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Original Article

ESTIMATION OF THE ANXIOLYTIC-LIKE EFFECT OF THE β-CARBOLINE ALKALOID HARMINE ON STRESSED PREGNANT RATS

RIMA BENATOUI*, ABDELMADJID BAIRI, ABDELKRIM TAHRAOUI

Laboratory of Applied Neuroendocrinology, Department of Biology, Faculty of Science, Badji Mokhtar University Annaba, BP12 EL Hadjar–Algeria
Email: benattouiryma@gmail.com

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ABSTRACT

Objective: During the last decade, the role of the β-carboline alkaloid harmine has essentially been studied with regard to its anxiolytic effect, as it was done in our laboratory; therefore, this study has been progressed to cover the effect of this alkaloid on pregnant wistar rats.

Methods: The molecule was used at doses of 10 mg/kg, 15 mg/kg, pregnant female rats were divided into three groups according to the stage of pregnancy: first, second, and the third week of pregnancy. Each group has been subdivided into seven subgroups: control group, two treated groups with harmine, acute footshock stress at 1,2mA, sub-acute footshock stress at 0,4mA, psychological stress, and the treated group that footshocked after with 1,2mA, all groups were carried out open field test, plus maze test and light/dark box test.

Results: Thigmotaxis is reflected by the significant increase in the traveled distance in peripheral area in the open field of the three groups 'weeks' at dose of 10 mg/kg, the enhancement in the number and time of rearing, at both doses, during the second and the last week, the significant increase in the number of entries 'in open arms' in plus-maze during the first and third weeks at 15 mg/kg, and the significant decreased in time spent in the light compartment of the light/dark box at the same dose of all groups 'weeks' were noticed, which confirm the anxiolytic effect of the alkaloid, even in the case of the footschock stressed pregnant rats of all groups 'weeks' that enhancement of number of enties into open arms during the plus maze test.

Conclusion: So we can conclude that the anxiolytic effect of harmine not shortening to male rats, but expands to female pregnant wistar rats, and establishes its effect by diminishing time in light compartment of light/dark box and number of entries in open arms of plus maze, in other hand, the increase in the number and the time of rearing reflects the enhancement of exploratory behavior.

Keywords: Harmine, Pregnant rats, Footshock stress, Anxiolytic-like effect

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INTRODUCTION

The animal studies seem to corroborate the results in humans, as reported by Santos et, al. (2007) [1], who showed that regular ayahuasca users scored lower on the scales for panic and hopelessness, which are indicators of a depressive state. In more recent, controlled studies for the determination of Ayahuasca pharmacokinetics, Harmine was the highest concentrated component [2, 3], a number of its compounds are inhibitors of monoamine oxidase (MAO) [4]. The potential neurochemical target is MAO-A, an enzyme involved in the metabolism of monoamines in the brain and other organs [5].

Harmine is a tricyclic β-carboline alkaloid that was first isolated from the seeds of the Asiatic *Peganum Harmala* L. of the Zygophyllaceae [6-9] by Fritsche (1847), cited by Gunn (1912)[10], it forms its highest alkaloid of roots and dry seeds [11].

Researches head to explore beneficial effects of natural products as the case of the study revealing the anti-oxidative stress effect of lutein [12]. Alkaloids can have anti-malarial activity [13], The β-carbolines are a class of pharmacological agents, which have been shown to both antagonize the actions of the benzodiazepines and possess intrinsic actions of their own these intrinsic actions are opposite to the actions of benzodiazepines and β-carbolines have therefore been termed "inverse agonists" [14]. Harmine may exert a protective effect on neuronal cells against a variety of insults [15], suppression of inflammatory cytokines in the hippocampus of the rat brain [16]; inflammation is considered as the body's response to injury and danger. This process occurs as a defensive response, which induces profound physiological adaptations triggered in an attempt to limit tissue damage and remove the pathogenic insult [17], immune modulator influences [18]. Harmine reduces lipid and protein peroxidation, and increased superoxide and catalase activities in the hippocampus and prefrontal cortex [19]. Moreover, it was able to bind to benzodiazepine, serotonine, and dopamine (DA) [20].

