Original Research Article

FEMALE RATS MAINTAIN PREGNANCY IN SHADE IMMUNOLOGIC PERTURBATIONS EXERTED BY HARMINE AND FOOTSHOCK STRESS ATTEMPTS TO LABOR

ABSTRACT

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. Harmine has enhanced the cognition during pregnancy, and exerted its anxiolytic effect too. Its decreasing effect of food consumption and enhanced implantation was the main reason to confirm its enhancing estrogen levels from previous works, raising thrombocytes due to its enhancement of serotonin via its effect of monoaminoxydase 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: Harmine . Footshock stress . pregnant rats . Monocytes .

INTRODUCTION

Treatment with ayahuasca did not affect the predictors of major depression, neither brain derived neurotrophic factor BDNF nor cortisol level, higher BDNF levels were detected[1]. *Peganum harmala* well known in traditional Bedouin medicine used as an emmenagogue and abortifacient[2]. 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[3]. 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'[3].

Harmine is a potent and selective inhibitor of MAO-A[4]. Thus, treatment with harmine would expected to produce a general increase in serotonergic activity[5]. 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[6]. In addition, evidence suggests that both inhibitory and facilitatory serotonergic effects are mediated postsynaptically[6]. However, in a later series of experiments the agonists have found to facilitate lordosis in females primed with estrogen alone[7,5].

Most research has focused on lymphocyte cytokine production 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[8]. Moreover, in line with human experiments, we observed recently that during rat pregnancy both monocytes and granulocytes have shown an activated phenotype[9].

Previous works had suggested that these rats exhibit greater basal impulsivity but not high reactivity to novelty[10]. Different cerebral structures participate in different types of memory, being the striatum related with procedural memory[11]. It is a dopaminergic area involved to

memory[12]. However, according to Kieras et al. (1999)[13] 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[14, 15]. 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[16].

Harmine produced an increase in dopamine release from rat striatal slices[17, 18]. In other hand footschock stress increased extracellular dopamine in the prefrontal cortex PFC[19, 20]. However, excessive MFC dopaminergic activity has a negative impact on the cognitive functions of primates, making them unable to select and process significant environmental stimuli[21]. 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[22], then harmine risks exerting its anti-stress effect.

Discussion

Neuronal modulation of harmine

The non-toxic effect of the ß-Carboline alkaloid 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 has previously described, as hallucinogenic drug, these mechanisms, to our knowledge, have not been[23], a study found that Harmine is a significant enhancer of short-term working memory in male rats[24]. 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[25].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 et al. (2014)[26] 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 mPFC. In the present study, the expression levels of inflammatory cytokines in the hippocampus of the rat brain have suppressed following treatment with harmine[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

In general, theta waves occur during stress, memory processing, orienting and exploratory[28, 29]. Our previous study revealed that inescapable footshock stimulation increases the power of low frequency L F (4–7 Hz) theta oscillations through the activation of GABAergic pathways in the medial rugh nuclei (MRN), which blocks the ability of 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

1,2mA affected significantly the exploration and locomotor behavior of pregnant rats. Similarly, footschock administration, before and after, passive avoidance facilitated learning, consolidation and evocation[31]. Similar to the case of 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.

Effects of harmine and footshock stress on spatial memory

It is conceivable that acute stress provoking physical pain decreases 5-HT2A receptormediated behavioral responses[32]. Of the three 5-HT2 subtypes, 5-HT2A receptors have equally expressed in the CA1 and CA3 hippocampal subfields, whereas 5-HT2C receptors predominated in CA1[33]. Acute pre-training systemic administration of aromatic β-carboline alkaloids, harmine and harmol, improved novel object recognition of mice[34]. It may, induced by interactions with serotonin (5-HT2A, 5-HT2C and 5-HT1A), dopamine and benzodiazepine receptors[34]. The treatment with harmine at 10mg/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[35]. Gamma oscillations in area CA3 have been implicated in hippocampal memory formation[36], and may serve as a mechanism by which CA3 output can coordinate CA1 activity during retrieval of spatial memories[37-39]. Because, activity in CA3 is required for association of objects with spatial location[36, 40]. 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[41]. In that case, we can suggest 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 during the second week of pregnancy (Figure 1a). Time spent with familiar object has decreased significantly 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 15mg/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, 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.

