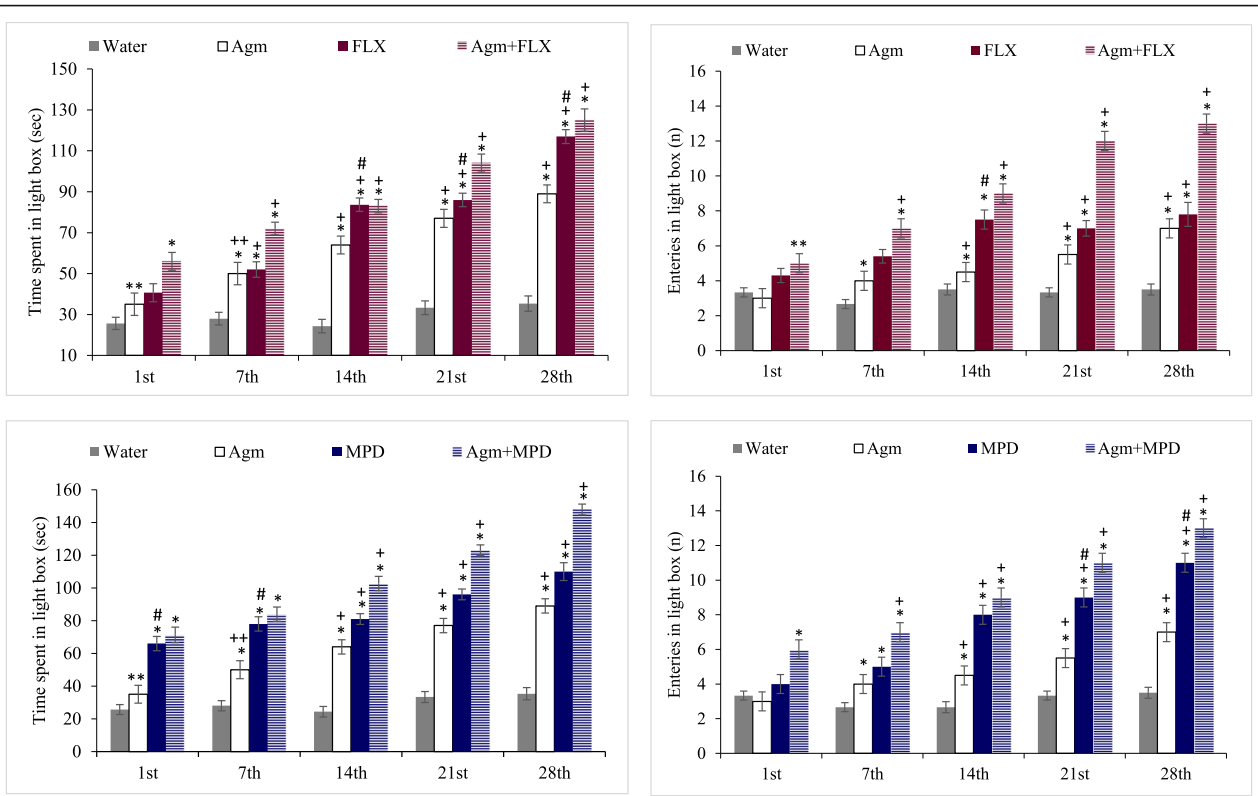

Fig. 5 a Light/dark transition test (time spent): values are expressed as means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.01, **p<0.05 from water-treated control group to drug-treated groups. +p<0.01, ++p<0.05 from drug's 1st administration. #p<0.01, ##p<0.05 from AGM-treated animals to FLX- and MPD-treated animals following two-way ANOVA. **b** Light/dark transition test (entries): values are expressed as means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.01, **p<0.05 from water-treated control group to drug-treated groups. +p< 0.01, ++p<0.05 from drug's 1st administration. #p<0.01, ##p<0.05 from AGM-treated animals to FLX- and MPD-treated animals following two-way ANOVA

after the 7th, 14th, 21st, and 28th (p<0.01) administration significantly increased entries in light compartment, while FLX after the 14th, 21st, and 28th (p<0.01) and MPD after the 7th, 14th, 21st, and 28th (p<0.01) administration increased entries in light box. Similarly, co-administration of FLX and AGM after the 1st (p<0.05), 7th, 14th, 21st, and 28th (p<0.01) and MPD + AGM after the 1st, 7th, 14th, 21st, and 28th (p<0.01) administration significantly enhanced entries in light compartment when compared with water-treated controls on same day.

