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Article History:

Submitted: 15.09.2022

Accepted: 10.10.2022

Published: 17.10.2022

#### ABSTRACT

Pregnancy is a complex physiological state, in which the effect of any stress multiplies its impacts: in this study, we are interested in the effects of electrical stress on pregnant rats and an attempt to mitigate them using an alkaloid. Pregnant rats were divided into seven groups: Control and two treated groups with harmine at 10, 15 mg/kg, psychological stressed group, two foot shocked groups 0, 4 and 1, 2 mA and a treated stressed group. Each group contains three sub-groups according to the stage of pregnancy. Rats have conducted to plus maze test and object recognition test. We marked increased time spent with new object and number of entries in all arms and a significant decrease in food consumption, the Red Blood Cells (RBC), Mean Corpuscular Hemoglobin Content (MCHC) have increased and a significant decrease

#### **INTRODUCTION**

Treatment with ayahuasca did not affect the predictors of major depression, neither Brain Derived Neurotrophic Factor (BDNF) nor cortisol levels, higher BDNF levels were detected even the existence of two types of BDNF (Almeida RN, *et al.*, 2019). *Peganum harmala* is well known in traditional Bedouin medicine used as an emmenagogue and abortifacient (Casey RC, 1960). In the case of an excess dose, the perfuse heart is arrested in diastolic phase and the contractions of smooth muscle are diminished with the exception of the uterus, which may be made to contract more powerfully (Jinous A and Fereshteh R, 2012). From a metabolic point of view, serotonin levels increase after MAO inhibition, stimulating the vagus nerve in the brain, which innervates the digestive tract; due to the toxic alkaloids characterized in the seeds of *P. harmala* 'Harmaline and harmine' (Jinous A and Fereshteh R, 2012).

Pletscher A and Gey KF, 1959 reported that harmine were responsible for inhibition of MAO *in vivo* on rat's brain. Then it has reported to be a potent and selective inhibitor of MAO-A (Buckholtz NS and Boggan WO, 1977). Thus, treatment with harmine would expect to produce a general increase in serotonergic activity (Mendelson SD and Gorzalka BB, 1986). A series of recent pharmacological studies indicate that the role of serotonin in the regulation of female sexual behavior may be considerably more complex than simple tonic inhibition of behavior (Aiello-Zaldivar *M, et al.*, 1992). In addition, evidence suggests that both inhibitory and facilitatory serotonergic effects are mediated postsynaptically (Aiello-Zaldivar *M, et al.*, 1992). However, in a later series of experiments the agonists have found to facilitate lordosis in females primed with estrogen alone (Clemens LG, *et al.*, 1980; Mendelson SD and Gorzalka BB, 1986).

The hemolytic effect of total alkaloids of *P. harmala* on erythrocytes of the animals could explained by the interaction of alkaloids with the compounds of the membranes of erythrocytes of animals (Mahdeb N, *et al.*, 2013); by this way harmine is prone if it were be the main cause.

Most research has focused on lymphocyte cytokine production

in monocytes and lymphocytes after treatment. The number of placentation and fetuses has increased significantly in treated group and treated stressed group even the significant decrease in stressed group. Harmine has enhanced the cognition of rats in object recognition test, and exerted its anxiolytic effect in the plus maze test even during pregnancy. It decreased food consumption and enhanced implantation, RBC and thrombocytes due to its enhancement of serotonin via its effect of Monoaminoxidase Inhibitor MAO I.

**Keywords:** Harmine, Footshock stress, Pregnant rats, Monoaminoxidase Inhibitor (MAO I)

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and we have previously shown that during pregnancy, the peripheral-specific immune response has shifted away from a type 1 (i.e. cellular) immune response towards a type 2 (i.e., humoral) immune response (van Nieuwenhoven AL, *et al.*, 2002). Moreover, in line with human experiments, we observed recently that during rat pregnancy both monocytes and granulocytes have shown an activated phenotype (Faas MM, *et al.*, 2000).

Previous works had suggested that these rats exhibit greater basal impulsivity but not high reactivity to novelty (Belin D, *et al.*, 2008). Different cerebral structures participate in different types of memory, being the striatum related with procedural memory (Squire LR, 2004). It is a dopaminergic area involved to memory (Ferretti V, *et al.*, 2010). However, according to Kieras DE, *et al.*, 1999 work memory model included components not only for auditory and visual information, but also for tactile and kinesthetic information. Female rats characterized by their sensibility to stress, and face to pregnant stress, the possibility of harmine to prevent physical stress during gestation, in short work memory task, has been excluded.

Repeated stress results in decreased dendrite length and density of dendritic spines of cortical and hippocampal neurons (Watanabe Y, *et al.*, 1992; Radley JJ, *et al.*, 2006). In our study, chronic foot-shock stress had no effect on the survival of hippocampal cells that were born several days before the start of the chronic stress protocol, but it cannot ensure that footshock stress does not affect the survival of cells that are born during the period of chronic stress (Dagytė G, *et al.*, 2009).