Antidepressant effect of harmine via 5-HT2 receptors

Although classical hallucinogens bind at multiple populations of 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 [42]. 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[43, 44]. According to Preskorn (2008)[45] 5-HT2A are involved in antidepressant pharmacotherapy. The plus maze test and object recognition test, confirmed

that harmine exerting its antidepressant effect activating the 5-HT2A receptors consequently, it enhanced locomotion.

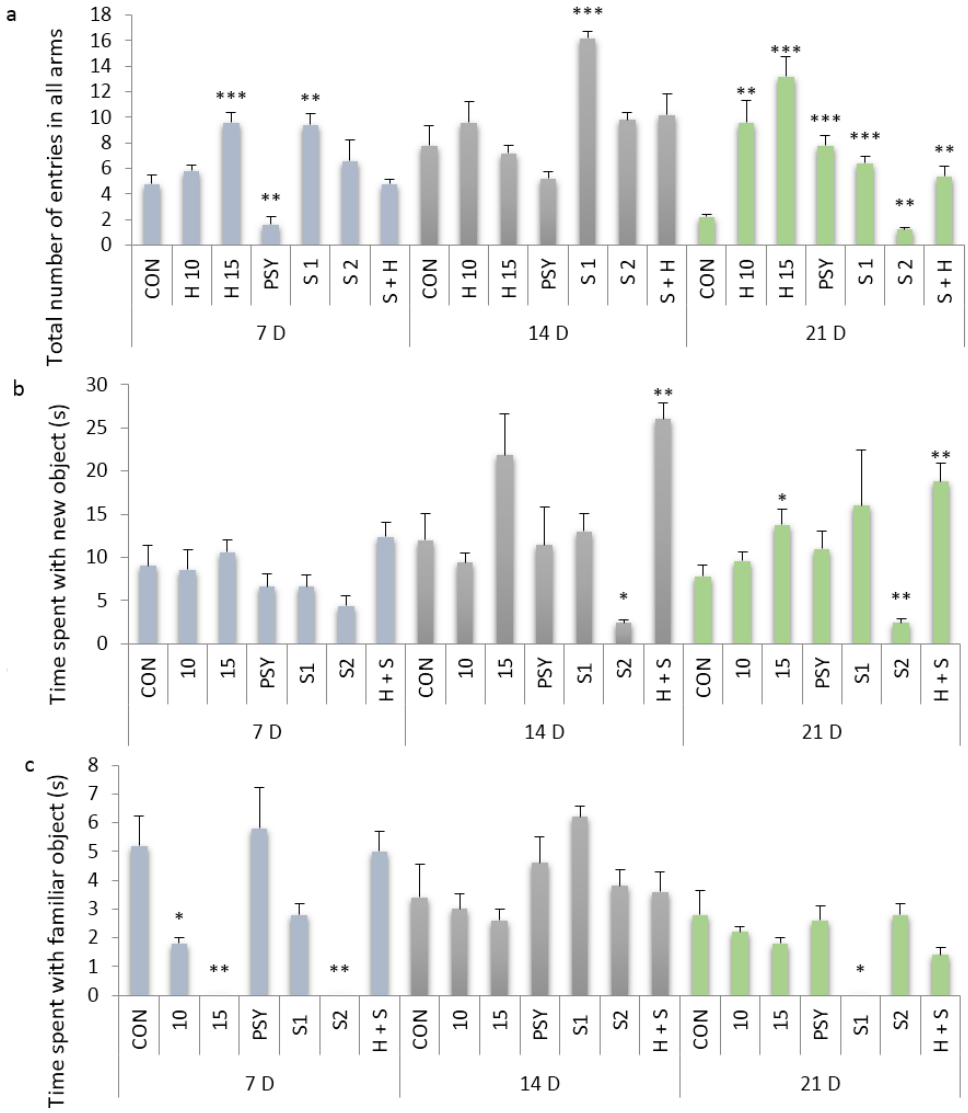

Figure 1: (a) Total number of entries in all arms during plus maze test, (b) Time spent with new object, (c) Time spent with familiar object of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0,4 and 1,2mA; respectively' (*p<0.05; p <0.01;*** p <0.001).**

Benign effects of harmine during pregnancy

It seemed that there were no remarkable relationships between hematological parameters and the doses of total alkaloid extracts from seeds of *P. harmala* TAEP[46]. The drastically decreased values in red blood cells R B C counts, hemoglobin concentration, and hematocrit strongly suggest that hemodilution (and consequently anemia) occurs in rat pregnancy[47]. It was even worsen 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.