Furthermore, AGM and MPD after the 14th, 21st, and 28th (p<0.01) administration; FLX after 21st and 28th (p<0.01) treatment and co-administration of AGM with FLX; and MPD after the 7th, 14th, 21st, and 28th (p< 0.01) administration increased entries significantly in light box when compared with their first administration. Entries were observed significantly increased after the 14th (p<0.01) administration of FLX and increased after the 21st and 28th (p<0.01) administration of MPD when compared with AGM.

3.4 Elevated plus maze

3.4.1 Elevated plus maze (time spent)

Data in Fig. 6a was analyzed by two-way ANOVA that explained the significant effects of days (F (4, 30) = 1870.219, p<0.01), treatment (F (5, 30) = 143.645, p< 0.01), and days × treatment (F (20, 30) = 168.143, p< 0.01). Post hoc analysis determined that AGM after the 7th and 14th (p<0.05) and 21st and 28th (p<0.01) administration and FLX after the 14th, 21st, and 28th (p< 0.01) administration increased significant time spent in open arms, while MPD after the 7th (p<0.05), 14th, 21st, and 28th (p<0.01) and co-administration of AGM with FLX after the 1st and 7th (p<0.05), 14th, 21st, and 28th (p<0.01) and AGM with MPD after the 1st (p<0.05), 7th, 14th, 21st, and 28th (p<0.01) administration significantly increased time spent in open arms when compared with water-treated controls.

When compared with first administration, AGM after the 7th and 21st (p<0.05) and 28th (p<0.01) administration whereas FLX, MPD, co-administration of AGM with FLX and MPD after 14th, 21st, and 28th

