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Cannabinoid receptor agonism suppresses tremor, cognition disturbances and anxiety-like behaviors in a rat model of essential tremor

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Running Title: CBR agonism suppresses tremor symptoms

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Abstract

Cognitive and motor disturbances are serious consequences of tremor induced by motor disorders. Despite a lack of effective clinical treatment, some potential therapeutic agents have been used to alleviate the cognitive symptoms in the animal models of tremor. In the current study, the effects of WIN55, 212-2 (WIN), a cannabinoid receptor (CBR) agonist, on harmaline-induced motor and cognitive impairments was studied. Adult rats were treated with WIN (0.5 mg/kg; i.p.) 15 min before harmaline administration (10 mg/kg; ip) after which exploratory and anxiety related behaviors, and cognitive function were assessed using open-field behavior and shuttle box tests. Rats that received harmaline only exhibited a markedly reduced number of central square entries when compared to harmaline vehicle-treated controls, whereas those treated with WIN and harmaline showed a significant increase in central square entries, compared to harmaline only treated. The passive avoidance memory impairments observed in harmaline treated rats, was reversed somewhat by administration of WIN. The neuroprotective and anxiolytic effects of WIN demonstrated in the current study can be offered cannabinoid receptor (CBR) agonism as a potential neuroprotective agent in the treatment of patients with tremor that manifest mental dysfunctions.

1. Introduction

Essential tremor (ET) is conventionally conceived of as a purely motor disease and some studies have revealed an association between ET and increased risk for cognitive impairment and dementia, which suggests that cognitive impairments in ET patients may be a consequence of an additional neurodegenerative disorder. However, other studies have identified cognitive deficits in ET patients as being frontosubcortical or corticocerebellar which are consistent with symptoms arising in whole or in part from ET itself and independent of medication used to treat ET symptoms (Janicki et al. , 2013). Furthermore, no pharmacotherapies to treat cognitive deficits in ET patients have been developed, revealing an unmet clinical need in this population.

Predictive animal models of symptoms and disease remain an important element of drug development. Systemic harmaline administration causes action tremor in mammals and has proved to be a useful animal model for the discovery of new therapies for primary symptoms of ET (Clifford, 1983a). Furthermore, in addition to harmaline causing agitation, cytotoxicity, delirium, paralysis, loss of coordination, tremor, visual disturbances and hallucinations (Khan et al. , 2013), it has also been reported to induce cognitive disturbances, most likely as sequelae to low harmaline doses (5-10 mg/kg) acting anxiogenically or higher doses (20 mg/kg) exerting reportedly anxiolytic effects in rodents (Hilber and Chapillon, 2005). It has also reportedly affected emotional reactivity in mice as decision making in an anxiogenic situation can be altered by harmaline treatment (Hilber and Chapillon, 2005) in addition to inducing cognitive disturbances that manifest as motor and spatial learning impairments (Hilber and Chapillon, 2005). Therefore, the symptoms exhibited by rodents following systemic harmaline administration are consistent with being predictive for drug effects upon cognitive domains of interest to human ET pharmacotherapy.

The endocannabinoid system is implicated in cognition and genetic deletion of cannabinoid type 1 (CB_1) receptors accelerates age-related cognitive decline in rodents (Jenniches et al. , 2015), accompanied by neuronal loss in the CA1 and CA3 regions of the hippocampus (Bilkei-Gorzo et al. , 2005). CB_1 receptors are presynaptically located where activation reduces presynaptic neuronal excitability and so inhibits neurotransmitter release (Howard et al. , 2013). CB_1 receptor expression is abundant in several brain regions including the hippocampus, prefrontal cortex, nucleus accumbens and amygdala where their modulation of neurotransmitter release exerts a variety of behavioral and cognitive effects (Khan, Maalik, 2013). Here, a substantial body of evidence from animal models and human studies has shown that CB_1 receptor agonists, frequently in the form of Δ^9-tetrahydrocannabinol which is the principal psychoactive component derived from *Cannabis sativa*, induce numerous and complex effects on cognitive functions including attention, learning, emotional reactivity, enhancement of the perceptions of the senses, and, idiosyncratically, impairment and improvement in short-term memory (Barzegar et al. , 2015, Razavinasab et al. , 2013, Shabani et al. , 2009, Shabani et al. , 2011). In the passive avoidance task, CB_1 receptor activation reversed opioid-induced memory impairment (Zarrindast, 2006) but in other reports have been shown to impair passive avoidance learning in addition to adversely affecting spatial and working memory (Hasanein and Teimuri Far, 2015, Shabani et al. , 2012). For example, the CB_1 receptor agonist, arachidonylcyclopropylamide (ACPA) induced memory acquisition impairment in mice which was reversed by co-administration of a CB_1 receptor antagonist (Nasehi et al. , 2015a). Interestingly, prenatal administration of the CB receptor agonist, WIN55,212-2 (0.5-1mg/kg) during embryonic days 5-20 can disrupt memory retention in offspring when assessed at P30-P35 using the passive avoidance task (Shabani, Divsalar, 2012). A role for CB_1 receptors in

