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Original Contribution

INVESTIGATION THE PROTECTIVE EFFECT OF VITAMIN C ON ANXIETY AND OXIDATIVE STRESS MODULATION IN MALE RATS TREATED WITH PROGESTERONE

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ABSTRACT

The prevalence of anxiety in women and men are different. This indicates the influence of sex hormones on anxiety. According to conflicting reports about the effects of Progesterone on anxiety, the aim of this study is to investigate the anxiogenic or anexiolytic effects of different doses of progesterone on absence or presence of vitamin C. Sixty-three male rats were used in nine groups including: control, sham, 3 groups of the progesterone (5, 10 and 30 mg/kg), Vitamin C (80mg / kg), combination group (different doses of progesterone plus vitamin C). Period of intraperitoneal injection was 5 days. Elevated plus maze was used for studying the anxiety related behavior. After termination of behavioral testing days, animals were decapitated and prefrontal cortex dissected to measure some of the oxidative stress markers,which are the indicator of lipid peroxidation and activities of antioxidative enzymes such as Superoxide dismutase (SOD), Catalase (CAT) and Peroxidase (POD) were determined in the rat's prefrontal cortex. Progesterone (5 mg/kg) had anxiogenic effect ($p<0.05$). While in other doses (10, 30 mg/kg) it had an anxiolytic effect ($p<0.05$). Vitamin C (80 mg/kg) alone and as a pretreatment of progesterone (5, 10, 30 mg/kg) reduced anxiety ($p <0.01$). The level of malondialdehyde (MDA) increased in progesterone 5 mg/kg ($p<0.001$) and decreased CAT activity ($p<0.05$). Progesterone (10 and 30 mg/kg) decreased MDA and CAT levels activity ($p<0.05$ and $p<0.01$). Vitamin C alone and vitamin C plus different doses of progesterone treatment groups caused decreasion in the MDA levels and SOD activities ($p <0.001$). It seems that progesterone (5 mg/kg) has anxiogenic effect and at the high doses, progesterone has anxiolytic effect. As an antioxidant, vitamin C can improve the anxiolytic effects of progesterone. Also different doses of progesterone have different effects on oxidative stress parameters.

Key words: Anxiety, Progesterone, Vitamin C, oxidative stress

INTRODUCTION

Anxiety is an adaptive response which detects and prepares an individual against a real or a potential threat (1). Anxiety disorders are 2–4 times more common in women than men. The increased vulnerability of women to anxiety disorders has been linked to the action of steroid hormones, in particular progesterone (and its related steroids) (2). The change in ovarian hormones level in a monthly cycle of menstruation has a significant effect on anxiety level (3). Progesterone is thought to produce its anxiety-modulating effects directly or via its derivative allopregnanolone (3α-5α-THP or 3α-hydroxyl-5β-pregnan-20-one), which modulates the ligand gated chloride channel GABAA receptors (4). There are contradictory reports about the role of ovarian hormones on anxiety. Some studies indicated that ovarian hormones have different effects including anxiolytic or anxiogenic effects on anxiety in rats and mice (5, 6). Several studies suggest a positive correlation between oxidative stress and anxiety-like behavior (7). Oxidative stress occurs when redox homeostasis is tipped towards an overbalance of free radicals, due to either their overproduction or deficiencies in antioxidant defense (8). A significant relationship has been found between trait anxiety and ROS formation in monocytes of hypertensive individuals (9). Patients with