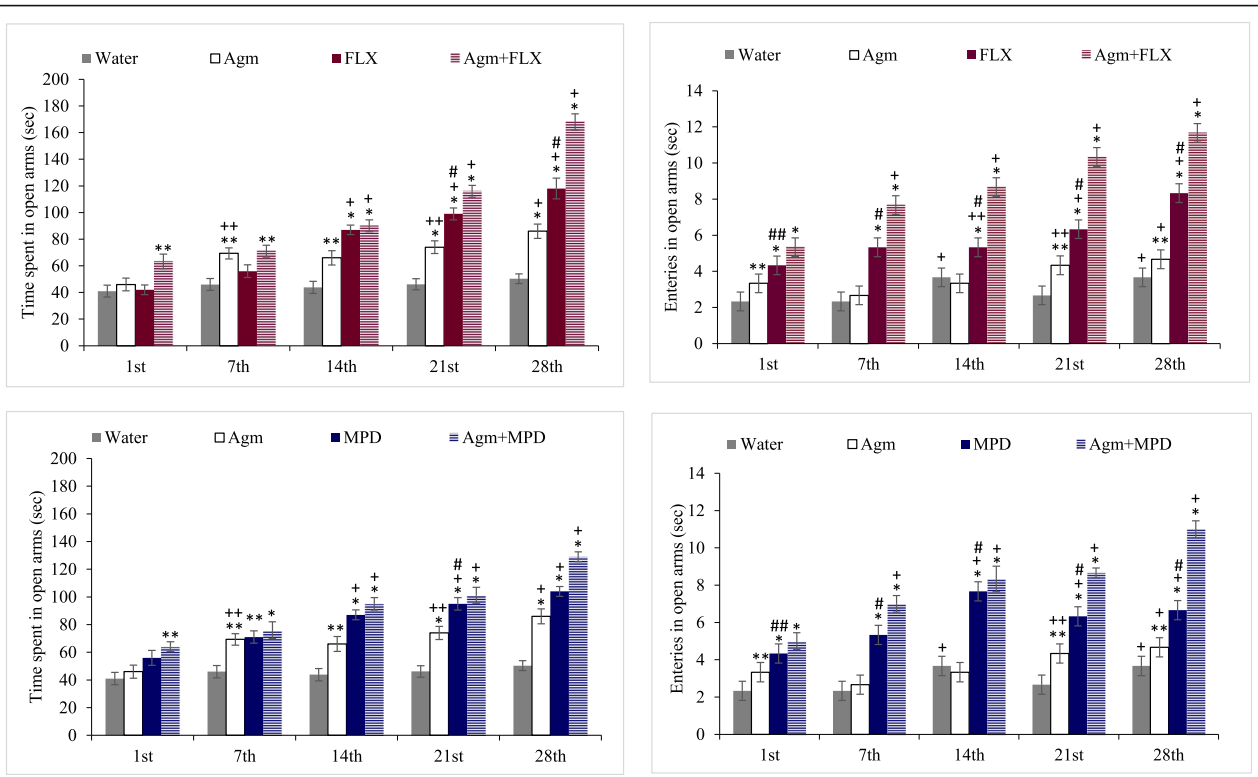

Fig. 6 **a** EPM (time spent): values are expressed as means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.01, **p<0.05 from water-treated control group to drug-treated groups. +p<0.01, ++p<0.05 from drug's 1st administration. #p<0.01, ##p<0.05 from AGM-treated animals to FLX- and MPD-treated animals following two-way ANOVA. **b** EPM (entries): values are expressed as means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.01, **p<0.05 from water-treated control group to drug-treated groups. +p<0.01, ++p<0.05 from drug's 1st administration. #p<0.01, ##p<0.05 from AGM-treated animals to FLX- and MPD-treated animals following two-way ANOVA

(p<0.01) days of administration increased time spent significantly in open arms. Time spent in open arms were observed significantly increased after the 21st and 28th (p<0.01) administration of FLX and after the 21st (p<0.01) day of MPD administration when compared with AGM-treated animals.

3.4.2 Elevated plus maze (entries)

Data in Fig. 6b was analyzed by two-way ANOVA that explained the significant effects of days (F (4, 30) = 282.783, p<0.01), treatment (F (5, 30) = 440.298, p<0.01), and days × treatment (F (20, 30) = 24.557, p<0.01). Post hoc analysis determined that entries in open arms increased after the 1st, 21st, and 28th (p<0.05) AGM administration, while FLX, MPD, and co-administration of AGM with FLX and MPD have shown significant increased time spent in open arms after the 1st, 7th, 14th, 21st, and 28th (p<0.01) administration when compared with control animals.

Number of entries increased in control after the 14th and 28th (p<0.01) days while in AGM after 21st (p<0.05) and 28th (p<0.01) administration when compared with first administration, whereas FLX after the 14th (p<0.05), 21st, and 28th (p<0.01) and MPD after the 14th, 21st, and 28th (p<0.01) days of administration have shown increased number of entries. Co-administration of AGM with FLX and MPD has shown significant increased entries after the 7th, 14th, 21st, and 28th (p<0.01) administration as compared to the first administration.

Entries in open arms were observed significantly increased after the 1st (p<0.05), 7th, 14th, 21st, and 28th (p<0.01) administration of FLX and MPD when compared with AGM-treated animals.