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memory consolidation was shown by treatment with the CB_1 receptor selective antagonist, rimonabant (0.1 mg/kg and 1.0 mg/kg; i.p.), which caused significant improvement in passive avoidance performance (Ágota S. Ádáma et al. , 2008). Moreover, alterations in the sleep–wake cycle, memory formation, locomotor activity and pain perception have been widely reported in studies of the effects of the endocannabinoid, anandamide (Arjmand et al. , 2015, Eric Murillo-Rodrígueza et al. , 1998).

In the present study, we examine the effect of harmaline at a reportedly low, anxiogenic dose (10 mg/kg; i.p.) upon tremor, gait, anxiety and associative learning and memory in rats before investigating the effects of cannabinoid receptor agonism upon harmaline-induced effects in these domains. Here, harmaline produced a moderate and persistent tremor, gait disturbances, increased anxiety and a significant impairment in the learning and recall capability in the passive avoidance task. While prior CBR agonist treatment had no effect upon harmaline-induced tremor or gait disturbances, the anxiogenic effects of harmaline were attenuated and some impairments of memory formation and retention were reversed.

2. Methods and materials

2.1 Animals

30 adult, male Wistar Kyoto rats (60-80 g) were used. Animals were kept in individual cages with access to food and water *ad libitum* and maintained on a 12 hours/12 hours dark/light cycle. Every effort was made to minimize animal suffering during all stages on the study. All procedures were approved by the Kerman Medical University Ethics Committee (EC/KNRC/92-63).

2.2 Drugs

The non-selective cannabinoid type 1 (CB_1) and type 2 (CB_2) receptor agonist, WIN55, 212-2 (WIN; Sigma, USA), was dissolved in dimethylsulfoxide (DMSO) before 100-fold dilution in normal saline. Harmaline hydrochloride dihydrate (Sigma) was dissolved in normal saline.

2.3. Behavioural tasks

2.3.1. Tremor scoring

Tremor was rated by two observers blinded to treatment. Intra- and inter-observer reliability were assessed via kappa coefficient (acceptance criterion: >80%). Tremor data were acquired during the open field test and quantitatively scored as follows: 0: No tremor, 1: occasional tremor affecting only the head and neck, 2: intermittent (occasional tremor affecting all body parts), 3: persistent (persistent tremor affecting all body parts and tail), 4: severe (persistent tremor rendering the animal unable to stand and/or walk) (Al-Deeb S1, 2002)

2.3.2. Gait analysis

The footprint test assesses animal walking patterns and gait kinematics. The hind paws of each animal were marked with a non-toxic ink and the animal allowed to traverse a clear Plexiglas tunnel (100 cm [L]×10 cm [H]×10 cm [W]) lined with white absorbent paper (100 cm × 10 cm) and ending in a darkened cage. The resulting tracks provide the spatial relationship of consecutive footfalls from which animal stride length and width were measured. Animals were habituated to the runway for 3 training runs before testing. Hind paw stride lengths were measured by distance (cm) between the respective paw prints to the successive ipsilateral prints to assess uni- or bi-lateral effects of treatment upon gait. Hind paw stride widths were measured

by distance between the centers of the respective paw prints to the corresponding contralateral stride length measurements at a right angle. Footprints at the beginning and end of each run were not considered in the analysis(Wecker L, 2013;).