Harmine produced an increase in dopamine release from rat striatal slices (Iurlo M, *et al.*, 2001; Schwarz MJ, *et al.*, 2003). In other hand footshock stress increased extracellular dopamine in the Prefrontal Cortex (PFC) (Sorg BA and Kalivas PW, 1993; Horvitz JC, 2000). However, excessive Medial Frontal Cortex (MFC) dopaminergic activity has a negative impact on the cognitive functions of primates, making them unable to select and process significant environmental stimuli (Pani L, *et al.*, 2000). A single footshock session acutely activates dopamine systems in the frontal cortex, hypothalamus, nucleus accumbens, and striatum, but daily application of footshock sensitizes dopamine release only in the cortex, nucleus accumbens, and striatum (Chrapusta SJ, *et al.*, 1997), then harmine risks exerting its anti-stress effect.

The present study aims to reveal the effect of harmine on memory and exploratory capacity of rats, blood quality and the fecundity parameters like immunity, number of fetuses after a treatment during three phases of pregnancy.

## 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), two to three months of age obtained from Pasteur Institute (Algiers, Algeria) were used in the present study. Rats have housed in a controlled temperature (22°C  $\pm$  1°C) with a dark/light cycle of 12 h/12 h, 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 has injected at doses of (10 mg/kg, or 15 mg/kg). Drug concentrations were prepared; immediately, prior to use, the necessary dose could injected in a volume of 0.1 mL per 100 g body weight of rats.

## Treatment

Pre-determined pregnant rats have classified into control, two groups receiving footshock. One group 'psychological stress' was putting in the same conditions of the stressed groups without receiving shocks (Endo Y, *et al.*, 2001). Two treated groups at a 10 mg/kg, and 15 mg/kg doses of harmine, injections were around 1 h before tests, and a group receiving 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<sup>th</sup>, 14<sup>th</sup>, and the last day of gestation. A day before decapitation pregnancy rats were travel tested.

## Foot shock procedure

The animals were placed individually in a Plexiglas shock cage  $(18 \times 12 \times 10 \text{ cm})$  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°C, and completely dried before placing each animal in the shock cage.

## **Object recognition**

Pregnant female rats, 24 h after open field test, have putted individually in the labyrinth to explore two similar objects placed in two opposite corners 10 cm from each of the nearest walls, for 5 min and 1 h later. In the second session, one of the familiar objects has replaced by a new one. The exploration of the objects has recorded during 5-min session (Ennaceur A and Delacour J, 1988). It's considered when the nose of the rat was near of the object at a distance less than 1 cm. After each passage, the labyrinth and the objects have cleaned with a solution of alcohol of 70°C.

## Elevated Plus Maze (EPM) test

Originally, Montgomery KC, 1955 created a so-called symmetrically elevated Y-maze for rats. Pellow S, *et al.*, 1985 into the Elevated Plus Maze (EPM) have modified it thirty years later. The test involves spontaneous exploration by rodents of the apparatus, in the absence of explicit reward. Rats have 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 considered as an external compartment in the fear situation. In general, EPM is used for assessment of anxiety-related behaviors in rats, the apparatus consists of two wooden arms of  $(110 \times 10 \text{ cm})$  interconnected by a square of 10 cm<sup>2</sup>, the plus-shaped platform contain two opposed open arms ( $50 \times 10 \text{ cm}$ ), and two opposed enclosed arms ( $50 \times 10 \text{ cm}$ ). A black Plexiglas covered the apparatus that was elevated 73 cm above the flour. 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, total number of arm entries, were taken as indices of overall activity (Acevedo MB, *et al.*, 2014), between two tests the maze must be cleaned with a solution of 70% of alcohol. The recorded video has analyzed to measure the number of entries into open arms, and enclosed arms.

## **Blood samples**

The day of decapitation blood of pregnant female rats has collected in EDTA (Ethylene Diamine Tetra-Aceticacid) tubes and tested.

## Statistical analysis

With using Minitab 17 statistical analysis, all data are presented as mean  $\pm$  Standard Error of the Mean (SEM). Differences among experimental groups in the Plus maze test object recognition, number of fetuses, blood parameters and the amount of food consumption were determined by one-way ANOVA, P values less than 0.05 were considered to be statistical significant.

#### **RESULTS AND DISCUSSION**

Our study has elucidated the non-toxic effect of the ß-Carboline alkaloid harmine on the pregnant female rats that has shown by its enhancement of cognition, locomotor activity, and standards of blood cells but the health status of the offspring remains the subject of research.