Footshock stress, which is primarily a physical stressor (pain) when used acutely, alters the firing rate of noradrenergic neurons of the locus coeruleus (LC) [21] and medial prefrontal cortex (mPFC) [22]. Moreover, in response to stress, there are also changes in dopamine and serotonin systems [23, 24], but at a considerable intensity, these parameters will be inverted, because, the serotonergic system plays a role in the regulation of the sympathetic nervous system and the hypothalamo–pituitary–adrenal axis (HPA A), and in the same way glucocorticoids and catecholamines affect central serotonergic systems [23, 25].

The acute footshock stress leads to changes in the activity of mPFC neurones, which could involve the LC-mPFC noradrenergic pathway [26]. Long-lasting nociceptive pulses in control animals, effectively, evoke the well-documented early LC response, corresponding to rapidly conducting peripheral nerve activation (A fiber) [27]. After a period of neuronal inhibition, this fast response was followed by the late response that appears to correspond to C-fiber activation [28]. Even the oral route of administration still continues to be the most preferred route due to its manifold advantages including ease of ingestion [29]. In this study, the route of the administration was intra-peritoneal due to the inactivation of the alkaloid after oral administration, and it seems likely that at least some unmetabolised harmine can enter the central nervous system following oral consumption [30].

Our study was designed for revealing the total effects of harmine on behavioral locomotion, measured with the traveled distance in open field test, in other hand, the time spent in the light compartment of the light/dark box that reflect the level of anxiety of psychological

stress and physical (pain) in comparison those treating before the stress during different pregnancy phases of female rats.

MATERIALS AND METHODS

Animals and housing

One hundred and five adult female wistar rats (180–210 g), after becoming pregnant, the experiment was repeated five times (n=5), 2-3 mo of age obtained from Pasteur Institute (Algiers, Algeria) were used in the present study. Rats were housed in a controlled temperature (22±1 °C) with a dark/light cycle of 12h/12h, initially, living in five in clear polyethylene cages with standard pellets food and water *ad libitum.*

Product

Harmine $C_{13}H_{12}N_2O$, CAS 442-51-3; (TCI, Japan). Harmine were injected at doses of (10 mg/kg, or 15 mg/kg). Drug concentrations were prepared; immediately, prior to use, the necessary dose could be injected in a volume of 0.1 ml per 100 g body weight of rats. To ensure its dissolution heating plate was been used, because dissolution may be the rate determining step for the onset of therapeutic activity [31]. The pretreatment time regarding behavioural tests was realized due to a maximal level of pharmacological activity.

Procedure

In order to induce pregnancy; rats were housed 1 male with 1 female, rats were mated overnight, the day on which spermatozoa were present in a vaginal smear was designated as the day 0 of pregnancy. Pre-determined pregnant rats were classified into control, two groups received footshock, one group 'psychological stress' was putting in the same conditions of the stressed groups without receiving shocks [32], and a group injected with harmine one hour before acute stress. Each group was divided into three groups according to the stage of pregnancy as follows: during (the first, second and last week); decapitation was respectively on the 7^{th}, 14^{th}, and the last day of gestation. One day before decapitation pregnant rats were tested. This study is the first to get differences in the anxiety-like effect of footshock and antidepressant effect of harmine during pregnancy in female rats.

Footshock procedure

The resistor was an instrument for maintaining the electric intensity (Ohmite mfg Co, Illinois) was used. The animals were placed; individually, in a Plexiglas shock cage (18 x 12 x 10 cm) with a metal grid floor, through which the shocks were delivered; it consisted of 4 stainless rods (each 2 cm in diameter) at 0, 5 cm intervals. Shock intensity was 0,4 mA and 1,2mA; respectively, Phy S1 and Phy S2, three shocks were delivered per minute, during 30 min. Both intensities were chosen by the experimenter according to the hoped results. They were applied between 9:00 and 13:00 h, the cage were cleaned with a solution of alcohol of 70°, and completely dried before placing another animal.

