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RESEARCH ARTICLE

MODULATORY EFFECT OF HARMINE ON SPATIAL MEMORY, FERTILITY VIA MAO INHIBITION, PREVENTING ANEMIA AND ANTI-NOCICEPTION UPON FOOTSHOCK STRESS AT THREE STAGES OF PREGNANT RATS

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Abstract

Aim and objective: Stress has overwhelmed the world, mortality due to stress is continually increasing, and pregnancy has known by its physiological modulation. This article has a clear explanation about the effect of the ß-carboline alkaloid harmine containing in the plant of *Peganum harmala*, it makes a huge debate in many countries as Iran, Brazil, Algeria, China, Australia about the main component that exerted abortion and until now there is no work resolved the matter.
Methods: In this study, we planned our experience, so that each sub-group treated with harmine, stressed with footshock or received the both, according to their pregnancy stages.
Results: Footshock stress as expected with its maximum intensity could be tolerated by the fetuses so could keep the rats pregnant along the main stage, damaged to several physiological parameters, but it makes a good pairing with harmine.
Conclusion: Harmine has enhanced the cognition during pregnancy, and exerted its anxiolytic effect too. Its decreasing effect of food consumption and enhancement of implantation was the main reason to confirm its raising estrogen levels from previous works, raising thrombocytes due to its enhancement of serotonin via its effect of monoamine-oxydase inhibitor MAO I. In other hand, footshock stress even its expected placentation defect but it seemed having an ameliorating effect on immune function during pregnancy.
Keywords: Footshock stress, harmine, monocytes, pregnant rats.

INTRODUCTION

Ayahuasca treatment does not affect any of major depression predictors, neither brain derived neurotrophic factor BDNF nor cortisol level, higher BDNF levels were detected[1]. In traditional Bedouin medicine *Peganum harmala* used as an emmenagogue and abortifacient[2]. In the case of an excess dose, all smooth muscles contractions diminished, or arrested as heart, expert the uterus, contract more powerfully[3]. From a metabolic point of view, administration of seeds of *P. harmala* 'Harmaline and harmine' can induce an increase of serotonin levels after MAO inhibition, affecting the digestive tract; due to its innervation by vagus nerve[3]. Harmine is a potent inhibitor of MAO-A[4]. Recent studies indicate that serotonin roles not shorten at a simple tonic inhibition of female sexual behavior[5]. In addition, serotonin affects postsynaptically to facilitate or inhibit behaviors[5]. During pregnancy peripheral-specific immune response focused on type 2 (i.e. humoral) immune response especially lymphocyte cytokine[6]. Moreover, rat's pregnancy as of humans does is characterized by activation of monocytes and granulocytes phenotype[7]. Previous works had suggested that these rats exhibit greater basal impulsivity but not high reactivity to novelty[8]. As each area of the brain has a crucial role in memory or performing tasks, striatum related with procedural memory[9]. It is a dopaminergic area involved to memory[10]. However, according to Kieras *et al.*,[11] 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[12,13]. Hippocampl cells born several days before chronic footshock stress did not affected but that does not prevent its effect on nascent cells during stress[14]. Harmine induced an increase in dopamine release in different rat striatal slices[15,16]. In other hand, footschock stress induce an increase in extracellular dopamine of the prefrontal cortex PFC[17,18]. However, cognitive functions of primates as selection, and reaction with their environment, disturbed by the excessive medial frontal cortex MFC dopaminergic activity[19]. Daily application of footshock elicits dopamine release in the cortex, nucleus accumbens, and striatum, on the contrary of a single footshock session; it activates more areas dopamine systems in the frontal cortex and hypothalamus[20], then harmine risks exerting its anti-stress effect.

MATERIALS AND METHODS

Animals and housing

One hundred and five adult female wistar rats (180-210 g), obtained from Pasteur Institute (Algiers, Algeria) were used in the present study, initially, living in five in clear polyethylene cages with standard pellets food and water *ad libitum*, after becoming pregnant, the experiment was repeated five times (n=5).

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 have dissolved in saline solution; immediately, prior to use, the necessary dose could injected in a volume of 0.1mL 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. 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 "according to the stage of pregnancy" was divided into three groups 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 pregnancy rats were travel tested.