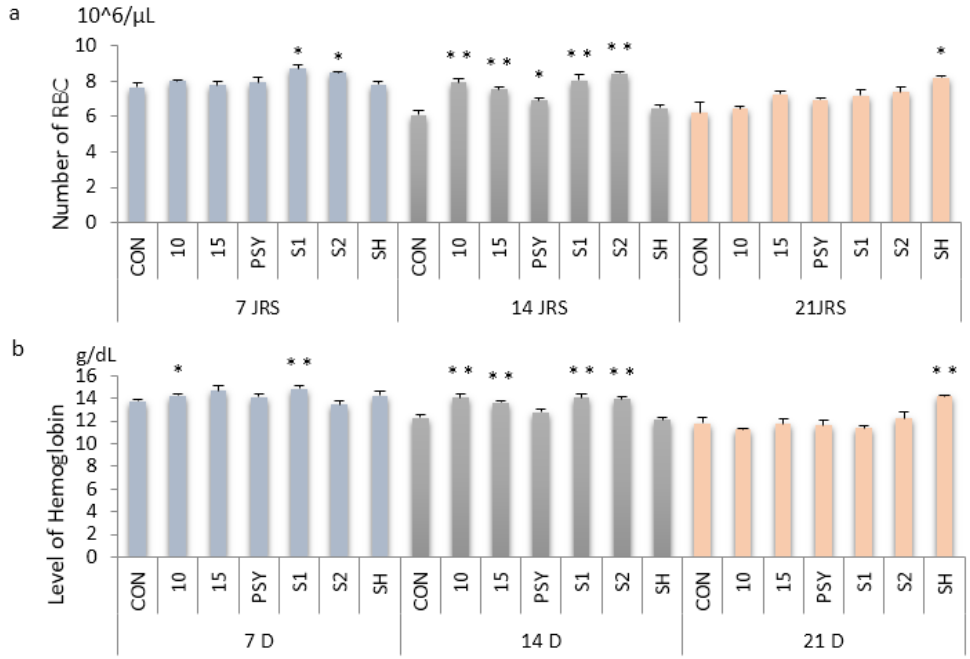

Figure 2. (a) level of red blood cells, (b) level of hemoglobin of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0,4 and 1,2mA; respectively' (*p<0.05; p <0.01;*** p <0.001).**

The underlying cause of anemia during pregnancy is 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 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[48, 49]. The results for the structure-activity relationships of harmine derivatives on osteoblast differentiation revealed that a double bond between C3 and C4 in the β-carboline structure might be essential for its osteogenic activity[50]. 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[50], the enhancement of red blood cells, hemoglobin, hematocrite could exerted by harmine even during pregnancy.

Immunological perturbations due to harmine treatment during pregnancy

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[51]. 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). We have previously shown that peripheral lymphocytes, monocytes, and granulocytes show an activated phenotype, especially in the last week of rat pregnancy[9]. 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 et al. (1995)[52] confirmed that footshock exposure markedly suppresses lymphocyte mitogenic activity.