Open field test (OFT)

OFT is a common measure of exploratory behaviour, general activity in rodents, and was originally used to investigate the emotionality of rats 'fear' [33]. Also, this test allows to the measure of the response action of the rats into a non-familiar environment. In addition, the open field model is based on rodent aversion to open spaces, and a tendency to walk close to walls, a behaviour known as thigmotaxis [34].

In order to assess possible effects of drug treatment, or stress on spontaneous locomotor activity; A square open field (70 x 70 cm), delimited by transparent plexiglass walls (45 cm high) was used, the maze was divided into squares (10 x 10 cm); the squares, in the border, '24' were considered as the peripheral area of the box. Rats were placed, individually, in the centre of the black box and allowed to explore freely for 5 min. Entry to a zone was defined as occurring when an animal placed all its four limbs into the compartment; travelled distance in the peripheral area, time and number of rearing were recorded, after every passage the box was cleaned by a solution of 70 ° of alcohol. After this test rats were placed in the elevated plus maze.

Elevated plus maze (EPM) test

Originally, a so-called symmetrically elevated Y-maze was created for rats by Montgomery (1955) [35]. It was modified after thirty years by Pellow *et al*. (1985) [36] into the EPM. The fact that the test involves spontaneous exploration by rodents of the environment, in the absence of explicit reward or consummatory behaviour, accounts for its classification as an etiological model [37]. Where rats faced to contradictory conditions for exploring the new space allocated to stretching; in a curious situation, or recession from the open, restricted, and elevated arms, which is considered as an external compartment in the fear situation.

Although EPM is used for assessment of anxiety-related behaviors in rats [38], the apparatus consist of two wooden arms of (110 x 10 cm) interconnected by a square of 10 cm^2, the plus-shaped platform contain two opposed open arms (50 x 10 cm), and two opposed enclosed arms (50 x 10 cm). The apparatus was covered by a black Plexiglas which was elevated 73 cm above the flour. Rats were placed, individually, on the centre of the platform and were allowed to freely explore the maze for 5-min of testing period. An entry was recorded when the animal entered the arm all its four paws, The total number of arm entries, were taken as indices of overall activity [39], between a rat and the next one the maze must be cleaned with a solution of 70% of alcohol. The recorded video was analyzed to measure the number of entries into open arms, and enclosed arms.

Light/dark box (LDB)

This test is used to assess anxiety of the rats. The conventional measures of anxiety-like behaviour in the LDB are; the number of transitions made between two compartments, and the time spent in the brightly illuminated area [40]. In other words, LDB is based on the innate aversion that rodents have of brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors [35, 36].

The labyrinth of open field was divided equally into two chambers one was covered by the dark cover to form the dark compartment, and the other chamber was illuminated by a lamp located approximately 120 cm above the apparatus, an opening of (9 x 12 cm) using by the rat to move between chambers. During the test, an individual rat was placed in the centre of the light compartment facing to the dark chamber to explore both compartments for a period of 5 min. The recorded video was analyzed for the measure of the time spent in light compartment and the dark one.

Statistical analysis

With using Minitab 17 statistical analysis, all data are presented as mean±SEM Differences among experimental groups in the open-field test, Plus maze and dark/light box tests were determined by one-way ANOVA, followed by Tukey test when ANOVA was significant; *P* values less than 0.05 were considered to be statistically significant.

Traveled distance in open field

Fig. 1 showed that treatment with harmine at a dose of 10 mg/kg increased the traveled distance in the open field significantly ($p<0,05$), during the three phases of gestation. The psychological stress increased it significantly during the first, second week ($P<0,01$), and the third week ($P<0,05$). While after a sub-acute stress the significant increase during the first week ($p<0,001$), the second, and the third week ($P<0,01$), the acute stressed rats are the alone group marked decrease in traveled distance, and significantly during second week ($P<0,05$); even though, treated stressed rats showed its significant increase during the three periods.