Footshock procedure

The animals were placed individually in a Plexiglas shock cage (18x12x10 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 test

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. In the second session "1h later", one of the familiar objects has replaced by a new one. The exploration of the objects has recorded during 5-min session[21]. 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

Pellow *et al.*,[22] has modified the Y maze test into the EPM. 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 (110x10 cm) interconnected by a square of 10 cm², the plus shaped platform contain two opposed open arms (50x10 cm), and two opposed enclosed arms (50x10 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 considered only when the rat enter his four paws into an arm, 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-Acetic) tubes and tested.

Statistical analysis

With using Minitab 17 statistical analysis, all data are presented as mean±S.E.M. 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 statistically significant

RESULTS AND DISCUSSION

Neuromodulatory effect of harmine

The non-toxic effect exerted by harmine on the pregnant female rats has shown by its enhancement of cognition, locomotor activity, and standards of blood cells but the health status of the offspring remains unclear. Harmine previously known as hallucinogenic drug, that is not been elucidated during this study[23], it has been shown that harmine ameliorate short term working memory in male rats[24]. Those results confirmed by current 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, striatal serotonin system participate in the consolidation of aversive task[25]. The inhibition of MAO-A, resulting in the enhanced memory exerted by harmine, owing to its increasing serotonin and

dopamine levels. Li *et al.*,[26] concluded that the activation of neurons linked to hippocampal theta rhythm enhanced working memory via 5-hydroxy-tryptamine 5-HT2A receptor, and involve of monoamine levels in the hippocampus and mPFC in other hand. Treatment with harmine suppress the expression of inflammatory cytokines in hippocampus rats[27]. It seemed that harmine exempt hippocampus from cytokines resulting from the inflammations during footshock stress, and in parallel with its inhibition of MAO-A allowed a better functioning of the hippocampus.

Footshock stress effects towards behavior

Theta waves happen during stress, orienting, exploratory and memory processing[28,29]. According to a previous study, inevitable footshock activate GABAergic pathways in the medial rugh nuclei MRN, increasing the power of low frequency LF (4–7 Hz) theta oscillations, resulting in disabling MRN to desynchronize theta waves[30]. 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, footschock exposure facilitated learning[31]. Similar to the case of pregnant female rats after an acute stress session. However, psychological stress enhanced caution in pregnant female rats.

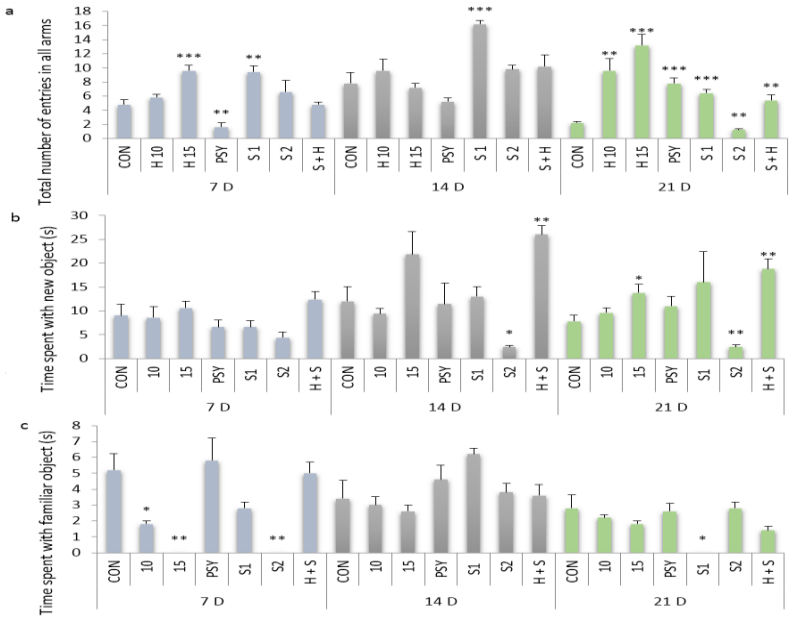

Figure 1: (a). Total number of entries in all arms during plus maze test, (b). Time spent with new object, (c). Time spent with familiar object of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0.4 and 1.2mA; respectively'.