Harmine has previously described, as hallucinogenic drug, these mechanisms, to our knowledge, have not been elucidated (Bergström M, et al., 1997), a study found that Harmine is a significant enhancer of short-term working memory in male rats (Mennenga SE, et al., 2015). Those results confirmed by our study because harmine did not show any sign of hallucinogenic effect on visual and tactile sensations of female pregnant rats, and, on the contrary, it ameliorates caution and memory. On the other hand, there is evidence of the participation of striatal serotonin system on the consolidation of aversive task (Prado-Alcalá RA, et al., 2003). These possibilities could explain the inhibition of MAO-A, increasing extracellular dopamine and serotonin levels, and therefore resulting in the enhanced memory exerted by harmine. Li LB, et al., 2015 conclude that the activation of medial septum-diagonal band of Broca complex and parvalbumin positive neurons linked to hippocampal theta rhythm enhanced working memory via 5-HT2A receptor, and involve monoamine levels in the hippocampus and median Prefontal Cortex (mPFC). In the present study, the expression levels of inflammatory cytokines in the hippocampus of the rat brain have suppressed following treatment with harmine (Zhong Z, et al., 2015). In our experience, harmine must exempt hippocampus from cytokines resulting from the inflammations during footshock stress.

In general, theta waves occur during stress, memory processing, orienting and exploratory (Buzsaki G, 2002; Vanderwolf CH, 1969). Our previous study revealed that inescapable footshock stimulation increases the power of Low Frequency (LF) (4-7 Hz) theta oscillations through the activation of GABAergic ( $\gamma$ -Aminobutyric acid) pathways in the Medial Raphe Nuclei (MRN), which blocks the ability of MRN to desynchronize theta waves (Hsiao YT, *et al.*, 2012). In this study, female pregnant rats, after a sub-acute stress show a significant increase in number of entries in all arms during three phases first (p<0.01) second and third week (p<0.001) (*Figure 1a*), and a slight change in exploratory behavior, but the acute stress at intensity of (1, 2) mA affected significantly the exploration and locomotor behavior of pregnant rats. Similarly, footshock administration before and after passive avoidance facilitated learning, consolidation and evocation (Kaneto H, 1997). That has shown in our study in pregnant female rats after an acute stress session. However, psychological stress (e.g., exposure to an adjacent room in which other subjects were exposed to footshock) enhanced caution in pregnant female rats.

It is conceivable that acute stress provoking physical pain decreases 5-HT2A receptor-mediated behavioral responses (Izumi T, et al., 2002). Of the three 5-HT2 subtypes, 5-HT2A receptors have equally expressed in the Carbonic Anhydrase 1 (CA1) and CA3 hippocampal subfields, whereas 5-HT2C receptors predominated in CA1 (Busceti CL, et al., 2015). Acute pre-training systemic administration of aromatic β-carboline alkaloids, harmine and harmol, improved novel object recognition of mice (Moura DJ, et al., 2006). It may, induced by interactions with serotonin (5-HT2A, 5-HT2C and 5-HT1A), dopamine and Benzodiazepine receptors (Moura DJ, et al., 2006). The treatment with harmine at 10 mg/kg has shown any significant increase of time spent with new object, even though the treated stressed rats demonstrated a significant increase (p<0,01) of time spent with the new object, during the second and third weeks (Figure 1b). Our results reflect the effect of harmine on the level of 5-HT2A that enhanced the exploratory behavior even after footshock stress, we suggest that harmine increased 5-HT2A that was decreased due to the physical pain of footshock. The entorhinal cortex input to CA1 is necessary and sufficient for novelty detection (Kesner RP, 2007). Gamma oscillations in area CA3 have been implicated in hippocampal memory formation (de Almeida L, et al., 2007), and may serve as a mechanism by which CA3 output can coordinate CA1 activity during retrieval of spatial memories (Colgin LL, et al., 2009; Montgomery SM and Buzsaki G, 2007; Shirvalkar PR, et al., 2010). Because, activity in CA3 is required for association of objects with spatial location (de Almeida L, et al., 2007; Lee I, et al., 2005). In our study, the detection of novelty in spatial area confirm the implication of caution and spatial memory tasks especially after the frequent visits successively of the new object and the examination of the near area indicates the zonal repairing of the objects in a space area. In other hand, Neurosteroids, particularly estradiol, progesterone, dehydroepiandrosterone and pregnenolone, are mainly located in astrocytes and neurons in the CA3-CA1 region of the hippocampus, playing a leading role in the learning process and storing in the rat brain (Shibuya K, et al., 2003). In that case, we can suggest that connections between CA3-CA1 corresponding of object's exploration, and, their memorization in the space inevitably, affected during such period of physiological activation.

Rearing has long considered as an exploratory behavior, pregnant rats have shown increasing number of entries in arms during the second week of pregnancy (*Figure 1a*). Time spent with familiar object has decreased sig-

nificantly after treatment during the first week of pregnancy (*Figure 1c*), while the time spent with new object has increased significantly after the acute treatment at 15 mg/kg during the third week (p<0,05) (*Figure 1b*). In other hand, the number and time of rearing of the pregnant female rats during Open Field Test (OFT) (Benatoui R, *et al.*, 2017). Therefore, we can conclude that the exploratory behavior of the rats shown during the exploration of the objects and OFT and plus maze test is coherent.