RESULTS

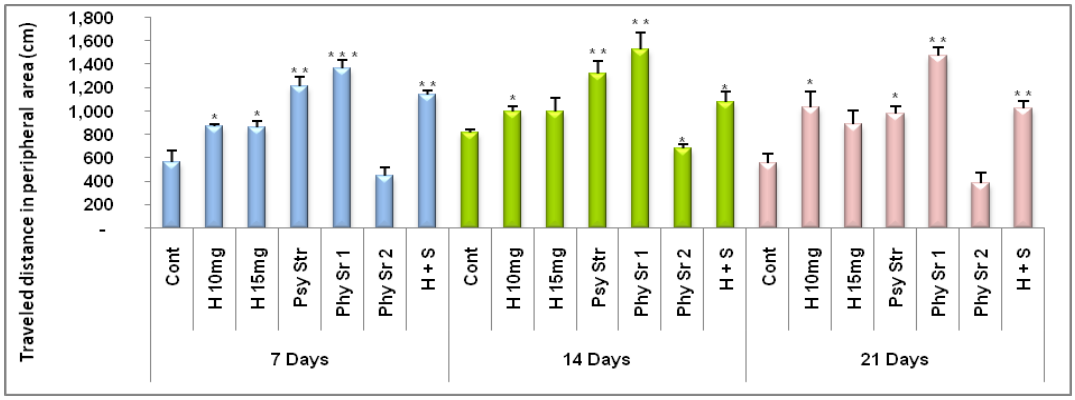

Fig. 1: Effects of psychological, physical stress and its treatment with harmine on traveled distance (cm) in the peripheral area of open field arena during three phases of pregnancy. 'Phy S1 and Phy S2, 0,4 mA and 1,2 mA; respectively' where n=5 (*p<0.05; p<0.01;*** p<0.001)**