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anxiety disorders compared to healthy controls, have higher activity levels of the antioxidant enzymes SOD and glutathione peroxidase (GSH) as well as higher lipid peroxidation activity. Oxidative metabolism is being also regarded as a plausible pathway that can affect the regulation of anxiety (10). Progesterone may reduce oxidative stress by its membrane stabilizing effect as well (11). GSH is an endogenous antioxidant scavenging free radicals and protecting against oxidative stress. Progesterone administration was effective to protect GSH levels (12). Studies suggest that progesterone reduces lipid peroxidation and oxidative stress, most likely by decreasing the generation of free radicals and enhancing endogenous free radical scavenging systems. Ascorbic acid (Vitamin C) is a low molecular weight antioxidant that scavenges the reactive oxygen species (ROS) through electron transfer rapidly and prevents lipid peroxidation (13). Vitamin C has also been implicated in anxiety and psychological stress-related behavior (14). These antioxidant properties might also be responsible for vitamins' anxiolytic effects, because it has been shown to modulate immobilization-induced oxidative stress in the rat brain as indicated by increased activity of (SOD), glutathione-S-transferase and CAT, and decreased lipid peroxidation (15). A candidate Braine region that underlie anxiety is the medial prefrontal cortex (mPFC). Medial regions of the PFC exhibit increased activation in anxious versus non anxious youth in response to emotional faces (16).

According to contradictory effect of progesterone on anxiety as being ineffective, worsening or improving of this phenomen, in the present study the anxiolytic or anxieogenic effects of different doses progesterone as well as oxidative stress parameters were investigated in the presence or absence of antioxidant vitamin C.

MATERIALS AND METHODS

Animals

The Regional Ethics Committee of Tabriz University of Medical Sciences approved all experimental procedures. Every effort was made to minimize the number of animals used and their suffering. Animals were obtained from the colony of Tabriz University of Medical Sciences. The experiments were performed on adult male Wister rats weighing 200-250g and 3-4 months old at the start of the experiments. The animals were housed in a temperature (23±1°C) and humidity-controlled room. The animals were maintained under a 12:12 h light/ dark cycle, with lights off at 8:00 p.m. Food and water was provided ad libitum except for the periods of behavioral testing. The behavioral testing was done during light phase. Drug or saline were injected intraperitoneally in a volume of 1 ml/kg. Test was preformed 30 min after injection. The experiments were performed on sixty -three rats randomly divided into 9 groups (n=7) as follows: 1 (Control (saline) group as solvent), 2 (sham group, sesame Oil as solvent), 3 (Progesterone 5 mg/kg), 4 (Progesterone 10 mg/kg) , 5 (Progesterone 30 mg/kg), 6 (Vitamin C 80 mg/kg), 7 (Progesterone 5 mg/kg + Vitamin C), 8 (Progesterone 10 mg/kg+ Vitamin C), 9 (Progesterone 30 mg/kg + Vitamin C).

Behavioral testing

Elevated plus maze test (EPM)

Behavior in the elevated plus maze is utilized to assess anxiety behavior (17). The plus maze was elevated 50 cm off the ground and consisted of four arms (49 cm long and 10 cm wide). Two arms were enclosed by walls 30 cm high and the other two arms were exposed. We measured four behavioral variables: the number of entries into open arms, the amount of time spent in open arms, the number of entries into enclosed arms and the amount of time spent in enclosed arms. Anxious animals are expected to make fewer entries into open arms and to spend less time in open arms than are non-anxious animals. Rats were placed at the juncture of the open and closed arms and the number of entries into and the amount of time spent on, the open and closed arms were recorded during a 5 min test. A rat was considered to have entered the new area when all four legs were in this area. The floor of each box was cleaned between sessions. Time spent on the open arms is an index of anxiety and the total number of arm entries is measure of motor activity. The testing session was performed 30 min after the drug injection.

Tissue preparation and measurement of oxidative stress markers in the prefrontal cortex:

Following behavioral testing, animals were sacrificed by decapitation and the prefrontal cortex were quickly removed and kept at -80^{o}C until used for preparation of homogenates. At the day of analysis the prefrontal cortex tissues were homogenize in cold KCL solution (1.15%) to give a 10% homogeny suspension and used for biochemical assay.