3.5 Activity box test

Data obtained from Fig. 7 was analyzed by two-way ANOVA that explained the significant effects of days (F (4, 30) = 3271.290, p<0.01), treatment (F (5, 30) = 326.036, p<0.01), and days × treatment (F (20, 30) = 130.137, p<0.01). Post hoc analysis determined that cage crossings increased significantly after the 1st and 7th (p< 0.05), 14th, 21st, and 28th (p<0.01) administration of AGM and the 1st (p<0.05), 7th, 14th, 21st, and 28th (p< 0.01) administration of FLX. Similar increase in cage crossings were observed in MPD as well as co-administration of AGM with FLX and MPD and after the 1st, 7th, 14th, 21st, and 28th (p<0.01) administration when compared with controls. Furthermore, increased

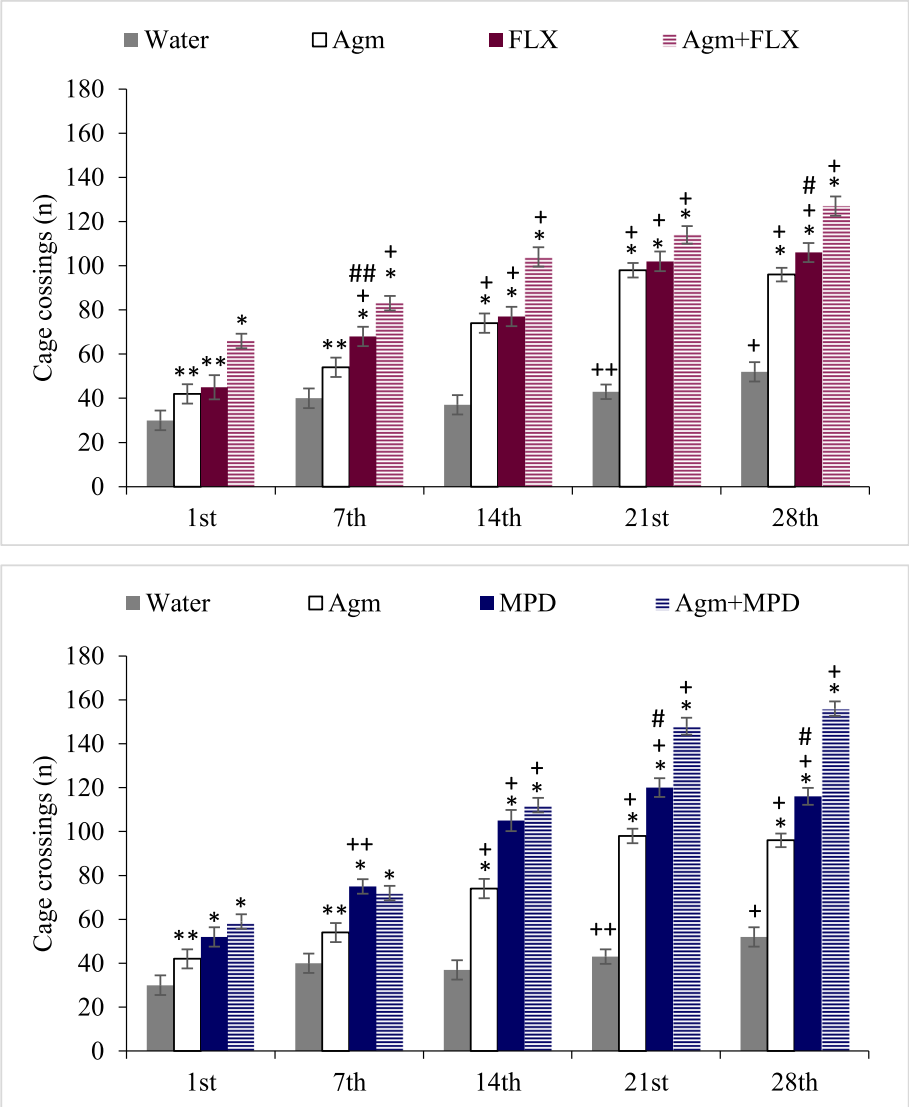

Fig. 7 Activity box test: values are expressed as means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.01, **p<0.05 from water-treated control group to drug-treated groups. +p<0.01, ++p<0.05 from drug's 1st administration. #p<0.01, ##p<0.05 from AGM-treated animals to FLX- and MPD-treated animals following two-way ANOVA

numbers of cage crossings were observed in controls after the 21st (p<0.05) and 28th (p<0.01) administration and in AGM after the 14th, 21st, and 28th (p<0.01) administration. Animals treated with FLX and FLX + AGM combined doses has shown increased cage crossings after the 7th, 14th, 21st, and 28th (p<0.01) administration, while MPD after the 7th (p<0.05), 14th, 21st, and 28th (p<0.01) administration and co-treatment of MPD and AGM after 14th, 21st, and 28th (p<0.01) day increased numbers of box crossed significantly when compared with their first administration. Number of cage crossings was observed significantly increased after the 7th (p<0.05) and 28th (p<0.01) administration of FLX and 21st and 28th (p< 0.01) administration of MPD when compared with AGM-treated animals.