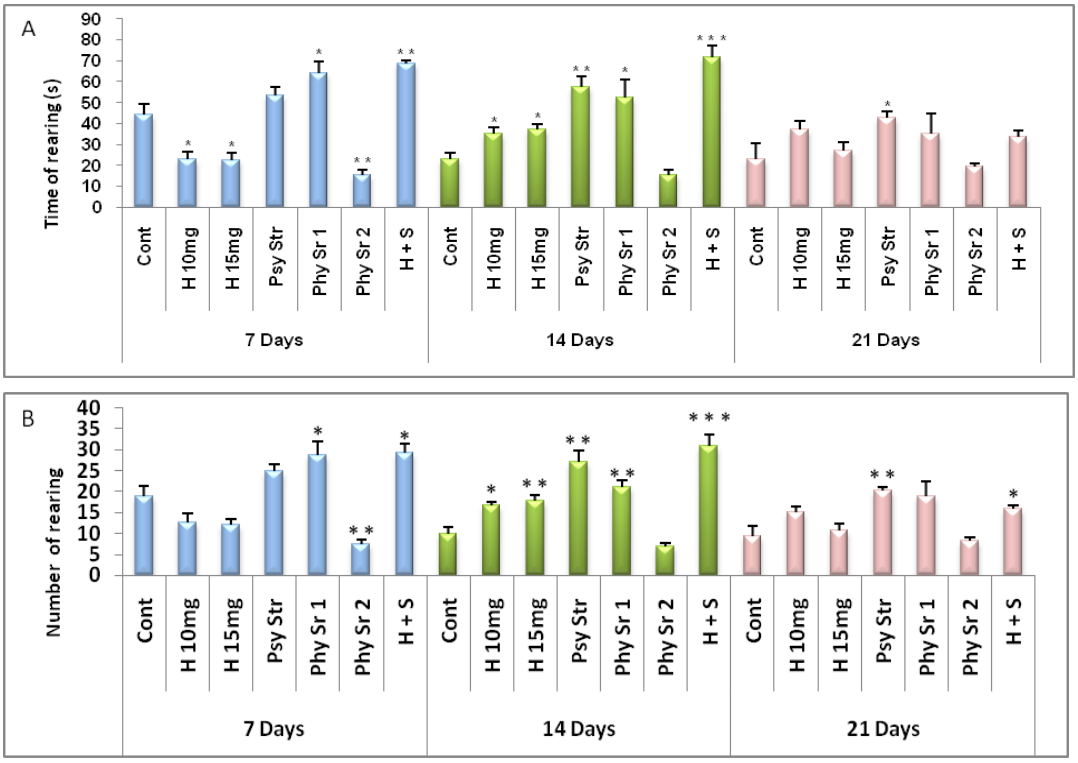

Fig. 2: Effects of psychological, physical stress and its treatment with harmine on (A) time of climbing (s) and (B) number of climbing in open field arena during three phases of pregnancy. 'Phy S1 and Phy S2, 0,4 and 1,2mA; respectively' where n=5 (*p<0.05; p<0.01;*** p<0.001)**

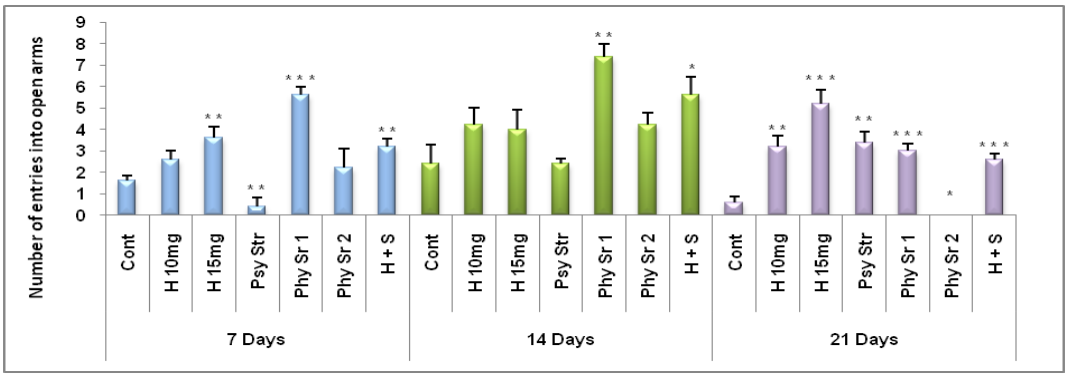

Fig. 3: Effects of psychological, physical stress and its treatment with harmine on a number of entries in open arms, in plus maze test, during three phases of pregnancy. 'Phy S1 and Phy S2, 0,4 and 1,2mA; respectively' where n=5 (*p<0.05; p<0.01;*** p<0.001)**

Time and number of rearing in open field test

During the second week of pregnancy, the acute treatment with harmine 10 and 15 mg/kg increased them significantly, but the psychological stress increased them significantly during the second and third weeks. The sub-acute footshock stress increased significantly (p<0,05) the time of rearing in the first and second week (fig. 2A), the acute stress was the alone factor that decreased them and significantly (P<0,01) during the first week (fig. 2A. B); even though, the treated stressed groups increased them significantly during the first and second week (fig. 2A. B).

Number of entries into open arms

Female rats had a few possibilities to visit the open arms, during the third week of pregnancy, even though, the acute treatment with harmine at 10 or 15 mg/kg increased it significantly (fig. 3) and respectively (P<0,01; P<0,001), psychological stress induced a decrease during first and second week, but increased it significantly during the third week (P<0,01), and a significant increase after the sub-acute stress during the different stages of pregnancy, and a significant decrease after the acute stress during the third week, the treated stressed groups increased significantly the number of entries into the open arms during the three periods differently (1st P<0,01; 2nd P<0,05; 3rd P<0,001).

