



Corticotropic and Behavioral Response of Stressed Pregnant Rats Treated with Harmine

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ABSTRACT

Harmine was drawn increasing attention in recent years as an antidepressant in male rats; in this regard our study has the merit to clone and characterize, for the first time, the effect of harmine on pregnancy and its treatment to footshock stress, the alkaloid were injected intraperitoneally, at two doses of 10mg/kg and 15mg/kg during seven days in three different periods of pregnancy. In other hand the footshock stress was applied at 2 intensities 0,4 mA and 1,2 mA; a psychological stress, and the stressed treated group, all groups were controlled by a group of pregnant rats, a set of behavioural tests were applied during the last day of injection, the 6th, 13th and the last day of pregnancy, Ethylenediaminetetraacetic acid (EDTA) plasma samples were used to be analyzed for adrenocorticotrophic hormone (ACTH); the significant increase in time spent in open arms in plus maze test (PMT) in the case of treatment of the stressed groups, and the significant reduction in immobility during open field test (OFT) and forced swim test (FST) were noticed in treated pregnant rats, accompanied with the decreased levels of ACTH, that's confirm the antidepressant effect of harmine applied to the pregnant rats and pregnant stressed rats.

Keywords: Harmine, pregnant rats, footshock stress, antidepressant effect.

INTRODUCTION

During last decades the opportunity of stress applied by environment conditions on animals increased. So, as experiments designed to detect synthesis of β -carboline in mammalian brain from abundant indole precursors such as serotonin and tryptophan, may yield negative results if the possibility of further metabolism by brain P450s is not considered¹, and based on increasing importance in the field of synthesis of heterocyclic compounds, a series of researchers has been concentrated on development of heterocyclic compounds having therapeutic high potential toward a variety of human diseases^{2,3}.

Recently, a growing body of evidence has indicated that harmine presents antidepressant-like actions in rodents subjected to an animal model of depression^{4,5}.

The monoamine neurotransmitters such as serotonin, noradrenaline and dopamine in the central nervous system play a key role in the pathophysiology of depression⁶. An alternative explanation is that the functional site of monoamine oxidase A (MAO-A) inhibition is distal to the striatum and inhibition may be due to an indirect mechanism of modulation via augmented levels of serotonin⁷. As described previously, harmine penetrates the brain very rapidly, most likely due to its highly lipophilic structure⁸⁻¹⁰. It acts preferentially on MAO-A at a nanomolar concentrations (IC₅₀=2-5 nM)¹¹, while there is additional water molecules involved in the binding of harmine with MAO-A¹²; that's responsible of the reversion and the mimic stabilization of the link.

In fact, prominent mechanism by which the brain reacts to acute and chronic stress is activation of the hypothalamo-pituitary-adrenal HPA axis¹³. The stress hormones, including ACTH, cortisol (corticosterone in rodents), adrenaline and noradrenaline, are critical to the acute stress responses¹⁴.

Such responses are mediated via the paraventricular nucleus of the hypothalamus, which stimulates the release of ACTH from the anterior pituitary gland, which, in turn, stimulates the release of corticosterone from the adrenal cortex into the circulatory system¹⁵.

The infusion of estradiol into the brain increases corticosterone responses^{16,17}.

In other hand, Neumann¹⁸ had reported that, during the last week of pregnancy HPA axis responses to stress are progressively reduced.

Moreover, the effects resulting from some prenatal stress regimens cannot be attributed only to 'prenatal' factors since it has been shown that stress experimented during pregnancy alters future maternal behavior in rats¹⁹.

The aim of the present study is to discern the antidepressant-like effect of harmine in pregnant female rats, and those subjected to footshock stress during three different periods of pregnancy.

MATERIAL AND METHODS

Animals and Experimental Design

Animals and Housing

One hundred and five adult female Wistar rats (180–210 g), 2-3 months 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*.

Drugs

Harmine $C_{13}H_{12}N_2O$, CAS 442-51-3; obtained from TCI (Tokyo, Japan) was 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. The pretreatment time regarding behavioral tests was realized due to maximal level of pharmacological activity.