3.6 Forced swim test

Data in Fig. 8 was analyzed by two-way ANOVA that explained the significant effects of days (F (4, 30) = 1305.458, p<0.01), treatment (F (5, 30) = 104.353, p< 0.01), and days × treatment (F (20, 30) = 20.990, p<0.01). Post hoc analysis determined that AGM after the 28th (p<0.01) administration significantly increased struggling time. Similarly, AGM co-treatment with FLX and MPD after the 1st, 7th, 14th, 21st, and 28th (p<0.01) administration increased duration of struggling in FST when compared with controls. Struggling time increased after

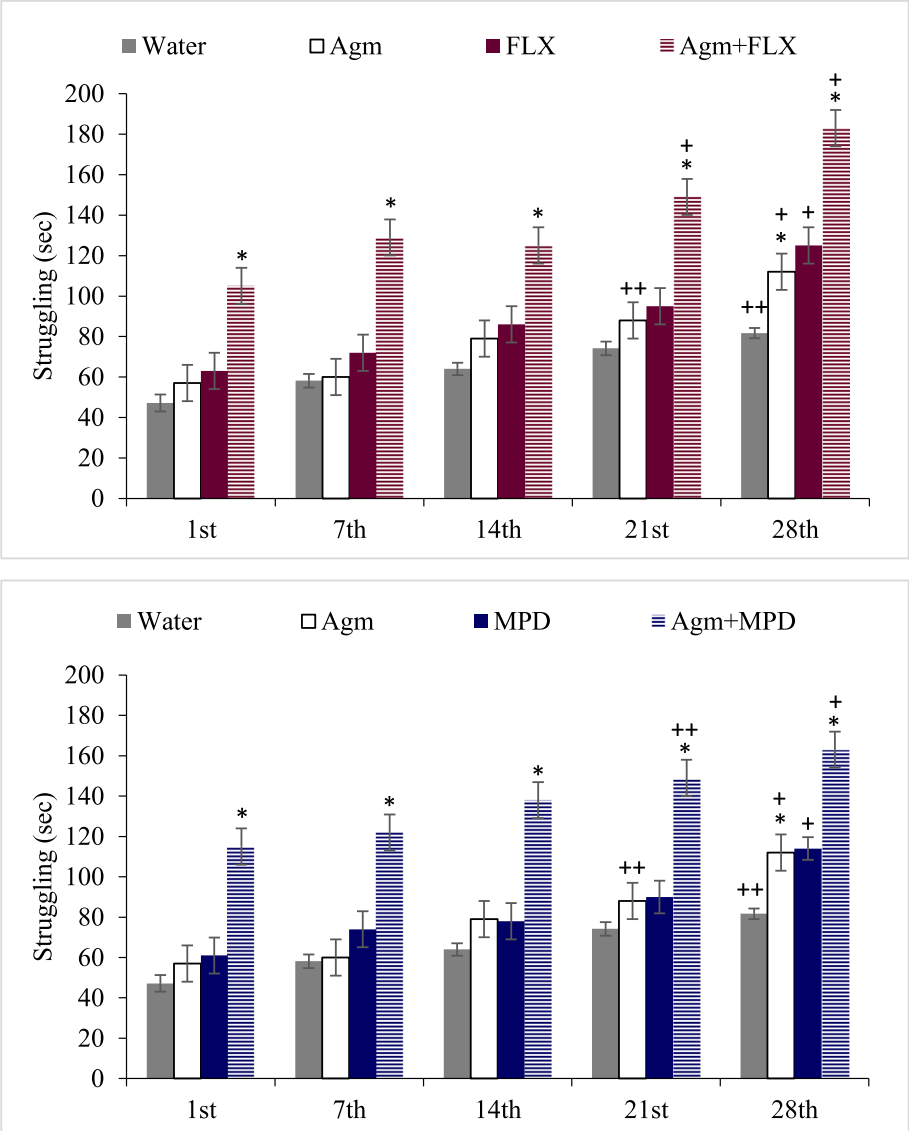

Fig. 8 FST: values are expressed as means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.01, **p<0.05 from water-treated control group to drug-treated groups. +p<0.01, ++p<0.05 from drug's 1st administration. #p<0.01, ##p<0.05 from AGM-treated animals to FLX- and MPD-treated animals following two-way ANOVA

the 28th (p<0.05) day in controls and after the 21st (p< 0.05) and 28th (p<0.01) AGM administration when compared with first administration. FLX and MPD has shown increased struggling after the 28th (p<0.01) administration while co-administration of FLX and AGM after the 21st and 28th (p<0.01) administration and MPD and AGM after the 21st (p<0.05) and 28th (p< 0.01) administration increased struggling significantly when compared with their first administration.

3.7 Open field test

All results obtained from Fig. 9 were analyzed by two-way ANOVA that explained the significant effects of days (F (4, 30) = 2444.642, p<0.01), treatment (F (5, 30) = 2093.791, p<0.01), and days × treatment (F (20, 30) = 99.996, p<0.01). Post hoc analysis determined that box crossed increased in all groups of animals (p<0.01) including AGM, FLX, MPD, and co-administration of AGM with FLX and MPD after the 1st, 7th, 14th, 21st, and 28th (p<0.01) when compared with water-treated controls.

Increased number of boxes crossed were observed in animals treated with AGM, FLX, and combined FLX and AGM after the 7th (p<0.05), 14th, 21st, and 28th (p<0.01) administration. While MPD treatment has shown increased box crossed after the 7th, 14th, 21st,