Number of entries into closed arms

As the case of the number of entries into the open arms, number of entries into closed arms characterized by an increase during the second week, and a fall during the third week of pregnancy, and with the treatment of harmine at dose of 15 mg/kg, rats showed a significant increase (fig. 4) during the first and the third week (P<0,05; P<0,001), the psychological stress decreased it during the second week, and increased it significantly during the third week, while the sub-acute footshock stress increased it significantly during the both periods (P<0,05).

The acute stress decreased it only during the third week, but not significantly with no significant changes in treated stressed groups.

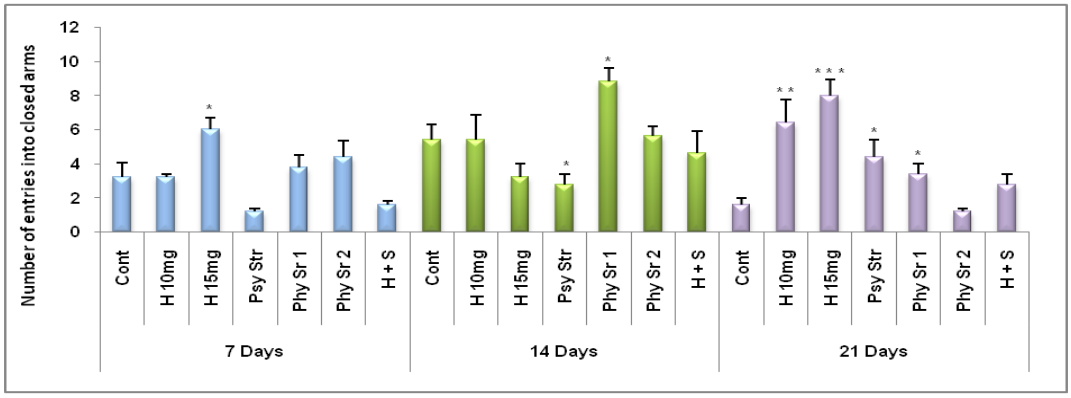

Fig. 4: Effects of psychological, physical stress and its treatment with harmine on a number of entries in closed arms, in plus maze test, during three phases of pregnancy. 'Phy S1 and Phy S2, 0,4 and 1,2mA; respectively' where n=5 (*p<0.05; p<0.01;*** p<0.001)**