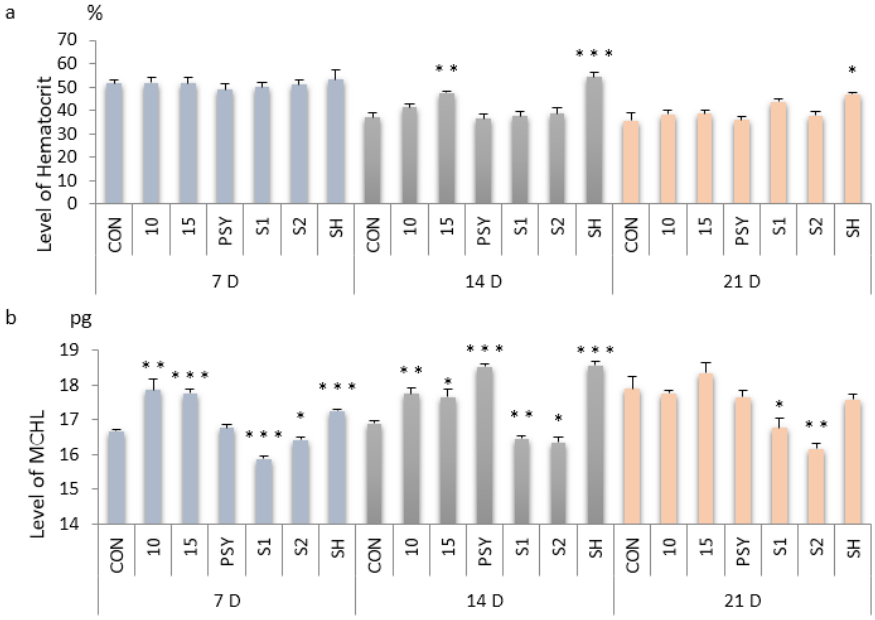

Figure 3. (a) Level of hematocrite, (b) level of M C H L of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0,4 and 1,2mA; respectively' (*p<0.05; p <0.01;*** p <0.001).**