Treatment

In order to induce pregnancy; rats were housed 1 male with 1 female so rats were mated overnight, 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 receiving footshock, one group 'psychological stress' was putting in the same conditions of the stressed groups without receiving shocks²⁰, two treated groups at a 10 mg/kg and 15 mg/kg doses of harmine, injections were around 1 h before tests²¹, and a group receiving harmine one hour before acute stress. Each group (n=5) 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 7th, 14th, and the last day of gestation. A day before decapitation pregnant rats were traveled test.

Footshock Procedure

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 1,2 mA and 0,4 mA, four shocks were delivered per minute, during 30 min. It were applied between 9:00 and 13:00 h, cages were cleaned with a solution of alcohol of 70°, and completely dried before placing another animal in the shock cage.

Behavioral Tests

OFT

The OFT is a common measure of exploratory behaviour and general activity in rodents, and was originally used to investigate the emotionality of rats 'fear'²². Also this test allow too 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 thigmotaxis²³.

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). Division of areas was onto two

zones distinguished: 20 squares at the border were considered as the peripheral zone, the center contains 25 squares, the corners were four squares of 10cm²; pregnant rats were individually placed in the center 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 four limbs into the compartment, after every passage the box was cleaned by a solution of 70° of alcohol. Directly after this test rats were placed in the elevated plus maze.

FST

Immobility is reduced by various clinically effective antidepressant drugs²⁴. The antidepressant effect of MAO inhibitors in this model was first described by Loomer²⁵.

The aquarium was a box of glass with (35 x 35 x 60 cm). The basic FST involves two sessions; animals were placed individually in the tank filled to 35 cm of water with a temperature (22-23°C). The first session was 15 min; followed by a test session of 5 min 24 h later.

Generally rats climb during the first minute to avoid the tank. The best immobility posture of rats is characterized by a floating in the tank with minor movement of posterior paws to keep their nose above the surface of water.

The importance of this test is to assess depression of the psychological stressed rats in comparison to physical stressed rats and the aptitude of antidepressant effect of harmine on pregnant rats.

PMT

Originally, a so-called symmetrically elevated Y maze was created for rats by Montgomery²⁶. That was modifying thirty years after by Pellow²⁷ into the elevated plus maze (EPM). The fact that the test involves spontaneous exploration by rodents of the environment, in the absence of explicit reward or consummatory behavior, accounts for its classification as an ethological model²⁸. Where rats faced to contradictory conditions for exploring the new space permitted to stretching; in curious situation, or recession from the open, restricted and elevated arms; that regards as an external compartment in fear situation.

Although, EPM is used for evaluating anxiety-related behaviors in rats²⁹, the apparatus consist of two wooden arms of (110 x 10 cm) interconnected by a square of 10 cm², the plus-shaped platform contain two opposed open arms (50 x 10 cm), and two enclosed arms (50 x 10 cm). The apparatus was covered by a black Plexiglas which was elevated 73 cm above the floor. Rats were placed individually on the center 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. Similarly, the percentage of time spent in the open arms in the elevated plus-maze test is increased by treatment with clinically effective anxiolytic agents but is decreased by anxiogenic agents³⁰, between



a rat and the next one the maze must be cleaned with a solution of 70% of alcohol. The recorded video was analyzed for measuring time spent in open and closed arms.

Measurement of ACTH

Collecting of blood samples of rat groups were collected by tail bleeding in EDTA tubes to determine the ACTH, the plasma samples were used to determine ACTH using a commercially available radio immune assay (RIA) kit according to the manufacturer’s instructions.

Statistical Analysis

With using Minitab 17 statistical analysis, all data are presented as mean± S.E.M. Differences among experimental groups in the, open-field test and Plus maze were determined by one-way ANOVA; *P* values less than 0.05 were considered to be statistical significant.