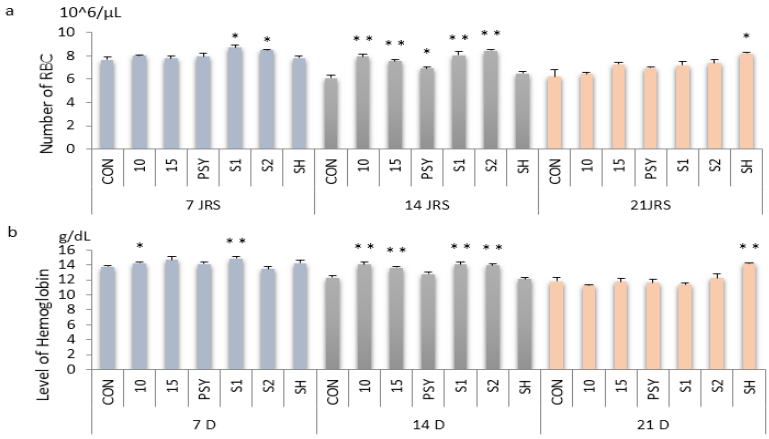

Figure 2: (a). Level of RBC, (b). level of hemoglobin of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0.4 and 1.2mA; respectively'.

Effects of harmine and footshock stress on spatial memory

Physical pain resulting from an acute stress decreases 5-HT2A receptor-mediated behavioral responses[32]. 5-HT2A receptors have an equal effect in the cornuammonis CA1 and CA3 hippocampal areas, whereas 5-HT2C receptors abound in CA1[33]. Acute pre-training administration of harmine improved novel object recognition of mice[34]. Moura *et al.*,[34] suggested that it is due to an interaction between serotonin (5-HT1A, 5-

HT2A and 5-HT2C), benzodiazepine and dopamine receptors. The treatment with harmine at 10 mg/kg has shown any significant increase of time testing the new object, even though the treated stressed rats demonstrated a significant increase (p<0.01) of time examining the new object, during the second and third weeks (Figure 1b).

Current 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 innervation with CA1 has a major role for novelty detection[35]. CA3 hippocampal subfield has implicated in hippocampal memory formation by gamma oscillations[36], and it is the main coordination between CA1 and CA3 recuperation of spatial memories[37-39]. Because, activation of CA3 related with localisation of the objects with spatial location[36,40].

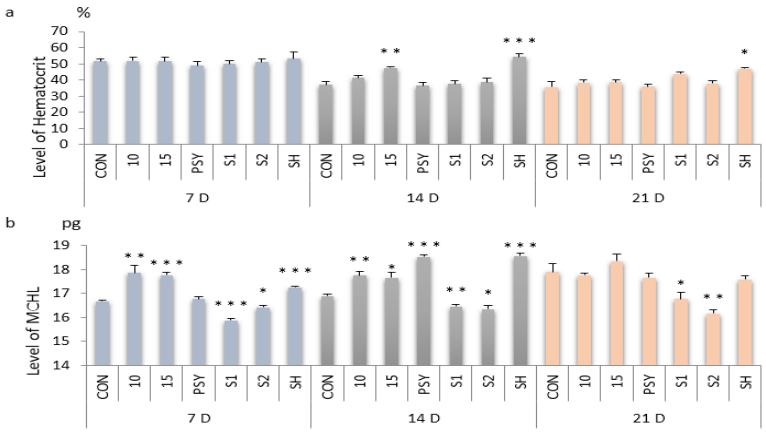

Figure 3: (a). Level of hematocrite, (b). level of M C H L of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0.4 and 1.2mA; respectively'.