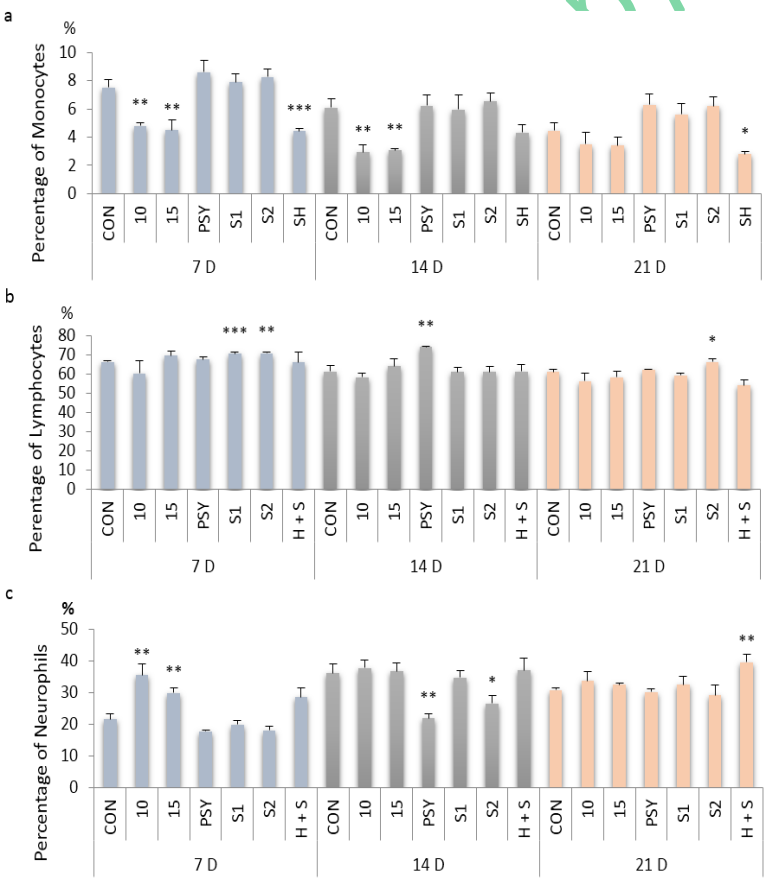

Figure 4. (a) Percentage of monocytes, (b) Percentage of lymphocytes, (c) Percentage of neutrophils of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0,4 and 1,2mA; respectively' (*p<0.05; p <0.01;*** p <0.001).**

The percentage of leukocyte and neutrophil increased from the first to the third trimester, whereas lymphocyte decreased from the first to the second trimester. Neutrophil degranulation leads to astrocyte death, which in turn causes oligodendrocyte death and axonal degeneration[53]. 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 level 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*-α (TNF-α), cyclooxygenase-2 (COX-2), and prostaglandin E2 in amnion-derived cells, which prevent the onset of labor[54, 55]. 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, as these hormones stimulate the influx of leukocytes (including macrophages) into the uterus[56, 57]. 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[51]. According to Luppi et al. (2002)[51] 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[58]. Circulating monocytes and granulocytes show an activated phenotype[59]. 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[60]. The increase in granulocyte number may also suggest a pro inflammatory state of the inflammatory system during the post-implantation phase of rat pregnancy[61]. The increased number of neutrophils in treated groups confirms a good implantation of fetuses generating proinflammation. 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. The increase in neutrophil counts at first stages of pregnancy is a toxicological mark. Elevation of white blood levels in treated rats with *P. harmala* directly indicates a strengthening of the immune system[62]. 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[63]. The increasing in neutrophils exerted by the plant was due to harmine alkaloid. 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 et al.

(2002)[47], 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[47].

The thrust of MAO effect of harmine on fertility

The number of thrombocytes increased slightly during rat pregnancy[47], (Figure 5a) elucidates a slower rise of thrombocytes during pregnancy, between 850 and 1050 10^3/µL in rats. Serotonin is also present in blood platelets, chromaffin cells of the intestinal mucosa and in the central nervous system[64]. Tranzer et al. (1972)[65] 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[66]. The precise mechanism by which estrogen decreases food intake h a s 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, who stated that the daily dietary intake of the rat reached its peak on day 14 of pregnancy and then declined gradually[67]. 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[68]. However, the ventromedial hypothalamic nucleus, which is considered to be the "center of satiety", its electrical stimulation induces inhibition of food intake[69]. It has suggested that CR F in PVN plays a role in the inhibition of food intake by stress, since CR F microinjected in para ventricular nuclei PVN alone inhibits food intake in rats[70]. However, estrogen does not affect feeding behavior by CR F neurons of PVN under emotional stress because emotional stress using a communication box did not increase expression of CR F mRNA in PVN[71]. 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[72]. Hotta et al. have shown that food intake is inhibited by electrical shock and emotional stress induced by a male rat communication box[73]. Psychic stress resulted in a 14% weight loss in stress group rats compared to control group rats in 4 weeks[74]. One study has shown that psychosocial stress induced dysfunction of the intestinal barrier has related to the release of acetylcholine[75]. 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).