RESULTS

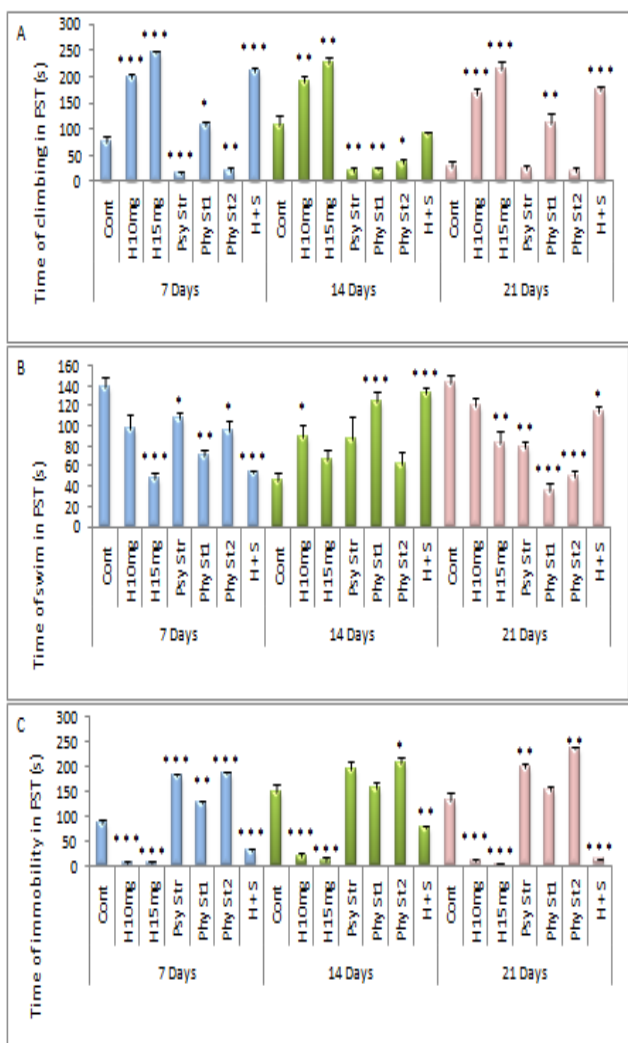


Figure 1: Effects of psychological, physical stress and its treatment with harmine on (A) time of climbing (s), (B) time of swim (s) and (C) time of immobility in FST, during three phases of pregnancy. Psychological stress ‘Psy Str’, Phy St1 and Phy St2 ‘0,4 mA and 1,2mA respectively’ (n=5) (**p*<0.05; ** *p* <0.01; *** *p* <0.001).

FST

Time of Climbing

Figure 1A showed an increase in time of climbing during the second week of pregnant female rats, after a treatment of harmine at 10 and 15 mg/kg, it increased significantly during the first, the third (*P*<0,001), and the second week (*P*<0,01), psychological stress decreased it significantly during the first and second week respectively (*P*<0,001; *P*<0,01), sub-acute stress could increase it significantly during the first and third week (*P*<0,05; *P*<0,01), while the acute stress decreased it significantly during the first and second week (*P*<0,01; *P*<0,05), the treated stressed groups showed a significant increase in time of climbing during the first and last week (*P*<0,001) of pregnancy.

Time of Swim

During the first and third week, of pregnancy, it decreased significantly after a treatment of 15 mg/kg (*P*<0,001; *P*<0,01) (Figure 1 B), but during the second week it increased significantly (*P*<0,05), at dose of 10 mg/kg. The psychological stress decreased it significantly during the first and the last week (*P*<0,05; *P*<0,01), the sub-acute stress decreased it significantly during the first and the third week (*P*<0,01; *P*<0,001), while it increased it significantly (*P*<0,001), during the second week. The acute stress induced a significant decrease during the first and third week (*P*<0,05; *P*<0,001), the treated stressed groups showed a significant increase during the second and the last week, while, during the first week it decreased significantly (*P*<0,001).

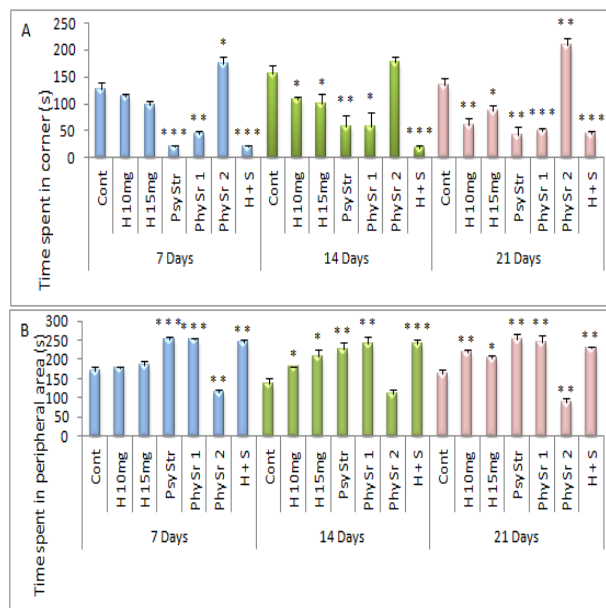


Figure 2: Effects of psychological, physical stress and its treatment with harmine on (A) time spent in corner (s) and (B) time spent in peripheral area in open field test, during three phases of pregnancy.