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, dehydroepiandro-sterone and pregnenolone, are mainly located in the CA3-CA1, playing a leading role in the learning process and storing in the rat brain[41]. In that case, it can be suggested that connections between CA3-CA1 corresponding of object' 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 in the second week of pregnancy (Figure 1a). Time spent with familiar object has decreased significantly after treatment during the first week (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 OFT. Therefore, it can be concluded that the exploratory behavior of the rats shown during the exploration of the objects and OFT and plus maze test is coherent. In general, hallucinogens own more than one type of 5-HT2 receptors, evidence outbalanced that 5-HT2A receptors are the main site responsible for hallucination[42]. When 5- HT2A receptors have activated locomotor activity increased, but 5-HT2A agonists (hallucinogens) examined had produced decreases in locomotor activity[43,44]. According to Preskorn *et al.*,[45] 5-HT2A in pharmacotherapy used as antidepressants. The plus maze test and object recognition test, confirmed that harmine exerting its antidepressant effect activating the 5-HT2A receptors.

Benign effects of harmine during pregnancy

Total alkaloid extracts from seeds of *P. harmala* TAEP has not affected the hematological parameters[46]. It will be obvious that, during mammal's pregnancy, decreased values in red blood cells R BC counts and especially hemoglobin concentration (anemia) are causing either, by hemodilution or nutritional deficiency. It was even worse after sub-acute and acute stress, inducing a decrease in hemoglobin level in RBC, it has corrected with harmine treatment (Figure 2a, b). 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. In this study, underlying cause of anemia during pregnancy refer to hemodilution rise. Pregnant female rats have marked a decrease in red blood cells, hemoglobin and hematocrite during the second week (Figure 2a, b, 3a), due to organogenesis 'neurulation and turning, optic and otic vesicles, kidneys, lung and forelimb bud formation'.

The treatment with harmine at both doses induced a significant increase of R BC (p<0.01) (Figure 2a), the mean corpuscular hemoglobin level MCHC levels (Figure 3b) has shown its enhancement induced by harmine during the first and second week.

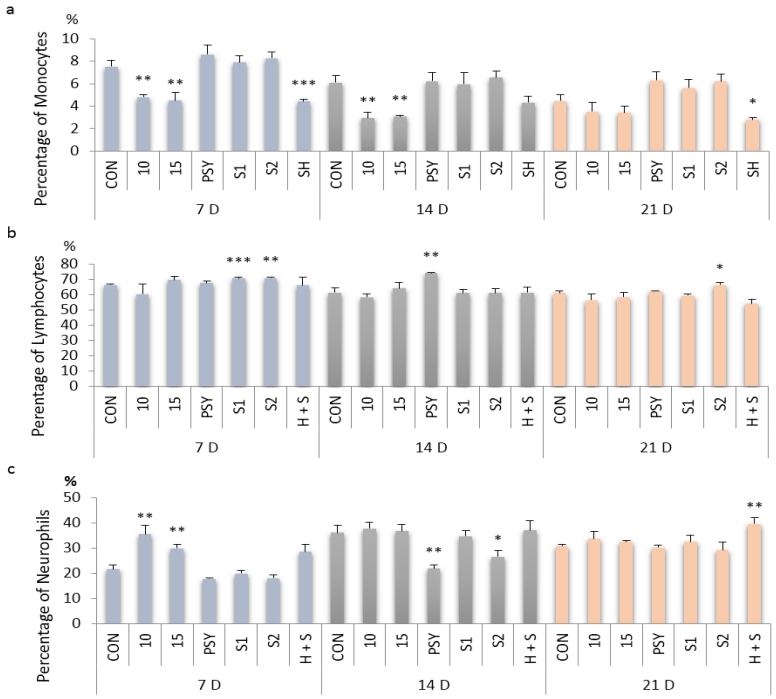

Figure 4: (a). Percentage of monocytes, (b). Percentage of lymphocytes, (c). Percentage of neutrophils of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0.4 and 1.2mA; respectively'.

The plant of *P. harmala* enriches the blood and is useful in weakness of muscles and brain[47,48]. The link doubled between C3 and C4 in the β-carboline structure as harmine and its derivatives is essential in estrogenic activity especially osteoblast differentiation[49], the enhancement of red blood cells, hemoglobin, hematocrite could exerted by harmine even during pregnancy. Current study report that throughout gestation, rats had an increasing in granulocytes and decreasing in lymphocytes, but monocytes level remains stable[50]. That case is not constant, 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). In the last week of rat pregnancy, lymphocytes, monocytes, and granulocytes show an activated phenotype[7].