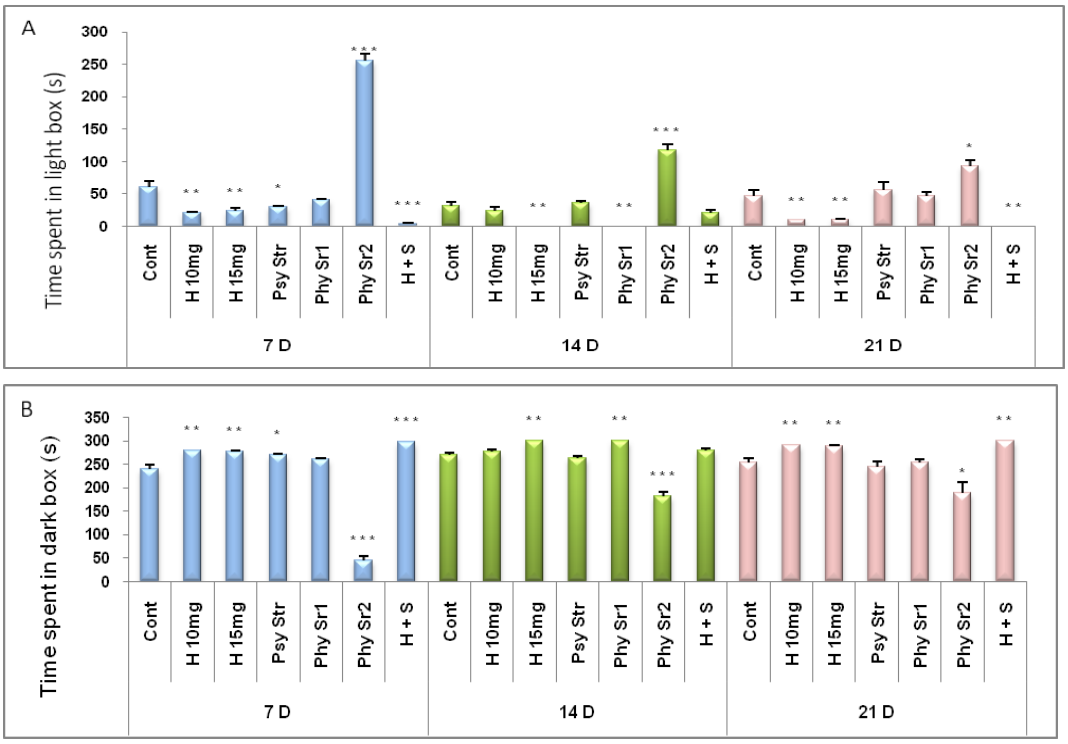

Fig. 5: Effects of psychological, physical stress and its treatment with harmine on (A) time spent in light box, (B) time spent in dark box, during three phases of pregnancy. 'Phy S1 and Phy S2, 0,4 and 1,2mA; respectively' where n=5 (*p<0.05; p<0.01;*** p<0.001)**

Time spent in LDB

Time spent in lightbox (fig. 5A) was significantly decreased by acute treatment at 15 mg/kg, in each period of treatment, while the treatment at 10 mg/kg decreased it significantly only during the first and third week (P<0,01), the acute treatment increased it significantly during the first, second week (P<0,001), and the third week (P<0,05), the treated stressed groups showed its significant decrease during the first (P<0,001), and the third week (P<0,01); Consequently, the decrease and increase of time spent in the dark box (fig. 5B) inversely changed for each group with the same rhythm of significance of the time spent in the light box.

DISCUSSION

Exposure to harmine treatment during 6 d, during the first week of pregnancy, enhanced the locomotor activity and exploratory capacities of rats in all behavioral tests; plus maze, open field and light/dark box; So, its effect during other different phases with the same period of 7 d, was similar to its antidepressant effect in male rats [43].

The ventral pallidum (VP) is a basal forebrain structure that is interconnected with motor and limbic structures and may be considered as an interface between motivational and effector neural signals [12]. Stimulation of the DA receptors in the VP through direct and indirect DA agonists increases locomotion [44, 45]. Interestingly, pretreatment with β-carbolines decreases in firing rate of neurones in the VP followed by apomorphine administration after inhibiting of dopaminergic projection conducted to the VP [12].

Despite the significant increase in extracellular of DA levels induced by harmine, it had no changes in locomotor activity [46] in the OFT [47]. While it is possible that the MAO-A inhibition of harmine has some functional effect on DA efflux in the striatum *in vivo*. The evidence presented here indicates that such activity does not modulate DA efflux at pre-synaptic terminals within the nucleus accumbens shell [48]. Only, the treatment at a dose of 15 mg/kg during the second and third week is in accord with these works. Moreover, our results could reflect the effect of harmine on DA efflux in the striatum, during all phases of pregnancy, at a dose of 10 mg/kg.

The increase in motor behaviour after a session of footshock stress was explained as a lesion of medial rugh nuclei (MRN), devoid of any change in vertical exploratory behaviour [49-51]. While in this study, female pregnant rats, after a sub-acute stress show a significant increase in both, travelled distance and vertical exploration, in another hand, during plus maze test the number of entries into open arms increased, which form the excellent sign of the anxiolytic-like effect of the sub-acute stress.