2.3.3 Open-field test

The open field apparatus consisted of a square Plexiglas arena (90 [W] ×90 [L] ×45 [H] cm), the floor of which was divided by lines into 16 squares to define central and peripheral regions. Each animal in turn was placed in the middle of the open field apparatus and vertical (rearing) and horizontal activity video recorded for a five-minute period. Video recordings were analyzed offline using EthoVision (Noldus Information Technology, Netherlands) video tracking software for automated classification of behavioral paradigms and the following parameters recorded for each animal: total distance moved (cm) and time spent in peripheral and central regions (seconds). At the end of each test, the animal was removed from the chamber and the field cleaned with 70% ethanol (Nazeri et al. , 2014).

2.3.4. Passive avoidance test

The passive avoidance task is a fear-aggravated test used to evaluate associative learning and memory in rodents. The animal learns to avoid an environment in which a prior aversive stimulus has been delivered. Here, passive avoidance learning was assessed using an inhibitory passive avoidance paradigm as described hereafter. Briefly, a shuttle-box device with dimensions of 100 [L] x 25 [W] x 25 [H] (cm) and consisting of two compartments (light and dark) separated by a door was used. In the learning phase of the test, each animal was first habituated to the test

equipment by placement in the light chamber (door closed) for 5 minutes before return to the home cage. The next day, the animal was returned to the light compartment, the door opened and the animal allowed to move to the dark chamber before the door was closed and the animal returned to the home cage. This process was repeated once and if an animal failed to move into the dark compartment, it was removed from the study. Finally, one hour after the previous exposure to the apparatus, the animal was placed into the light compartment, the door opened and, on entering the dark compartment, given an electric shock (0.5A, 2ms; via wires embedded in the dark chamber floor). This final part of the process was repeated up to five times at 1 hour intervals until the animal learned to avoid the dark compartment (remains in light compartment for at least 300s) and the number of shocks required for learning recorded. The assessment phase of the test was undertaken 24 hours after the learning phase. The animal was placed in the light chamber (door closed) and, after 30s, the door opened and the time until the animal entered the dark chamber recorded as the step-through latency (STL). The total time spent in the dark compartment (TDC) during a period of 5 minutes after door opening was also recorded.

2.2. Experimental design

Animals were divided into three groups (n=10/group). The control group (harmaline vehicle plus WIN vehicle) received WIN vehicle (normal saline; 0.5 ml; i.p.), 15 minutes before harmaline vehicle (normal saline; 0.5 ml; i.p.). The harmaline only group (WIN vehicle plus harmaline) received WIN vehicle (normal saline; 0.5 ml; i.p.), 15 minutes before harmaline (10 mg/kg; i.p.). The harmaline plus WIN group (WIN plus harmaline) received WIN (0.5 mg/kg; i.p.), 15 minutes before harmaline injection (10 mg/kg; i.p.). Each group began behavioural testing as described hereafter 30 minutes after receiving harmaline vehicle (control) or harmaline

(harmaline only and harmaline plus WIN groups). Each group undertook four behavioral tests: open field test, tremor score evaluation and gait analysis which were administered sequentially with 15 minute rest interval between each test. The learning phase of the passive avoidance task was undertaken 3 hours after gait analysis when tremor symptoms had subsided, while assessment of memory retrieval in the task was performed 24 hours after the learning phase.

2.3. Statistical analysis

SPSS (IBM, USA) and GraphPad Prism 6 (GraphPad Software, USA) were used for statistical analysis of data and figure production. All data were first assessed for normality using a Kolmogorov-Smirnov test. Results found to be normally distributed ($p>0.05$ in K-S test) were expressed as mean±SEM and analyzed using a one-way ANOVA test. Where a main effect was seen in ANOVA tests, pairwise comparisons between groups were then made using Tukey's *post-hoc* tests. Results that were not normally distributed ($p<0.05$ in K-S test) were expressed as median and interquartile range (expressed as median (interquartile range)) and analysed using a Kruskal-Wallis test. Where a main effect was seen in Kruskal-Wallis tests, pairwise comparisons between groups were then made using Dunn's multiple comparisons test. In each case, $p<0.05$ was considered statistically significant.