Psychological stress ‘Psy Str’, Phy Sr1 and Phy Sr2 ‘0,4 mA and 1,2 mA respectively’ (n=5) (**p*<0.05; ** *p* <0.01; *** *p* <0.001).



Time of Immobility

According to Figure 1C, female pregnant rats demonstrated the highest level of depression during the second week of pregnancy; but after an acute treatment with harmine, in every phase, it decreased significantly ($P < 0,001$), psychological stress increased the immobility significantly during the first and last week ($P < 0,001$, $P < 0,01$), the sub-acute stress induce a significant increase during the first week ($P < 0,01$); but acute stress, during each trimester, rise significantly the immobility; even though, treated stressed groups reduce it significantly during each phase.

OFT

Time Spent in Corner and Peripheral Area (s)

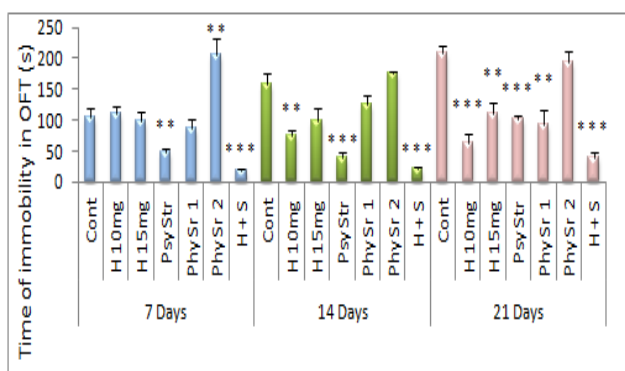


Figure 3: Effects of psychological, physical stress and its treatment with harmine on time of immobility (s) in open field test, during three phases of pregnancy. Psychological stress 'Psy Str', Phy Sr1 and Phy Sr2 '0,4 mA and 1,2mA respectively' (n=5) (* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$).

Acute treatment with harmine induce a significant decrease in time spent in corner during the second and last week of pregnancy (Figure 2A), psychological and sub-acute stress decreased it during all periods, the acute stress increased it significantly during the first and third week ($P < 0,05$; $P < 0,01$), and the treated stressed groups decreased it significantly during each period ($P < 0,001$).

Inversely to the time spent in corner (Figure 2B) show an increase after each apply; but differentially, except the acute stress, which decreased it significantly during the first and third week ($P < 0,01$); thereafter, the treated stressed groups increased it significantly during the first, last week ($P < 0,01$), and the second week ($P < 0,001$).

Time of Immobility

Female rats proved an increase in time of immobility during pregnancy in open field test (Figure 3), treatment with harmine at 10 mg/kg, during the second and third week decreased it significantly ($P < 0,01$; $P < 0,001$); the psychological stress decreased it during the three stages (1st $P < 0,01$; 2nd and 3rd weeks $P < 0,001$), acute stress was rise significantly only during the first week, ($P < 0,01$); even though the treated stressed groups show a significant decrease in every period ($P < 0,001$), with a small increase in each period.

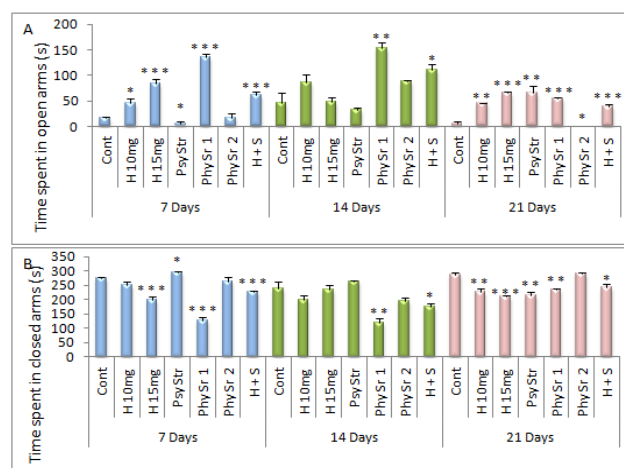


Figure 4: Effects of psychological, physical stress and its treatment with harmine on (A) time spent in open arms, (B) time spent in closed arms, during three phases of pregnancy. Psychological stress 'Psy Str', Phy Sr1 and Phy Sr2 '0,4 mA and 1,2mA respectively' (n=5) (* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$).