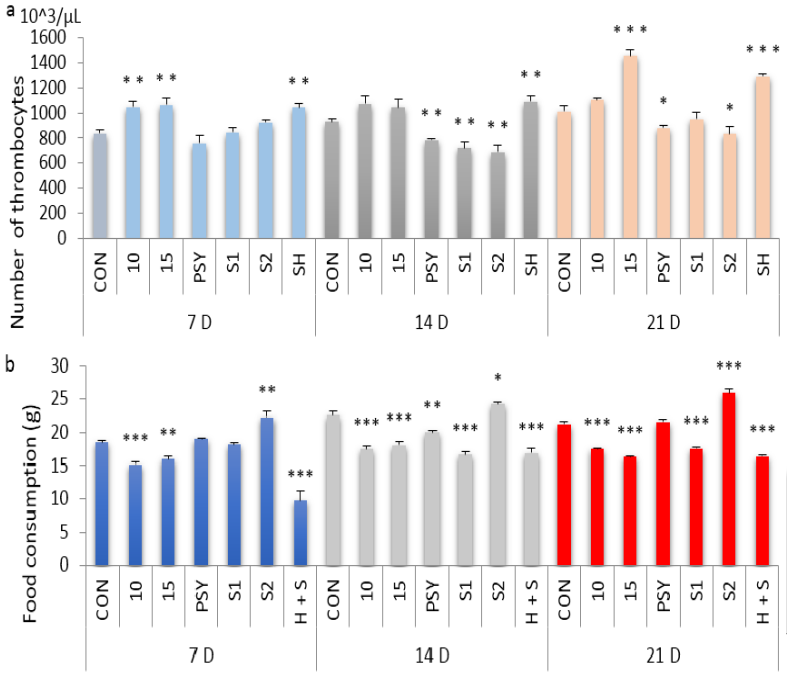

Figure 5: (a). Level of thrombocytes, (b). Food consumption of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0.4 and 1.2mA; respectively'.

Current 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 *et al.*,[51] confirmed that lymphocyte mitogenic activity suppressed by footshock.

The percentage of leukocyte and neutrophil increased throughout gestation, whereas lymphocyte decreased at second trimester only. Neutrophil degranulation causes astrocyte death, together with oligodendrocyte death leading to axonal degeneration[52]. Normal granulation was considered in current study 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 neuro-modulator effect through immunological adjustment of harmine during gestation. The high level of neutrophils indicate an increasing in cytokines. As anti-inflammatory cytokines inhibit Th1 cells, macrophages, and prostaglandin E2 preventing fetal rejection. Which confirm that the low level of monocytes 'macrophages' in the first trimester (Figure 4a) 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, stimulating the transit of leukocytes (including macrophages) from vessels and lymph to the uterus[53,54]. It can be suggested that the decrease of monocytes in the blood was due to the effect of harmine by increasing estrogens production who in turn allowing the current of leukocytes towards 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. As monocytes, respond to Gram-negative bacteria infections. According to Luppi *et al.*,[50] 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.

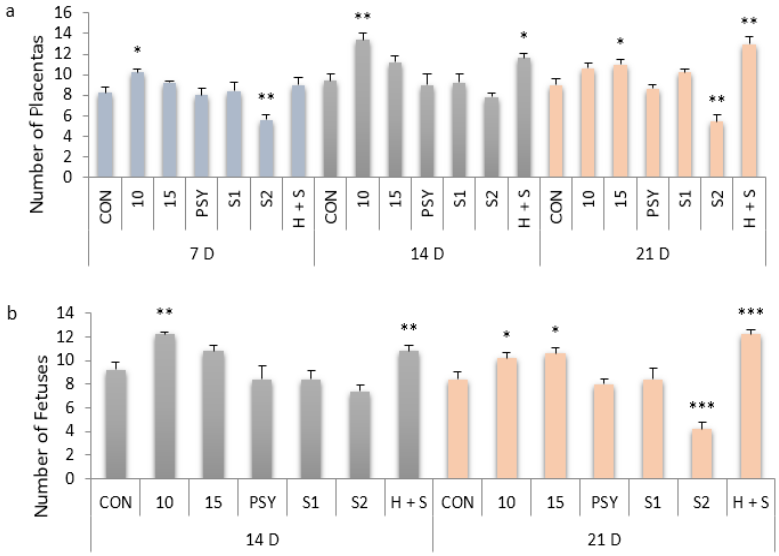

Figure 6: (a). Number of placentas, (b). Number of fetuses of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0.4 and 1.2mA; respectively.