3. Results

3.1 Effect of harmaline and CBR agonism on motor behaviours

When tremor was examined, a main effect of treatment was observed ($H(2)=24.4$; $p<0.001$; Fig.1A). Subsequent *post hoc* tests revealed that both the harmaline ($p<0.001$) and harmaline plus WIN ($p<0.01$) groups exhibited significantly greater tremor scores when compared to control animals although no significant difference was seen between harmaline and harmaline

plus WIN55,212-2 groups, suggesting that WIN55,212-2 treatment had no effect upon harmaline induced tremor.

In the gait analysis test, a main effect of treatment upon step width was detected ($F_{2, 27}$=8.1; p<0.001; Fig.1B). *Post hoc* pairwise comparisons revelaed that both harmaline only and harmaline plus WIN groups exhited significantly increased step width (both P<0.001 vs control) although a significant difference between harmaline only and harmaline plus WIN groups was also detected (P<0.05) demonstrating that WIN treatment was able to partly ameliorate harmaline effects upon this parameter. Moreover, a main effect of treatment upon left and right step lengths was also detected (H(2)=16.11; p=0.0003 and $F_{2,30}$=83.8; p<0.0001 respectively; Figs 1C&D). *Post hoc* paiwise comparisons revealed that left and right stride lengths were significantly decreased by both harmaline only (left & right: p<0.001) and harmaline plus WIN treatments (left: p<0.01; right: P<0.001) to reveal that WIN treatment has no effect upon harmaline-induced disturbances in stride length.

3.2 CBR agonism ameliorates harmaline-induced anxiety-like, but not exploratory behaviours

In the open field test, an overall effect of treatment upon total distance moved ($F_{2, 23}$=112.7; p<0.0001; Fig.2A), velocity ($F_{2, 27}$=149.2; p<0.0001; Fig.2B), time spent in the center ($F_{2, 24}$=52.3; p<0.0001; Fig.2C) and time spent in the perimeter ($F_{2, 24}$=51.4; p<0.0001; Fig.2D) was found. *Post hoc* pairwise comparisons revealed that harmaline only and harmaline plus WIN significantly reduced total distance moved (harmaline only & harmaline plus WIN: p<0.001 vs control) and velocity (harmaline only & harmaline plus WIN: p<0.001 vs control). Therefore, it

is clear that WIN treatment has no effect upon harmaline-induced changes in distance moved or velocity.

However, when *post hoc* pairwise comparisons were conducted between groups for measures of time spent in the center and perimeter of the apparatus, a different pattern of effects emerged. Here, time spent in the center was significantly decreased in the harmaline only group (p<0.001 vs control) but not the harmaline plus WIN group (p>0.5 vs control). Moreover, the harmaline-only group was signifciantly decreased compared to the harmaline plus WIN group (p<0.001) demonstrating that WIN treatment was able to reverse the harmaline-induced effects on this measure observed. Correspondingly, time spent in the perimeter was significantly increased in the harmaline only group (p<0.05 vs control) and decreased in the harmaline plus WIN group (p<0.001 vs control). Moreover, the harmaline-only group was signifciantly increased compared to the harmaline plus WIN group (p<0.001), again demonstrating a WIN-induced reversal of harmaline effects.

3.3 Harmaline-induced impairment of performance in the passive avoidance test can be partially reversed by CBR agonism

In the passive avoidance test, an overall effect of treatment was seen upon the number of shocks required before learning was achieved (H(2)=14.98; p<0.001; Fig. 3A) where subsequent pairwise comparisons revealed that while harmaline only treated animals required significantly more shocks (p<0.001 vs control), harmaline plus WIN treated animals did not differ from controls in their capacity to learn (p>0.5 vs control). In the assessment phase of the test which was undertaken 24 hours after learning, an overall effect of treatment upon step through latency

was found ($F_{2, 27}=35.93$; $p<0.0001$; Fig.3B) where post hoc pairwise comparisons revealed that this measure was signifciantly decreased in both harmaline only and harmaline plus WIN groups (both $p<0.001$ vs control) although the harmaline plus WIN group was signifciantly increased when compared with the harmaline only group ($p<0.01$) indicating that WIN treatment was able to partially ablate the harmaline-induced reduction in step through latency. Finally, when time spent in the dark compartment during the assessment phase was examined, an overall effect of treatment was detected ($F_{2, 24}=21.09$; $p<0.0001$; Fig.3C) and could be attributed to signifciantly increased values for this parameter exhibited by both the harmaline only and harmaline plus WIN groups (both $p<0.001$ vs control).