Stimulation of the dopamine receptors in the Ventral Pallidum (VP) through direct and indirect dopamine agonists increases locomotion (Klitenick MA, et al., 1992; Gong W, et al., 1996). Interestingly, pretreatment with  $\beta$ -carbolines decrease in firing rate of neurons in the VP followed by apomorphine administration causing first an inhibition of dopaminergic projection conducted to the VP, this protective effect could explained by interaction between ß-carbolines and dopaminergic mechanisms (Haghparast A, et al., 2012). VP innervated by dopaminergic and glutamatergic fibers from the Ventral Tegmental Area (VTA) (Klitenick MA, et al., 1992). Dopamine has a significant pallidal influence, for the majority of randomly recorded VP neurons are sensitive to electrical activation of the both VTA and substantia nigra (Maslowski-Cobuzzi RJ and Napier TC, 1994; Mitrovic I and Napier TC, 2002). It should be noted that in rats MAO-A inhibition also significantly augments levels of tissue dopamine (Garrett MC and Soares-da-Silva P, 1990). There is a grow body of evidence that the enhancemenet of the number of entries during our experience was due to the impact of harmine on dopaminergic receptors.

Biochemical studies in rats provided evidence that hippocampus, as well as corpus striatum shows an equal distribution of Benzodiazepine 1 (BDZ1) and BDZ2 receptor types (Longo VG, et al., 1988), the activation of BDZ1 receptor type had reduced the amplitude of the hippocampal theta rhythms. The  $\beta$ -carbolines are a class of pharmacological agents, which have 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  $\beta$ -carbolines have therefore been termed "inverse agonists" (Haghparast A, et al., 2012). Our findings provide evidence that ß-carbolines harmane, norharmane and harmine induce an antidepressant-like effect via stimulation of the Benzodiazepine receptor in an inverse manner (Farzin D and Mansouri N, 2006). Treatment with harmine at dose of 15 mg/kg increased number of entries significantly during the first and third week (p<0.001), the treated stressed group showed its enhancement, but significantly only during the third week (p<0.01) (Figure 1a). In this study, the increased number of entries in all arms revealing the anxiolytic-like effect of harmine at all stages of pregnancy, appeared resulting on the action of harmine on Benzodiazepine receptors.



Figure 1: (a) Total number of entries in all arms during plus maze test. (b) Time spent with new object. (c) Time spent with familiar object of stressed and treated rats with harmine during three stages of pregnancy. S1 and S2, 0, 4 and 1, 2 mA; respectively (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001)

Although classical hallucinogens bind at multiple populations of 5-hydroxytryptamine 2 (5-HT2) receptors (i.e. 5-HT2A and 5-HT2C receptors), evidence is mounting that 5-HT2A receptors are the primary targets of these agents (Nelson DL, *et al.*, 1999). The activation of 5-HT2A receptors can lead to an increase in locomotor activity, but 5-HT2A agonists (hallucinogens) examined had produced decreases in locomotor activity (Wing LL, *et al.*, 1990; Krebs-Thomson K and Geyer MA, 1996). According to Preskorn SH, *et al.*, 2008, 5-HT2A is involved in antidepressant pharmacotherapy. All our results, of the plus maze test and object recognition test, confirmed that harmine exerting its antidepressant effect activating the 5-HT2A receptors consequently, it enhanced locomotion.

An increase in the number of entries added to the lengthy time spent in the open arm apparatus indicates a lower level of anxiety (Pellow S, *et al.*, 1985; Hogg S, 1996). The lake of the 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 could be explained by the curiosity induced by the psychological stress (Benatoui R, *et al.*, 2017). The corticosterone levels of psychological stress induced by the communication box not directly related to the electrical intensity of the footshock in the physical stress group (Endo Y, *et al.*, 2001). If only the rat putted in a closed box (without adjacent box of footshock stress session) could change its locomotor behavior and mood anxiety. It seemed that there were no remarkable relationships between hematological parameters and the doses of total alkaloid extracts from seeds of Total Alkaloid Extract of *P. harmala* (TAEP) (Wang Y, *et al.*, 2019).

The drastically decreased values in Red Blood Cells (RBC) counts, hemoglobin concentration, and hematocrit strongly suggest that hemodilution (and consequently anemia) occurs in rat pregnancy (de Rijk EP, *et al.*, 2002). It was even worsen after sub-acute and acute stress, inducing a decrease in hemoglobin level in RBC, it has corrected with harmine treatment (*Figures 2a and 2b*). The treated stressed groups enhanced significantly the Red Blood Cells and hemoglobin during the third week, and hematocrite during the second (p<0.001) and third (p<0.05) week.