PMT

Time Spent in Open arms and Closed Arms

Pregnant rats increased time spent in open arms during the second week (Figure 4A), with a notable decrease during the last week, even though, the decreased of time spent in third week of pregnancy after a sub-acute stress was significant ($P < 0,05$), while harmine at 15mg/kg and acute stress induced a significant increase ($P < 0,001$); psychological stress and treatment of harmine increased significantly at 10mg/kg ($P < 0,01$), consequently; treated stressed group increased it significantly ($P < 0,001$), as like during the first week, and the second week too it increased significantly ($P < 0,001$; $P < 0,05$).

Concerning to, the treated groups at 15mg/kg, time spent in closed arms decreased significantly ($P < 0,001$) during the first and third week (Figure 4B), while the decreased time at 10mg/kg were significant only during the third week ($P < 0,01$), even though, treated stressed groups showed a significant decrease during the second and last week ($P < 0,05$), and the first week ($P < 0,001$). While the psychological stress shown a significant increase during the first week, and a significant decrease during the third week ($P < 0,01$), sub-acute stress reduced significantly the time spent in closed arms in each period of pregnancy.

ACTH Plasma Levels

The acute treatment with harmine at 15 mg/kg induced a significant decrease of ACTH during the first, third weeks ($P < 0,05$), and the second week ($P < 0,001$) (Figure 5); and 10 mg/kg dose too, induced a decrease of ACTH levels in every period of pregnancy; therefore, the treated stressed groups in deferent stages of pregnancy, showed a decrease in ACTH levels, despite, the significant increase after the acute stress during the first, third week ($P < 0,001$), and the second week ($P < 0,05$). Psychological stress decreased it during first and second week but not significantly.

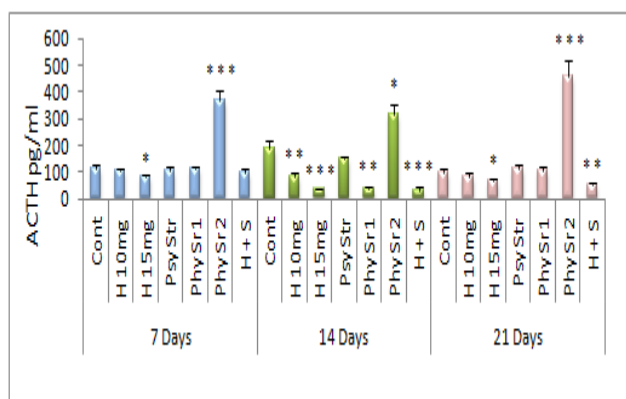


Figure 5: Effects of psychological, physical stress and its treatment with harmine on level of ACTH pg/ml, during three phases of pregnancy. Psychological stress 'Psy Str', Phy Sr1 and Phy Sr2 '0.4 mA and 1.2mA respectively' (n=5) (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

DISCUSSION

The acute treatment with harmine at 15 mg/kg induced a decrease of anxiety, in a different period of pregnancy, especially, during the second week that showed the highest level of anxiety in control groups at deferent points of measure.

Pregnancy or progesterone injected to ovariectomized rats reduced immobility behavior³¹, especially the 2nd week of pregnancy it marked by an increase of progesterone³². That is not in accord to our results that showed an increase in time of immobility spent in FST and ACTH level during the second week, in other hand the progressive increase in time of immobility in open field test reflect its accordance with oestradiol level.