3. Discussion

Affective and cognitive symptoms represent an emerging symptom domain in ET and for effective therapeutic interventions to be developed, require the development of, and examination in, appropriate animal models of symptoms and disease. Here, we evaluated the effect of a moderate, systemic dose harmaline, previously reported to produce some tremor and anxiogenic symptoms in mice, upon rats. Thereafter, we examined the effect of CB receptor agonist pretreatment upon harmaline-induced symptoms. Since CB receptor agonism has not only been reported to exert complex and dose dependent effects upon measures of cognition, but has also been anecdotally used to manage motor symptoms and affective disorders (Ware et al. , 2005).

Harmaline reliably induced moderate tremor consistent with previous behavioural studies which have shown similar effects such as ataxia, motor deficits and catatonia (Nasehi et al. , 2010, Vaziri et al. , 2015). Moreover, gait analysis revealed increased step width with

simultaneously decreased step length bilaterally. CB receptor agonist pretreatment did not affect harmaline-induced tremor or gait defects which is notable since CB receptor activation by the psychoactive plant cannabinoid, Δ^9-THC, typically produces motor disturbances in rodents (Taylor and Fennessy, 1982), dogs and humans yet has also been anecdotally claimed to exert beneficial effects in tremor disorders (Clifford, 1983b, Fitzgerald et al. , 2013). It is possible that the moderate tremor induced by 10mg/kg harmaline may not have been of a sufficient magnitude for any beneficial or detrimental effect of CB receptor agonism thereupon to be detected (i.e. inadequate effect size).

In the open field test, harmaline decreased both velocity and the total distance moved suggesting that treatment may be anxiogenic although harmaline-induced motor dysfunction (tremor and gait) may have directly impaired locomotor activity, independent of any effect upon anxiety. However, when the time spent in the different areas of the apparatus was assessed, harmaline treatment caused a decrease in time spent in the center and a corresponding increase in time spent in the perimeter suggesting that, irrespective of direct effects of harmaline upon motor function, treatment was anxiogenic, consistent with previously reported evidence in mice in the elevated plus maze (Hilber and Chapillon, 2005). Interestingly, while CB receptor agonism had no effect upon harmaline-induced deficits in velocity and distance moved, it did reverse harmaline's anxiogenic effects as assessed by time spent in the different areas of the open field apparatus. Therefore, our results indicate an important role of the endocannabinoid system in the modulation of stress-responses and these findings are consistent with the knowledge that an interrupted endocannabinoid signaling contributes to the progress of affective disorders, which is supported by clinical data like investigating the characteristics and patterns of cannabis and other drug use among long-term cannabis users in an Australian rural area, showed the most common

reasons for using cannabis were for relaxation or relief of tension and enjoyment or to feel good. The most commonly reported negative effects were feelings of anxiety, paranoia, or depression, tiredness, lack of motivation and low energy (Reilly et al. , 1998) and to examine the reasons for cannabis use among individuals with psychotic disorders, found that boredom, social motives, improving sleep, anxiety and agitation and symptoms associated with negative psychotic symptoms or depression were the most important motivators of cannabis use (Schofield et al. , 2006).

There is the hypothesis of fundamental and long-term changes in behavioral patterns due to cannabis consumption (Kowal et al. , 2011a, Shabani, Divsalar, 2012). They argue that such changes caused by the effects of cannabis on neurotransmitter systems, including opioidergic, GABAergic, glutamatergic dopaminergic and serotonergic systems, for example in the study of Mikael A. Kowal (Kowal et al. , 2011b), the results point to less efficient striatal dopaminergic functioning in chronic cannabis users. This finding seems crucial in understanding the suspected psychotic effects of long-term cannabis use and throws some doubts on the claim that cannabis-induced psychosis results from the combination of increased striatal and reduced prefrontal DA levels.