The underlying cause of anemia during pregnancy is hemodilution rise in plasma volume more as compared to increase in the red cell mass (Rodríguez-Dennen F, *et al.*, 2011). Pregnant female rats have marked a decrease in Red Blood Cells, hemoglobin and hematocrite during the second week (*Figures 2a, 2b, 3a*), due to organogenesis 'neurulation and turning, optic and optic vesicles, kidneys, lung and forelimb bud formation', but, after an acute treatment of harmine we could prevent this decrease of RBC. The treatment with harmine at both doses induced a significant increase of RBC (p<0.01) (*Figure 2a*), the MCHC levels (*Figure 3b*) has shown its enhancement induced by harmine during the first and second week.

The plant of *P. harmala* enriches the blood and is useful in weakness of muscles and brain (Chatterjee A and Pakrashi SC, 1997; Kritikar KR and Basu BD, 1995). The results for the structure-activity relationships of harmine derivatives on osteoblast differentiation revealed that a double bond between C3 and C4 in the  $\beta$ -carboline structure might be essential for its osteogenic activity (Yonezawa T, *et al.*, 2011). The importance of a methoxy or hydroxy group at position 7 is also suggested by a comparison of the effective concentrations for the Alkaline Phosphatase (ALP) activity (Yonezawa T, *et al.*, 2011), that's confirmed by our results showing the enhancement of Red Blood Cells, hemoglobin, hematocrite of treated groups.

We report that pregnant female rats had an increasing percentage of granulocytes and a decrease in lymphocytes. The proportion of monocytes remains stable throughout gestation (Luppi P, *et al.*, 2002). Which is not in accord to our results, monocytes was high at the beginning of gestation but decreased continuously until the end of gestation (*Figure 4a*). The percentage of lymphocytes deceased a little at the second stage and remains stable (*Figure 4b*), while neutrophils marked an increase at the second stage followed by a slight decrease at the third stage (*Figure 4c*).



Figure 2: (a) Level of red blood cells. (b) Level of hemoglobin of stressed and treated rats with harmine during three stages of pregnancy. S1 and S2, 0, 4 and 1, 2 mA; respectively (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001)



Figure 3: (a) Level of hematocrite. (b) Level of Mean Corpuscular Haemoglobin Level (MCHL) of stressed and treated rats with harmine during three stages of pregnancy. S1 and S2, 0, 4 and 1, 2 mA; respectively (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001)



Figure 4: (a) Percentage of monocytes. (b) Percentage of lymphocytes. (c) Percentage of neutrophils of stressed and treated rats with harmine during three stages of pregnancy. S1 and S2, 0, 4 and 1, 2 mA; respectively (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001)

We have previously shown that peripheral lymphocytes, monocytes, and granulocytes show an activated phenotype, especially in the last week of rat pregnancy (Faas MM, *et al.*, 2000). Our results indicate an intense increase of neutrophils of treated groups at all stages of pregnancy, and it was significantly at the first stage at both doses (*Figure 4c*). The study of Kusnecov AW, *et al.*, 1995 confirmed that footshock exposure markedly suppresses lymphocyte mitogenic activity, contrary to our results footshock stress enhanced the percentage of lymphocytes significantly at the first and third stages that was not expected. The high levels of RBC and hemoglobin marked in footshock stressed and psychological stressed groups confirmed that the main site of action of the both stresses was not the bone marrow.

The leukocyte and neutrophil counts gradually, and signifcantly increased from the first to the third trimester and that the lymphocyte count gradually decreased from the first to the second trimester (Karakus S, *et al.*, 2016). That is in accord to our results. Neutrophil degranulation leads to astrocyte death, which in turn causes oligodendrocyte death and axonal degeneration (Papadopoulos MC and Verkman A, 2012). We consider a normal granulation with the increased number of the neutrophil exerted by harmine, so it moderates the behavioral state shown during tests due to the preventive effect on oligodendrocyte death and axonal degeneration, which indicates the neuromodulator effect through immunological adjustment of harmine during gestation. The high levels of neutrophils indicate an increasing in cytokines.

Anti-inflammatory cytokines IL-4 and IL-10 inhibit Th1 cells and macrophages, which in turn prevent fetal allograft rejection. In addition, these cytokines also inhibit Tumor Necrosis Factors-a (TNF-a), Cyclooxygenase-2 (COX-2), and prostaglandin E2 in amnion-derived cells, which prevent the onset of labor (Piccinni MP, 2010; Goodwin VJ, et al. 1998). Which confirm that the low level of monocytes 'macrophages' in the first trimester (Figure 5a) is beneficial for preventing miscarriage so the treatment with harmine is beneficial only for the first trimester of pregnancy. Estrogens may affect the number of macrophages in uterus, as these hormones stimulate the influx of leukocytes (including macrophages) into the uterus (Zheng Y, et al., 1988; Kachkache M, et al., 1991). In the case of our experience, we can suggest that the decrease of monocytes in the blood was due to the effect of harmine by increasing estrogens production who in turn stimulate the influx of leukocytes into the uterus to contribute to placentation process. The disadvantage of lowering monocytes by harmine being in delaying decidua and makes it difficult. Therefore, the preferred use of harmine is limited in the first and second phases of gestation, which correspond to the first phase of human pregnancy, but at the end of the third trimester of gestation, it create a barrier to the triggering of the decidua, we should prefer not to take it.