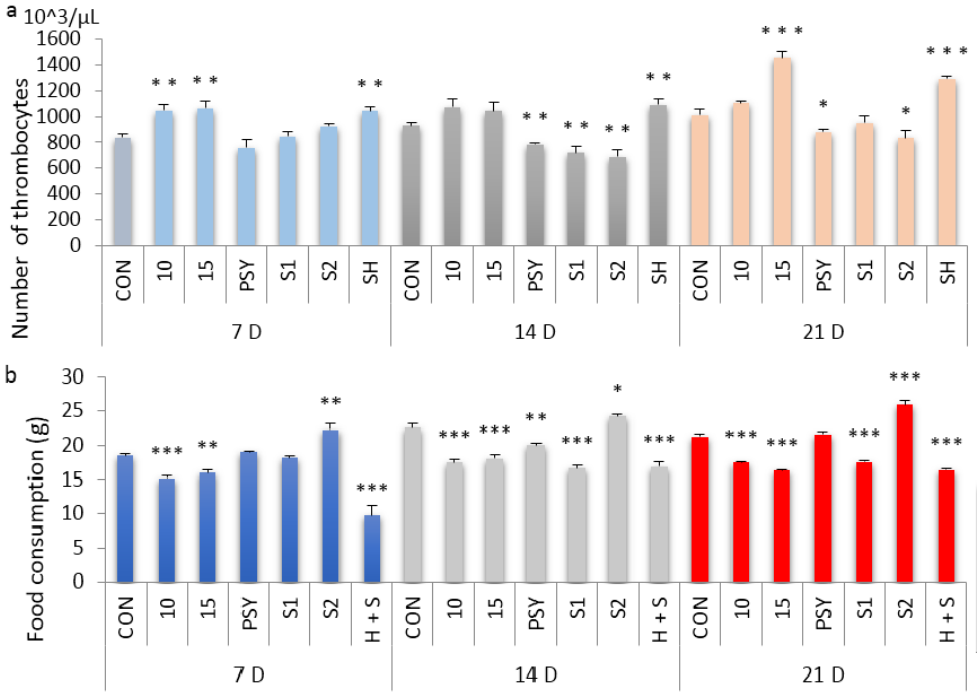

Figure 5. (a) Level of thrombocytes, (b) Food consumption of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0,4 and 1,2mA; respectively' (*p<0.05; p <0.01;*** p <0.001).**

As it mentionned before inhibition of MAO-A augments levels of tissue dopamine and attenuates levels of the dopamine metabolite DOPAC in the nucleus accumbens76. Other studies indicate that dopaminergic neurotransmission, particularly in the nucleus accumbens, could also participate in the reward mechanisms associated with food intake77. In other hand, it regulated indirectly by leptine secretion and, leptine decreases dopamine release
in the nucleus accumbens during food intake78. Which explain the decreased in consumption amount of pregnant rats. 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 uterus79. For example, human placenta expresses predominantly MAO A 80. 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 6b), the increase of placentation (Figure 6a) after the treatment have been significant (p<0.05) in the first and third week with dose 10 and 15mg/kg respectively, the second week with the 10mg/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. azastene81. 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.

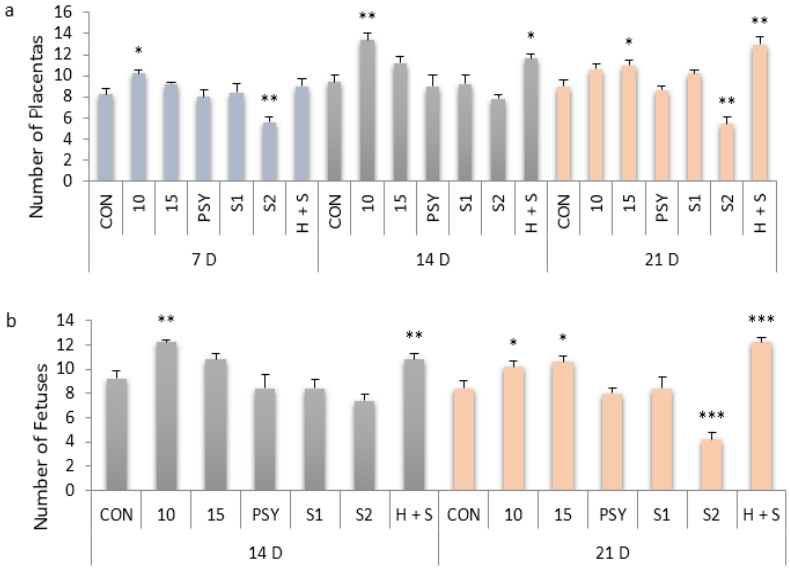

Figure 6. (a) Number of placentas, (b) Number of fetuses of stressed and treated rats with harmine during three stages of pregnancy. 'S1 and S2, 0,4 and 1,2mA; respectively' (*p<0.05; p <0.01;*** p <0.001).**