In the passive avoidance test, harmaline treatment impaired learned inhibition as indicated by the increased number of shocks required before animals met criterion and this impairment was not affected by CB receptor activation by WIN55, 212-2. When memory was assessed in the passive avoidance test, harmaline treatment impaired acquisition of passive avoidance as demonstrated by a shorter step through latency and longer time spent in the dark compartment. However, unlike learned inhibition, CB receptor agonism modulated this harmaline-induced impairment since WIN55,212-2 treatment partially reversed the harmaline-

induced reduction in step through latency although the harmaline-induced increase in time spent in the dark compartment was unaffected. Animal studies have demonstrated that an acute administration of CB1 agonists (e.g., natural agonist, Δ9-THC, and synthetic agonists, CP55940 and HU-210) and also pretraining administration of CB1/CB2 mixed agonist, WIN 55,212-2, attenuated acquisition of memory in various animal models (Abush and Akirav, 2010). In the water maze, systemic or local CA1 injections of AM251, WIN55, 212-2, and AM404 all impaired spatial learning, suggestion that targeting the endocannabinoid system may aid in the treatment of disorders associated with impaired extinction-like processes, such as post-traumatic stress disorder (Kruk-Slomka et al. , 2015). There are several evidences that harmaline has a number of diverse effects such as excitation, euphoria, motor tremor, alteration in associative and motor learning and calcium channel opening, with a resultant rise in neuronal excitability (Nasehi, 2015). It has reported increased caspase-3 activation in the lack of the CB1 receptors in knockout mice, indicating the neuroprotective potential of these receptors (Jackson et al. , 2005). Harmaline neurotoxicity could be mediated by glutamate excitotoxicity which could result in neuronal cell death. However, CB1 receptor activation in the brain inhibited the presynaptic release of glutamate, which has been shown to prevent excitotoxicity, leading to cell death (Shen et al. , 1996). Cannabinoids have been shown to inhibit voltage-activated Ca^{2+} channels (Daniel and Crepel, 2001) and cause a hypoglutamatergic condition by inhibiting the release of glutamate (Chemin et al. , 2001).

Previous behavioral studies such as step-down passive avoidance test, spontaneous eye blink rates, object recognition task, have shown a number of different effects for harmaline, such as alteration in associative memory and learning with a resultant increase in neuronal excitability (Dahhaoui et al. , 1992, Moura et al. , 2006, Nasehi et al. , 2015b, Nasehi, Piri, 2010). Harmaline

had previously been demonstrated to adversely affect memory retrieval, consistent with our findings here (Dahhaoui, Stelz, 1992). However, other studies have reported that harmaline treatment can enhance long term memory in adult Swiss mice received an intra-peritoneal injection of beta-carbolines alkaloids, harmine and harmol (1.0, 2.5 or 5.0 mg/kg) 30 min before training in an object recognition task. They induced an enhancement of short-term memory (STM) at all doses tested when compared to controls. (Moura, Rorig, 2006). These apparently contradictory results may be explained by harmaline effects acting through a number of brain areas when administered systemically. Harmaline has been revealed to a lower voltage-gated calcium channel currents, resulting in decreased neuron excitation (Handforth et al. , 2010). Calcium influx stimulates cellular signaling pathways involved in memory processes. Nasehi et al (2015) suggested harmaline through reduction of neuron excitation could decrease memory acquisition (Nasehi et al. , 2015c).

It was previously demonstrated that spatial learning and memory and passive avoidance learning and memory were negatively interfered with harmaline (Dahhaoui, Stelz, 1992). Our data did show administration of harmaline leads to impairments in memory retrieval of rats in contextual fear memory paradigms. The facilitative effect of WIN on memory extinction does not seem to be specific to contextual fear memory because it was also observed in the water maze reversal task (Hasanein and Teimuri Far, 2015).

In conclusion, the present study confirms that a moderate, systemic harmaline dose, in addition to inducing a classical tremor and motor disturbance, also elicits an anxiogenic response and impairs learned inhibition and acquisition of learned avoidance. Moreover, while systemic CBR agonism did not affect harmaline's motor effects in this model, anxiogenic and cognitive impairments were partially reversed.

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7. Figure Legend:

Figure 1: Effects of CBR agonist WIN on (A) tremor score, (B) step with, (C) left and (D) right step length after harmaline administration. (n=10 animals per group). Values show as means$\pm$SEM significantly different between harmaline versus control (** $p<0.01$ and ***$p<0.001$) and WIN versus harmaline (# $p<0.05$) group.