In pregnancy, monocytes are able to respond to infections, e.g., caused by Gram-negative bacteria, and thus protect the mother's and the baby's health from pathogens (Luppi P, *et al.*, 2002). According to Luppi P, *et al.*, 2002, the decrease in monocytes due to harmine treatment can deprive mother and fetus from protection against Gram-negative bacteria, the footshock stress seems beneficial in that point, and it confer more protection to the mother and fetus even the decreased number of fetuses.

It has been suggested that to maintain resistance to infection, the activity of the maternal innate immune system, represented by the increased of monocytes and granulocytes (Sacks G, *et al.*, 1999). Circulating monocytes and granulocytes show an activated phenotype (Sacks GP, *et al.*, 1998). That explained previously as a normal physiological behavior due to blastocyst in uterine tissue including in linking of the placenta with mother's uterine tissue. The leukocytosis is due to increased inflammatory response during normal pregnancy, which can be a consequence of selective immune tolerance, immunosuppression and immunomodulation of fetuses (Osonuga IO, *et al.*, 2011). The increase in granulocyte number may also suggest a pro inflammatory state of the inflammatory system during the post-implantation phase of rat pregnancy (Faas MM, *et al.*, 2003). The increased number of neutrophils in treated groups confirms a good implantation of fetuses generating pro-inflammation. The lowering effect of harmine on monocytes faced the high number of fetuses could be due to an anti-inflammatory effect of harmine, and we prefer the first suggestion that, anti-inflammatory cytokines IL-4 and IL-10 inhibit Th1 cells and macrophages, which in turn prevent fetal allograft rejection. As the cytokines had a crucial role for that part, further studies with cytokines assay will be helpful.

A significant increase in White Blood Cells (WBC), lymphocyte and monocyte count in pregnant women with high-risk for Neural Tube Defects (NTD) but only increase of monocyte count in pregnant women with high-risk for trisomy-21 has observed (Ozdemir AT, *et al.*, 2016). According to Ozdemir AT, *et al.*, 2016, the low level of monocytes in our study induced by harmine could prevent such disease but the footshock stress could induce this disease.

A significant increase in number of neutrophil counts after the first week of treatment, in treated rats compared to control rats indicate the toxicological effect of the plant (Muhi-eldeen Z, et al., 2008). In addition, elevation of GB levels in treated rats directly indicates a strengthening of the immune system (Hariri AT, et al., 2011). This suggests that the crude extract of P. harmala contains bioactive substances that boost the immune response by increasing the level of white blood cells: The first defensive level in the body (Atsamo AD, et al., 2011). Our study confirms that the increasing in neutrophils exerted by the plant has due to harmine. We can suggest that the desired effect of harmine lies in its enhancement of neutrophils, and that's needed especially during the second week according to de Rijk EP, et al., 2002, which could confirmed only with a post-partum study of prenatal treated rats with harmine. The increased need for neutrophilic granulocytes during rat pregnancy has reflected by numerous clustered neutrophilic granulocytes in placental blood vessels at days 8-12 of pregnancy and during late pregnancy, at sites of degenerating cells and necrotic areas (de Rijk EP, et al., 2002).

The number of thrombocytes increased slightly during rat pregnancy (de Rijk EP, *et al.*, 2002), that is in accord to our study elucidates a slower rise of thrombocytes during pregnancy, between 850 and 1050  $10^3/\mu$ L in rats. Serotonin is also present in blood platelets, chromaffin cells of the intestinal mucosa and in the central nervous system (Beekman M, 2004). Tranzer JP, *et al.*, 1972, Showed serotonin reuptake in megakaryocytes, immediate precursors of blood platelets. We can conclude that, harmine offer serotonin 'precursor of platelets' to the bone through its MAO inhibitory effect, it can help bone stem cells in platelet production.

Exposure to excess estrogen during pregnancy reduces pregnancy weight gain and food intake resulting in increased resorption or abortion (Zimmerman SA, *et al.*, 1991). The precise mechanism by which estrogen decreases food intake not identified. Many of the physiological changes taken place for the metabolism of nutrients, the required content has increased in order to ensure the genesis of placenta and the fetus, and the development of the uterus and mammary glands, then a hematopoiesis vigorous is essential.