A lot of contradictions regarding the mechanism of antidepressant effect of harmine, so it was sited first by³³, thus affinity of harmine at imidazoline I2 receptors might further strengthen the case for testing harmine as an antidepressant. Miralles³⁴ reported more moderate affinities of harmine for the I2 (Ki=630 nM). But, it was confirmed by Jackson³⁵, that the antidepressant-like effects of I2-ligands have also been reported in the rat forced swim test. It is thus, possible that the observed antidepressant like effects of β -carbolines may be mediated by I2-imidazoline receptors, but this possibility should be further tested⁵. This effect seems to be not mediated by presynaptic monoaminergic mechanisms, but appears to be induced by an inverse-agonistic mechanism located in the benzodiazepine receptors⁵.

Ayahuasca-treated group rats showed significantly more swimming activity³⁶. The swimming behavior in the forced swimming test is strongly related to the serotonergic activity³⁷.

Several research, have been shown recently, harmine in different doses and manner of treatment reduced the immobility time and enhanced the climbing time of forced swimming test in rats^{4,38,39}. Similar to our results, that's show a significant increase, in time of climbing,

after an acute treatment during each phase of pregnancy, and its aptitude to diminish significantly the immobility time during FST.

The latency to immobility is a parameter used to characterize antidepressant-like activity⁴⁰; in more cases, it marked by the time of climbing, which followed by the first fraction of immobility during the FST. Our study has demonstrated that, harmine interacts with MAO-A⁴¹, several cell-surface receptors, including serotonin receptor 2A (5-HT_{2A})⁴², which are involved in antidepressant pharmacotherapy⁴³.

The BDZ-induced anxiolytic effect at the beginning of the session, specifically during the first 3 min, since both percentage of open arm entries and time spent in open arms were increased⁴⁴, and an enhancement of curiosity of the female rats in the circumstances of novelty⁴⁵, in addition, the decrease of stopover in the closed arms explained by a lack of anxiety. After a treatment of harmine, pregnant female rats showed an increase in time spent in open arms, as the case, with male rats studied in our laboratory⁴⁶.

Other reports relate an increased anxiety in the plus-maze test by the end of gestation¹⁸. Intermittent footshock in rats reduces substance P content in the ventral tegmental area⁴⁷, and several hypothalamic nuclei⁴⁸.

Oestradiol, with optimal levels of corticosterone, reduces anxiety and depression indicators in ovariectomized rats subjected to open field, elevated plus-maze, or FST⁴⁹. In other hand, enhanced sensitivity to ACTH as a result of high estrogen levels in pregnancy can explain such lack of fidelity between the ACTH and corticosterone responses^{50,51} is in contradictory position and prove that the capacity of the anterior pituitary to generate an ACTH response to stress is not reduced in late pregnancy. Possibly, due to negative glucocorticoid feedback⁵², at the end of pregnancy, HPA axis responses to a range of psychological and physical stressors are markedly suppressed and, in some cases, completely abolished^{18,53,54}, and chronic (21 d) treatment with estradiol increases the corticosterone response to footshock⁵⁵. According to results of Saddick³², that showed the highest level of progesterone in 14th day of pregnancy, we can suggest the anxiolytic effect of progesterone.

These results approve the earlier issues claiming that electric shock stress increases the immobility time in FST^{56,57}. It was shown that glutamatergic NMDA receptors are involved in stress responses^{58,59}. The acute footshock stress leads to changes in the activity of mPFC neurons which could involve the LC-mPFC noradrenergic pathway⁶⁰. In this sense, we have recently shown that long-term neuropathic pain leads to anxio-depressive-like behaviour that is temporally accompanied by an impairment of the LC⁶¹. In addition, electrophysiological recordings of LC neurons showed a low tonic but

exacerbated nociceptive-evoke activity when the injured paw was stimulated⁶². Subsequent studies have shown that acute footshock stress increased the mean firing frequency of neurons in the LC⁶³. Moreover, antidepressants that inhibit the reuptake of noradrenaline can normalize the nociceptive-evoked responses of LC neurons in rats that experience neuropathic pain⁶⁴.

CONCLUSION

In this study, antidepressant-like effect of harmine has been exerted in female pregnant rats, the results of either the behavioural tests or the ACTH level during the three stages of pregnancy showed its beneficial effect even during pregnancy of mammals. The therapeutic effect of harmine on rats stressed by acute footshock of intensity 1,2mA has been established during pregnancy.

The effect of how the on the molecular level when preventing the harmful effects of stress will be studied.

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