The anti-nociceptive effect of harmine toward footshock pain

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)[82]. In present study, we have confirmed that GLT-1 mRNA and protein expression is remarkably elevated in harmine treated group[83]. 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[83]. Because, the elevation of 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[84]. For a long time *P. harmala* has been used in traditional medicines for the relief of pain and as an antiseptic agent[3]. We can conclude that the enhancement of the number of fetuses was 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[85]. The study of Schwarz et al. (2003)[18] 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 through to its MAO A inhibition too.

The impact of *Peganum harmala* on fertility during seasons

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, due to the high levels of potassium which activates enzymes. Abortion is frequent in animals that digest this plant in a dry year[86]. 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* traditionally has been used as an abortifacient agent, due to its stimulatory effect on uterine, apparently through the release of prostaglandins[87], 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[88], which containing Harmalol, harmaline and harmine in a very important portions[89]. 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 article has interested in one of the most important physiological processes in the animal's body, in accordance with this concept, effect of footshock stress on pregnant rats depending on fetuses' development have elucidated.

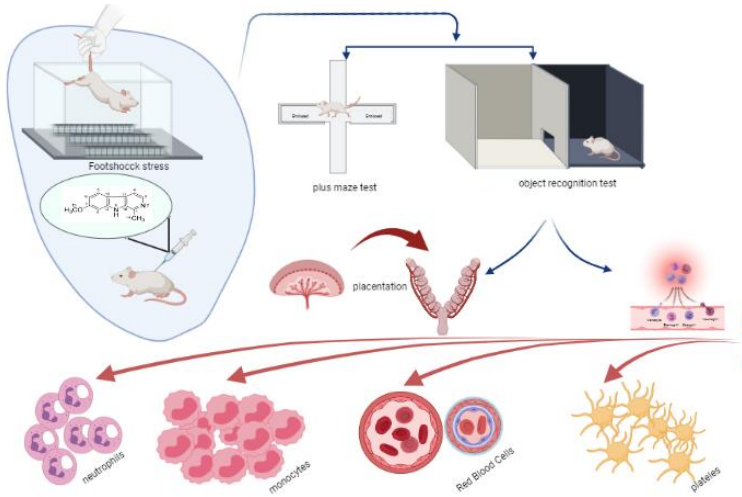

Figure 7: main steps followed for this study

It has confirmed that harmine improve caution and memory which denied its hallucinogenic effect. 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-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, we can explain its disturbing effect on white cells has just due its acting on the uterus' tissue. On the other hand, decreasing number of fetuses and neutrophils faced the increasing number of monocytes in footshocked 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.

As the number of fetuses was not sufficient to confirm the beneficial effect of harmine faced its enhanced effect of neutrophils, a prolonged study of the physiological state of female rats during breastfeeding with their pups will be necessary.

Abbreviations

ALP alkaline phosphatase, **BDNF** brain-derived neurotrophic factor, **CA** cornu ammonis, **COX-2** cyclooxygenase-2, **CRF** corticotropin-releasing factor, **DOPAC** dihydroxyphenylacetic acide, **GCI** Global cerebral ischemia, **GLT** glutamate transporter, **5-HT** 5-hydroxytryptamine **MAO** monoamine-oxidase, **MCHC** mean corpuscular hemoglobin level, **MFC** medial frontal cortex, **MRN** medial rugh nuclei, **PFC** prefrontal cortex, **PVN** paraventricular nucleus, **RBC** red blood cells, **TNF-α** *tumor necrosis factors-α.*

Competing interests

None declared

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