Our findings are similar to those of Paul PK, who stated that the daily dietary intake of the rat reached its peak on day 14 of pregnancy and then declined gradually (Paul PK, 1974). Indeed, the lesion of the lateral hypothalamic area leads to a decrease in food intake as well as in body weight, while electrical stimulation of this nucleus causes an increase in food intake, even in satiated animals (Williams G, *et al.*, 2001). However, the ventromedial hypothalamic nucleus, which is considered to be the "center of satiety", its electrical stimulation induces inhibition of food intake (King BM, 2006). It has suggested that Corticotropin-Releasing Factor (CRF) in Paraventricular Nucleus (PVN) plays a role in the inhibition of food intake by stress, since CRF microinjected in para ventricular nuclei PVN alone inhibits food intake in rats (Krahn DD, *et al.*, 1988). However, estrogen does not affect feeding behavior by CRF neurons of PVN under emotional stress because emotional stress using a communication box did not increase expression of CRF mRNA in PVN (Makino S, *et al.*, 1999). Nevertheless, it seems that estrogen inhibits food intake in several species, although the precise mechanism by which estrogen decreases food intake has not identified.

Neuroinflammation derived from obesity shown to affect other brain structures such as hyppocampus, cortex, brain stem or amygdala. In addition, obesity has been associated with the increased occurrence of central disorders such as depression and cognitive impairment (Guillemot-Legris O and Muccioli GG, 2017). Hotta M, *et al.*, 1999, have shown that food intake is inhibited by electrical shock and emotional stress induced by a male rat communication box (Hotta M, *et al.*, 1999). Psychic stress resulted in a 14% weight loss in stress group rats compared to control group rats in 4 weeks (Thilander B, *et al.*, 2011). One study has shown that psychosocial stress induced dysfunction of the intestinal barrier has related to the release of acetylcholine (Saunders PR, *et al.*, 1997). Animals exposed to the acute footshock stress had a significant increase in food intake compared to controls (*Figure 5b*); the decrease in food intake of treated stressed group was significant during at three phases (p<0.001).

As it mentionned before inhibition of MAO-A augments levels of tissue dopamine and attenuates levels of the dopamine metabolite 3,4-Dihydroxyphenylacetic Acid (DOPAC) in the nucleus accumbens (Garrett MC and Soares-da-Silva P, 1990). Other studies indicate that dopaminergic neurotransmission, particularly in the nucleus accumbens, could also participate in the reward mechanisms associated with food intake (Bassareo V and di Chiara G, 1999). In other hand, it regulated indirectly by leptine secretion and, leptine decreases dopamine release in the nucleus accumbens during food intake (Krügel U, *et al.*, 2003) which explain the decreased in consumption amount in our experience.

The two types of uterine mitochondrial MAO may be located in different types of adrenergic neurons with distinct roles in the mechanisms responsible for regulating the physiological activities of the uterus (Grosso DS and Gawienowski AM, 1975). For example, human placenta expresses predominantly MAO A (Grimsby J, *et al.*, 1990). In that case, we can suppose that harmine can exert the similar effects in human.

The number of fetuses of the pregnant rats have been between 7 and 11 (*Figure 6a*), the increase of placentation (*Figure 6b*) after the treatment have been significant (p<0.05) in the first and third week with dose 10 and 15 mg/kg respectively, the second week with the 10 mg/kg dose the increase have been also significant (p<0.01). The relation between abortion and the progesterone fall has confirmed by the abortive effects of progesterone synthesis inhibitors in the rat at mid-pregnancy, e.g. azastene (Creange JE, *et al.*, 1978). It is known that, estradiol increases progesterone levels in the rat hypothalamus; the both concentrations of harmine are appropriate and any elevation of them we suppose may be harmful, because exposition to high levels of estrogen can alter conditions maintained gestation. In other hand, the decreased ratio of food intake in treated rats indicated an increase of estrogen level, which induced the production of progesterone, and subsequently ensures good conditions of gestation.



Figure 5: (a) Level of thrombocytes. (b) Food consumption of stressed and treated rats with harmine during three stages of pregnancy. S1 and S2, 0, 4 and 1, 2 mA; respectively (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001)



Figure 6: (a) Number of placentas, (b) Number of fetuses of stressed and treated rats with harmine during three stages of pregnancy. S1 and S2, 0, 4 and 1, 2 mA; respectively (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001)