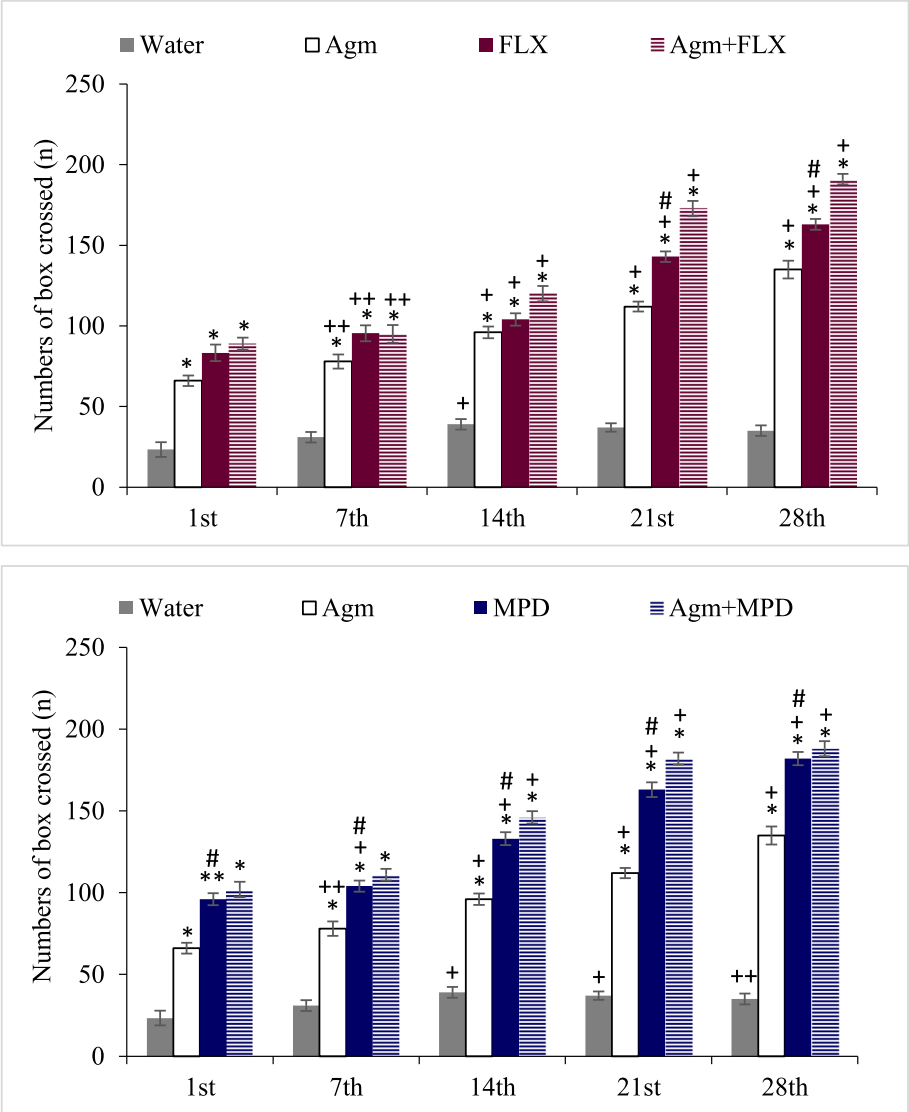

Fig. 9 Open field test: values are expressed as means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.01, **p<0.05 from water-treated control group to drug-treated groups. +p<0.01, ++p<0.05 from drug's 1st administration. #p<0.01, ##p<0.05 from AGM-treated animals to FLX- and MPD-treated animals following two-way ANOVA

and 28th (p<0.01) administration and MPD and AGM after the 14th, 21st, and 28th (p<0.01) days when compared with the first administration. Square crossed was observed significantly increased after the 21st and 28th (p<0.01) administration of FLX and 1st, 7th, 14th, 21st, and 28th (p<0.01) administration of MPD when compared with AGM-treated animals.

4 Discussion

The present study was designed to determine the comparative effects of AGM with standard antidepressant and psychostimulant drugs namely FLX and MPD respectively in various behavioral paradigms with specific doses for 28 days. Various preclinical studies validated FLX impact on 5-HT neurotransmission that involves the modulation of physiological mechanisms including food intake, sleep, aggression, body temperature, vomiting, fear, and sexual behaviors [12]. MPD inhibits the reuptake of norepinephrine and dopamine at presynaptic neuron. It blocks the respective transporters of neurotransmitters and consequently concentration of dopamine and norepinephrine in the synaptic cleft [30]. Present study described the effects of AGM on body weight in comparison with other selected drugs. Significant increase in body weight was observed in AGM-administered group from their first dose and in comparison with FLX and MPD in weekly administration while maximum increase in body weight was demonstrated by