Figure 2: The effect of WIN55, 212-2 on explorative and anxiety like behavior changes induced by harmaline. Total distance moved (A) and velocity (B) decreased in harmaline and WIN groups. Anxiety like behaviors (C: time spent in the center; D: time spent in perimeter) altered by the harmaline administration while this effect reversed with 0.5 mg/kg WIN. $^{*}p<0.05$, $^{***}p<0.001$ as compared to the control group; $^{###}p<0.001$ as compared to the harmaline group.

Figure 3: The effect of harmaline - induced tremor and pretreatment with WIN on the fear learning in passive avoidance learning paradigm. (A): number of shocks received in the training day was altered in harmaline only group, which implies that fear learning, is altered by harmaline. (B): decreased step through latency (STL) indicates an impaired fear memory. Administration of WIN counteracted these impairments. (C): time in dark compartment was significantly increased by both the harmaline only and harmaline plus WIN groups.

***$p<0.001$ as compared to control group; ## $p<0.01$as compared to harmaline group.

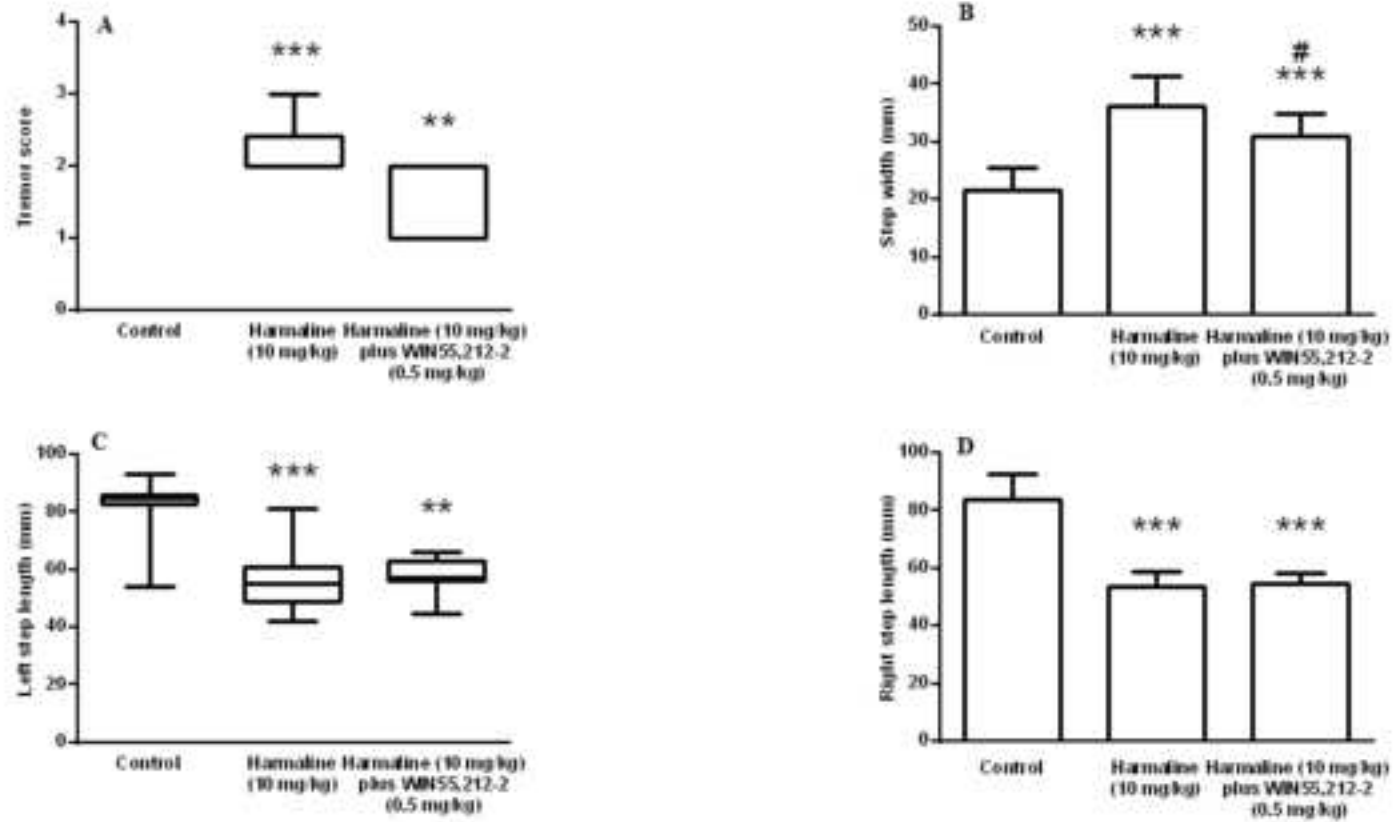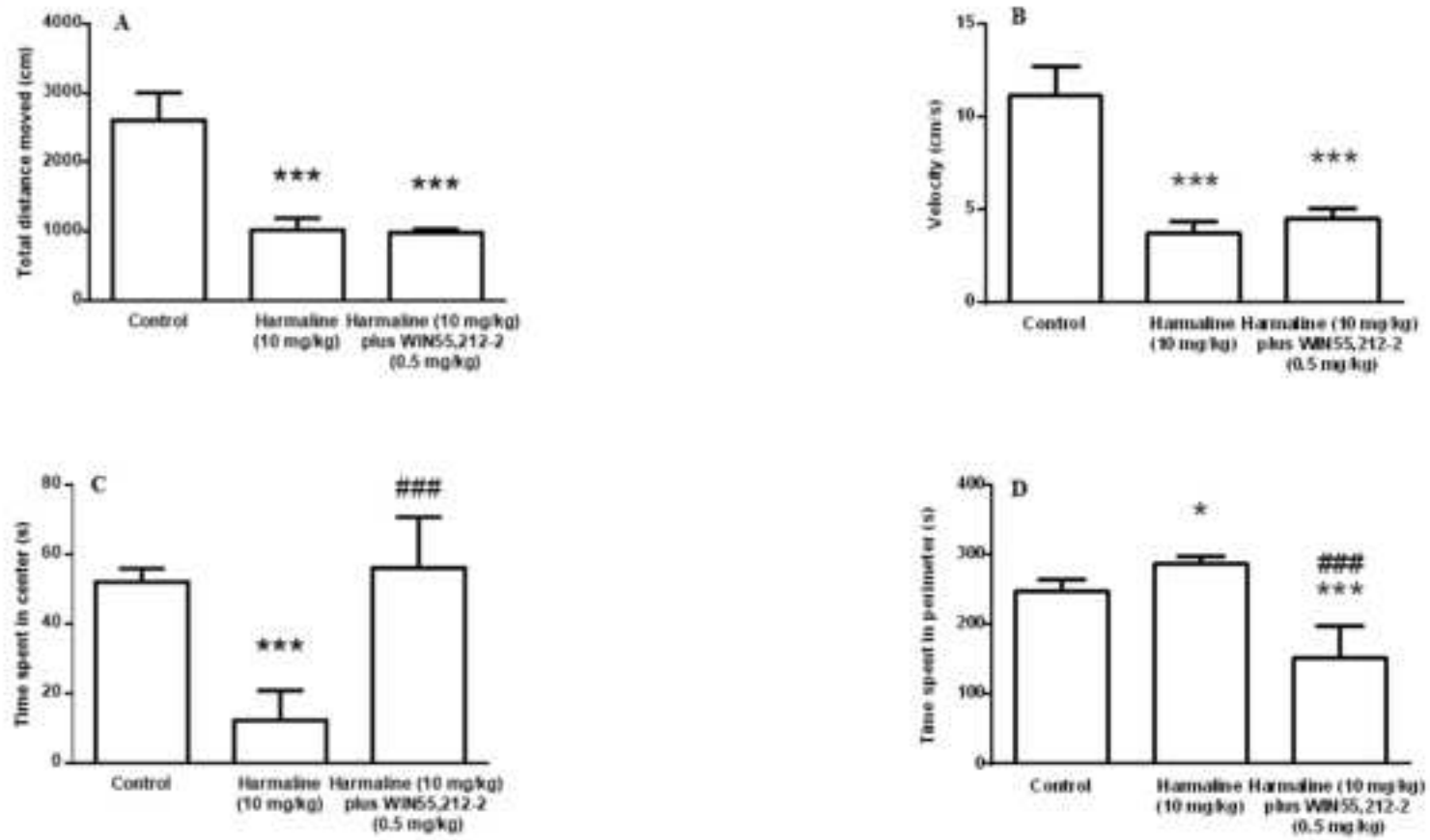## Fig 1

Fig 2

Fig 3