Previous studies suggested that increased of monocytes and granulocytes constitute the main way for maternal immune system to resist infection. It considered previously, as a normal physiological behavior due to blastocyst in uterine tissue to link placenta with mother's uterine tissue. In the case of normal pregnancy, immunosuppression and immunomodulation of fetuses increased inflammatory response leading to leukocytosis[55]. The increase in granulocyte number may also suggest a pro inflammatory state of the inflammatory system during the post-implantation phase of rat pregnancy[56]. 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 antiinflammatory effect of harmine, and we outbalance the first suggestion that, anti-inflammatory cytokines as IL-4 inhibit macrophages, preventing in its turn fetal allograft rejection. As the cytokines had a crucial role for that part, further studies with cytokines assay will be helpful. The increase in neutrophil counts at first stages of pregnancy is a toxicological mark. Rats treated with *P. harmala* had high levels o f white blood cells that indicated an establishment of the immune system[57].

Atsamo *et al.*,[58] suggested that bioactive substances of the crude extract *P. harmala* boost increase blood cells boosting in result the immune response. The increasing in neutrophils exerted by the plant was due to harmine alkaloid. It can be suggested 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 *et al*.,[59], which could confirmed only with a post-partum study of prenatal treated rats with harmine. At second stage of pregnancy, 8-12 days and late pregnancy, the increased need for granulocytes generate their clustering in placental blood vessels, at sites of necrotic areas[59].

The thrust of MAO effect of harmine on fertility

The number of thrombocytes increased slightly during rat pregnancy[59], (Figure 5a) elucidates a slower rise of thrombocytes during pregnancy, between 850 and 1050 10^3/µL in rats. In addition to its main cerebral sites, Serotonin spread in blood platelets, the intestinal mucosaand chromaffin cells[60]. Tranzer *et al*.,[61] Showed serotonin reuptake in megakaryocytes, immediate precursors of blood platelets.

It can be concluded 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[62]. The main action of decreasing food intake by estrogen has not identified yet. 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. Current findings are similar to those of Paul, who stated that the daily dietary intake of the rat reached its peak on day 14 of pregnancy and then declined gradually[63]. Indeed, the lesion of the lateral hypothalamic area leads to a lake in food intake in consequence low body weight, while electrical stimulation of this nucleus conducts to an increase in food intake, even in satiated animals[64]. However, electrical stimulation of the center of satiety "ventromedial hypothalamic nucleus" induces inhibition of food intake[65], Since, corticotrophin releasing factor CR F microinjected in para ventricular nuclei PVN play an anorexic role in rats, it has suggested that, CR F in PVN an inhibitor of food intake by stress[66]. Whereas, feeding amounts had not affected by estrogen via PVN neurons under emotional stress that did not show any increase expression of CR F mRNA in PVN[67]. Nevertheless, it seems that estrogen inhibits food intake in several species. Only its mechanisms decreasing effect on food intake amounts has not revealed yet. Neuro inflammation derived from obesity affect several brain regions such as hippocampus, cortex or amygdala. In general, obesity has been associated with central disorders such as cognitive impairment[68]. Hotta *et al*., have confirmed that either electrical shock or emotional stress could lessen food intake at rats[69]. Psychic stress of rats resulted in weight loss by 14% in a month[70]. One study has shown that psychosocial stress acting via acetylcholine and training intestinal barrier dysfunction[71]. Animals exposed to the acute footshock stress induced a significant rise 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 has mentioned before, inhibition of MAO-A attenuates levels of the dopamine metabolite DOPAC in the nucleus accumbens, so enhancing levels of tissue dopamine[72]. Other studies indicate that dopaminergic neurotransmission, particularly in the nucleus accumbens, could also participate in the reward mechanisms associated with food intake[73]. In other hand, it regulated indirectly by leptine secretion and, leptine decreases dopamine release in the nucleus accumbens during food intake[74]. Which explain the decreased in consumption amount of pregnant rats. The two types of uterine mitochondrial MAO located adrenergic neurons including their various types to regulate uterus functions[75]. For example, human placenta expresses predominantly MAOA[76]. In that case, it can be supposed that harmine can exert the similar effects in human.