both co-administered groups of AGM with FLX and with MPD. Body weight was also observed to be enhanced in MPD and AGM co-treated animals when compared with FLX and AGM co-administrated groups. MPD decreases hunger and appetite that results in reduced food intake and weight loss in rats and humans [31, 32]. Increased food intake and appetite was observed in AGM-administered animals in weekly assessment as observed in Fig. 4. MPD-induced loss of appetite was also revealed in the present study while co-administered FLX and AGM resulted in increased food intake when compared with AGM, FLX, and co-treated MPD and AGM groups. MPD-treated animals exhibited hyperactivity assessed by distance traveled in open field test; furthermore, effects of MPD increased in late weeks that described sensitization impact of MPD. Results have shown the effects of AGM on locomotion and exploration in open field paradigm in comparison with FLX and MPD and co-administration. Increased total numbers of square crossed were observed in AGM-treated animals from first administration while total number of boxes crossed was enhanced in co-treated groups of AGM with FLX significantly. BALB/c mice demonstrated that chronic FLX treatment increased time spent in the central region of the open field box specifically in the first few minutes of test cutoff time [33]. Increase in crossings and rearing behaviors were observed in rats treated with AGM that leads to significant behavioral alternations [34]. In the present study, significant increase in cage crossings was observed in AGM-treated animals, whereas animals treated with AGM and FLX produced most significant effects when compared with any other drug-treated animal group.