In previous studies, we have showed that footshock stress, together with enhancing depolarization-dependent release of endogenous glutamate, increases excitatory postsynaptic currents amplitude (measured immediately after the stress session) (Musazzi L, et al., 2010). In present study, we have confirmed that GLT-1 mRNA and protein expression is remarkably elevated in harmine treated group (Sun P, et al., 2014). It is worth noting that our results demonstrated that harmine remarkable attenuation of astrocytes activation in the cortex region of the brain. That we admit as a physiologic response to protect neurons from insults derived by GCI (Global Cerebral Ischemia), and acting with a synergic mechanism together with GLT-1 (Sun P, et al., 2014). Because, the elevation of Glutamate Transporter-1 (GLT-1) activity and expression is possible to decrease glutamate accumulation in the synaptic cleft, consequently reduction of the over-activation of postsynaptic glutamate receptors, thus reduce the death of neurons (Rauen T and Kanner BI, 1994). For a long time P. harmala has been used in traditional medicines for the relief of pain and as an antiseptic agent which was confirmed by our study, according to the enhancement of the number of fetuses due to the stabilization of polarization of muscular tissues and preventing of pain sensation. In summary, the effectiveness of tricyclic antidepressants in neuropathic pain is most likely multimodal, with contribution of monoamine reuptake inhibition and blockade of N-methyl-D-aspartate receptors, sodium channels and calcium channels (Mika J, et al., 2013). The study of Schwarz MJ, et al., 2003 showed the inhibition by harmine of MAO B in rat liver its intensity was similar to this exercised by clorgyline, so harmine only can disturbs MAO B and have a lowering effect on MAO B but it inhibit MAO A. We can conclude that the anti-nociceptive effect exerted by harmine was due to its MAO A inhibition.

Shedding light on the mechanism of the plant, we supposed that the abortifacient effect of P. harmala is due to the existence of harmaline and other components. During the early summer months, animals attracted to plants, when they are green. Because rootstocks of spring, been active photosynthetically during summer (Ahmad I, et al., 2013), due to the high levels of potassium which activates enzymes. Abortion is frequent in animals that digest this plant in a dry year (Mahmoudian M, et al., 2002; Fathiazad F, et al., 2006). When the levels of potassium were low (Ahmad I, et al., 2013), that can be the direct cause of residing of inactivated enzymes and harmaline, which we supposed responsible of abortion in animals. Maximum alkaloids were associated with spring and autumn (Ahmad I, et al., 2013), we supposed that the toxic effect of the plant during the summer when photosynthesis were high, is only, due to the subsistence of harmaline which responsible at the emergence of harmine. As vasicine alkaloid of Adhatoda vasica traditionally has been used as an abortifacient agent, due to its stimulatory effect on uterine, apparently through the release of prostaglandins (Gupta OP, et al., 1978), it can be considered as an agent reinforce abortion in P. harmala plant.

The fruit and seeds have also used as digestive, hallucinogenic, and uterine stimulant (Bown D, 1995), which containing Harmalol, harmaline and harmine in a very important portions (Herraiz T, *et al.*, 2010). Harmine in our study has not shown its abortifacient effect, further studies will conducted on the effect of other alkaloids of the plant.

### CONCLUSION

This study has interested in one of the most important physiological processes in the body of the animal, in accordance with this concept, effect of footshock stress on pregnant rats depending on fetuses' development have elucidated. Our results have shown that harmine improve caution and memory which denied the hallucinogenic effect of harmine. The MAO inhibitory effect of harmine has raised the level of serotonine with the anti-inflammatory effect in hippocampus were the main reason of enhancement of memorization in pregnant rats. On the other hand, as CA1 known by its development in female rats is sufficient for novelty detection, and CA3 responsible for spatial memory had the same distribution of 5-HT2A receptors, harmine has acted its utter effect. Inhibitor effect on MAO A enhancing serotonine has a dual aspects, as its increased estrogens production stimulate the influx of leukocytes into the uterus to contribute to placentation process. The fact that harmine increase 5-HT2A and footshock stress decreased it inducing pain, confirmed that harmine had an anti-no-ciceptive effect. In addition, exempting hippocampus from cytokines due to the anti-inflammatory, adding its effect on Benzodiazepine receptors, harmine confirmed its anxiolytic effect even during pregnancy. The treated group has shown an enhancement in neutrophils that we consider has a normal granulation. Consequently, it indicates the neuromodulator effect through immunological adjustment of harmine during gestation. Treated groups with harmine showing the decreasing in monocytes with significant increase in neutrophils was worrying even the raise in fetuses' number and implantation.

As harmine acted positively on bone marrow and enhanced the RBC preventing anemia, we can explain its disturbing effect on white cells has just due it's acting on the uterus' tissue. On the other hand, decreasing number of fetuses and neutrophils faced the increasing number of monocytes in foot-shocked groups indicate that the nociceptive effect of stress decreasing 5-HT2A receptors decrease implantation. All our results boost the idea that harmine could exerted its whole effects during pregnancy. except its effects on blood cells that threaten the progress of implantation due to the damaged effect exerted by neutrophils on uterus tissue and the depriving effect of monocytes on the mother and fetuses immunity from bacteria.

#### ETHICAL APPROVAL

The whole methods used in this experiment have carried out, according to guidelines for ethical conduct in the care and use of nonhuman animals in research of APA 20002-4242, 2012.

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