The number of fetuses of the pregnant rats have been between 7 and 11 (Figure 6b), the increase of placent-ation (Figure 6a) after the treatment have been significant (p<0.05) at first and last stages 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). Former studies set the relation between the lack of progesterone levels, synthesis of their inhibitors and abortion at rats. 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.

The anti-nociceptive effect of harmine toward footshock pain

Musazzi *et al*., have shown that footshock stress, enhance endogenous glutamate immediately after the stress session[77]. In a study by Sun *et al*., it has confirmed that GLT-1 m-RNA multiplied after harmine treatment[78]. As harmine attenuate astrocytes activation, and acting with glutamate transporter GLT 1 to protect neurons from insults derived by Global cerebral ischemia GCI[78]. Glutamate accumulation in synapses lowered by boosting GTL- 1 activity, so that resulting in attenuation in postsynaptic glutamate receptors activation, consequently, reducing the death of neurons[79]. It can be concluded that the enhancement of the number of fetuses was due to the stabilization of polarization of muscular tissues and preventing of pain sensation. Tricyclic antidepressants have multimodal action to attenuate neuropathic pain as monoamine reuptake inhibition and N-methyl-D-aspartate blocking receptors[80]. The study of Schwarz *et al*.,[16] 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. It worth notice that the anti-nociceptive like-effect exerted by harmine was through to its MAO A inhibition too.

***P. harmala* physiology during seasons and its impacts on animal' fertility**

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. At the beginning of summer, plants overfilled with vivacity, so due to their emitting odors and molecules with plants attract the animals. Because

rootstocks of spring, been active photosynthetically during summer, due to the high levels of potassium which activates enzymes. Animals consuming the plant show frequent abortion in water scarcity year[81].

When the levels of potassium were low, 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, 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* stimulatory effect on uterine, via the release of prostaglandins, it was used as an abortifacient agent[82], 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[83], which containing Harmalol, harmaline and harmine in a very important portions[84]. Harmine in current study has not shown its abortifacient effect, further studies will conducted on the effect of other alkaloids of the plant.

CONCLUSIONS

This article has interested in the reaction of rats towards a multipotent molecule, in accordance with this concept, effect of footshock stress on pregnant rats depending on fetuses' development have elucidated. This study confirmed that harmine improves alertness and memory, and the study denied its hallucinogenic effect. The inhibitory effect of MAO increased the level of serotonin with the anti-inflammatory effect in hippocampus was the main reason for strengthening 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 induce the entrance of leukocytes from blood vessels to 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-nociceptive effect. In addition, exempting hippocampus from cytokines due to the anti inflammatory effect, adding its anxiolytic effect via benzodiazepine receptors 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 R BC preventing anemia, it can be explained by its disturbing effect on white cells has just due it's acting on the uterus' tissue. The decreased number of fetuses' placentation, and neutrophils on the one hand, and the increasing number of monocytes on the other hand in foot-shocked groups indicate that the nociceptive effect of stress decreasing 5-HT2A receptors decrease implantation. All studies 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. Since the number of fetuses was not sufficient to confirm the beneficial effect of harmine and its neutrophil enhancing effect was countered, a prolonged study of the physiological state of female rats during breastfeeding with their young will be necessary.

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AUTHORS' CONTRIBUTION

Benatoui R: experiments design, experiments conduction, provision of study materials and equipment, study validation, writing and reviewing. **Bairi A:** conceptualized and refined research ideas, experiments design, provision of study materials and equipment, data analysis, study validation, supervision, writing and reviewing. **Tahraoui A:** study validation, supervision, animal demands and administrative agreements. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

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