Acute administration of AGM (80 mg/Kg) or chronic treatment (20 mg/kg or 10 mg/kg, for 3 days) significantly improved light–dark transitions in rats in the light–dark transition test [35]. Chronic treatment of FLX and buspirone in teleost species demonstrated improved time spent in light compartment of light/dark transition box [36]. Present study described the effects of AGM in comparison with other drugs on anxiety assessed in light/dark transition box. Increased time spent in light box was observed in animals treated by AGM and its co-administered animal groups. Improved time spent in light compartment is observed after weekly assessment in FLX and co-treated FLX and AGM groups. Data obtained has described enhanced effects of both co-administered drugs with AGM on the total numbers of entries in light box, while MPD significantly improved entries in light compartment when compared with FLX- and AGM-treated animals. Present study revealed the significant anxiolytic effects of both FLX and MPD co-administration with AGM in light/dark transition test. Study showed that chronic AGM treatment increased percentage of entries and time in open arms of EPM paradigm [34]. MPD treatment revealed anxiolytic effects in EPM test [22] while FLX acute administration induces anxiogenic-like activity in EPM that is typical clinical effect of the FLX first phase treatment [37]. Similar results were observed in EPM test that described the effects of AGM in comparison with FLX and MPD and their co-administration with AGM. Improved time spent in open arms was observed in AGM-treated animals from first day of administration. FLX and MPD increased time spent significantly when compared with AGM administration whereas, co-administration of FLX and AGM demonstrated most enhanced activity of animals in EPM test. Present study revealed significant increased entries in open arms of EPM in both co-treated groups of AGM with FLX and MPD, while treatment with FLX and MPD exhibited comparatively improved entries in open arms of EPM. Studies validated the anxiolytic effects of AGM in mice exposed to chronic stress by decreasing the duration of immobility and enhancing the swimming interval in stressed mice [34]. Present study revealed anxiolytic and antidepressant effects of AGM and comparative drugs in FST. Struggling duration increased in AGM-treated animals from first administration while AGM co-treatment with FLX and MPD animals demonstrated enhanced struggling in animals compared with other animal groups. Study showed antidepressant effects of FLX in FST proposing that enhanced serotonin neurotransmission may increase swimming behavior as it also decreases immobility time [38].

5 Conclusion

Present study revealed the anxiolytic, locomotor, and antidepressive behavioral effects of AGM compared with FLX and MPD. However, rats when treated with combined doses of AGM with FLX or MPD showed improved behaviors along with food intake and body weights. Study determined the safe and efficient administration of AGM along with conventional antidepressant and psychostimulative drugs. This study has also proposed the potential mechanism of action of AGM at monoamine receptors that may lead to inhibition of monoamine reuptake transporters. This may lead to increase in 5-HT, D, and NE concentrations at synaptic level.

Abbreviations

AGM: Agmatine; FLX: Fluoxetine; MPD: Methylphenidate; ADHD: Attention deficit hyperactivity disorder; EPM: Elevated plus maze; FST: Forced swim test; SERT: Serotonin reuptake transporter; DAT: Dopamine reuptake transporter; NET: Norepinephrine reuptake transporter; 5-HT: 5-Hydroxytryptamine; D: Dopamine; NE: Norepinephrine; ADC: Arginine decarboxylase

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Authors' contributions
All authors have read and approved the manuscript. HR Study concept and design. Data collection. Data analysis or interpretation. Writing the paper. HaR Data collection. MF Research supervisor.

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Availability of data and materials
All relevant data are within the manuscript.

Declarations

Ethics approval and consent to participate
All experiments were approved by the Institutional Advanced Studies and Research Board (BASR/03367/Sc.) and performed in strict accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publication no. 85–23, revised 2011).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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