The VP innervated by dopaminergic and glutamatergic fibres from the ventral tegmental area [45, 52]. So, pregnant female rats stressed by an acute footshock showed a diminished motility, they can enhance mobility after an acute footshock stress prevented by an injection of 10 mg/kg of harmine.

Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety [53]. Thiébot *et al.* (1980) [54], had advised caution by concluding that the anxiolytic effect of benzodiazepines depends exclusively on a selective effect on serotonergic neurotransmission. The predictive validity of the EPM is good with regard to the effectiveness of benzodiazepine/gamma-aminobutyric acidergic drugs, but inconsistent evidence has been shown for various neuropeptides and serotonergic drugs [55], and an inverse activation of the benzodiazepine receptor plays only a minor role, if any, in the effects of harmine on dopamine release and metabolism in the rat striatum [46]. The significant increase in the number of entries into open arms by pregnant female rats established the effect of harmine on benzodiazepine receptors. Furthermore, harmine in female rats can exert well its anxiolytic effect due to the highest number of serotoninergic receptors, compared to male rats.

Sedation and anxiety are primarily mediated in the CNS by the GABA-A receptor complex, which is also involved in other physiological and neurological disorders, such as, epilepsy, depression, Parkinson syndrome, and Alzheimer's disease [56]. Depression can be described as a mood, a state of being or energy level that includes lack of motivation, a sense of hopelessness and a lack of physical energy [57]. Rosa *et at.* (2000) [58] revealed that the reduction in motor activity and enclosed arm entries exhibit a considerable level of anxiety. In this study, the number of entries into open arms, and enclosed arms demonstrated the anxiolytic-like effect of harmine that corresponds with all measures revealing the anxiolytic-like effect of harmine during each phase.

Increased open arm time of stressed rats in the EPM has also been noted following inactivation of either the hippocampus [59], or mPFC [60]. And physical stressors include disturbances of the internal environment and psychological stressors are stimuli that affect emotion and induce fear, anxiety, or frustration; stressors may be work in combination [61].

The lake of a number of entries into all arms after a psychological stress, during the first and second week, it is a sign of its anxiogenic effect, but, the enhancement of traveled distance during OFT confirms the curiosity induced by the psychological stress.

The total traveled distance was indicative of locomotor activity and the number of rearing was indicative of exploratory behavior [38, 62]. In this case, the exploratory behavior follows the enhancement of the anxiolytic-like effect of the molecule applied on pregnant female rats; especially, during the second and third week.

This decrease in latency to enter the dark compartment may represent an overall increase in locomotion in the face of novelty, or it could be interpreted as a measure consistent with increased caution, or “anxiety-like” behaviors [42, 43]. During LDB test, control female rats showed a less anxiety during the second week, which in accord to results of plus maze test, as the increase in number of entries into open arms.

The essential oil extracted from *Vitex agnus-castus* produced antinociception in the acute corneal pain through mechanisms that involved an opioidergic system and/or the cholinergic system, but not via L-arginine/NO/cyclic GMP pathway, supporting the folkloric usage of the plant to treat various painful processes [63]. With to the analgesic effect of harmine on the footshocked pregnant rats under study, pain has not been established yet, so, in further studies, we have to pay important to the NO group and see if it included in this mechanism.

CONCLUSION

All the results approve that the anxiolytic-like effect of harmine not narrowed to male rats as it has been found in previous studies, but it can preclude the anxiety of pregnant rats in the case of the significant decrease in time spent in light compartment of the dark/light box at the dose of 15 mg/kg, of the treated pregnant rats, and its capacity enlarged to prevent the anxiogenic effect of the foot-shock stress in groups showed a significant increase in the number of entries in open arms of the elevated plus-maze test, and the significant decrease in time spent in light compartment of light/dark box of pregnant rats concerning the three phases of pregnancy, in other hand, its enhancement to the exploratory capacities elucidated by the elevation of the number and time of rearing during the second and the third week of pregnancy.

CONFLICT OF INTERESTS

All authors have none to declare

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