Measurements of oxidative stress markers in the prefrontal cortex

Lipid peroxidation assay

MDA results from degradation of polyunsaturated fatty acids. The production of this substance is used as a biomarker to measure the level of lipid peroxidation. MDA reacts with Thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to form a 1:2 MDA-TBA adduct, which absorbs at 532 nm. Thus, the quantity of TBARS is proportional to the amount of MDA. Concentration of TBARS is determined according to the method of Mihara and Uchiyama (1978). Briefly, 3 ml of 1% phosphoric acid and 1 ml of 0.6% w/v TBA aqueous solution were added to 0.5 ml of homogenate supernatant and heated for 45 min in a boiling water bath. After cooling, 4 ml n-butanol was added; the mixture was shaken and then centrifuged at 3,000×g for 10 min. Then, the absorbance of the samples at 532 nm was measured. The concentration of TBARS was calculated using the MDA standard curve and is represented as nmol/mg of protein (18)

Antioxident enzymes activity assay

Superoxide dismutase (SOD) assay

SOD activity in the prefrontal cortex homogenates was assayed using a method based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. Briefly, 1 ml of Tris-HCl (45 mM) buffer containing EDTA was mixed with 5 µl of homogenate supernatant 10 and was placed in the spectrophotometer. The unit was auto-zerod at 420 nm and then 50 µl of pyrogallol (0.2 mM) was added to the above solution and quickly the absorbance of samples was measured at 420 nm every 15 seconds, up to two minutes. The inhibition of pyrogallol autoxidation is proportionate to the activity of SOD present in the sample. Enzyme inhibitory capacity is defined as one unit of SOD and was placed in the spectrophotometer. The unit was auto-zerod at 420 nm and then 50 µl of pyrogallol (0.2 mM) was added to the above solution and quickly the absorbance of samples was measured at 420 nm every 15 seconds, up to two minutes. The inhibition of pyrogallol autoxidation is proportionate to the activity of SOD present in the sample. Enzyme inhibitory capacity is defined as one unit of SOD (19).

Catalase (CAT) assay

CAT activity was determined according to Briefly, hydrogen peroxide (20 mM) in a phosphate buffer (50 mM, pH 7.0) was used as hydrogen peroxide substrate and decrease of the substrate in H2O2 concentration at 25o C was followed spectrophotometrically at 240 nm for 1min. Activity of the enzyme was expressed in units per mg of protein, with 1 unit equaling the amount of enzyme that degrades 1µM H2O2 in a minute (20).

Peroxidase (POD) assays.

Steady-state measurements for peroxidase activity were carried out spectrophotometrically using guaiacol as electron donor substrate. The increase in the absorption as a result of the formation of the oxidized product (tetraguaiacol) was measured at 470 nm using the extinction coefficient of 26.6 mM-1 cm-1. Initial rates were calculated from time-dependent absorbance changes and were used for detection of enzyme activity. Assays were carried out at room temperature (~ 20-25ºC) using Pharmacia, Biotech Ultrospec 1000 spectrophotometer (Sweden). Reaction mixture contained 0.1 M citrate-phosphateborate buffer system, pH 7, 15 mM guaiacol, 3.3 mM H2O2 and 25 µl extract. The total volume of reaction mixture was 3 cm3 and assays were carried out in a quartz cuvette with 1 cm path length. All reactions were started by addition of H2O2 to the reaction mixture (21)

Statistical analysis

Data are expressed as the means ± SEM. The statistical analysis of the data was carried out by one-way ANOVA-followed by student newmans keuls test. In all comparisons, $P < 0.05$ was considered significant

RESULTS

1) Effects of Progesterone and vitamin C alone and in combination on anxiety like behaviors:

A) The one–way ANOVA results revealed that there was no significant difference between progesterone 5 mg/kg with control group ($p>0.05$), there was a significant difference between progesterone (10 and 30 mg/kg) groups with control group ($p<0.05$), on the time spent in open arm (OAT). According to **Figure 1 (A),** there is a significant difference between vitamin C (80 mg/kg) with control group ($p<0.05$), on the time spent in OAT. Pretreatment of vitamin C with different doses of progesterone significantly increased the time spent in the open arm when compared to the control group ($p<0.05$). In combination therapy, Pre-treatment of vitamin C with progesterone (5, 10 and 30 mg/kg) significantly increased the OAT when compared to the control group ($p<0.05$, $p<0.05$ and $p<0.001$ respectively). There was a significant difference between progesterone 5 mg/kg group with vitamin C plus progesterone 30 mg/kg group ($P<0.05$), on the time spent in **OAT.**

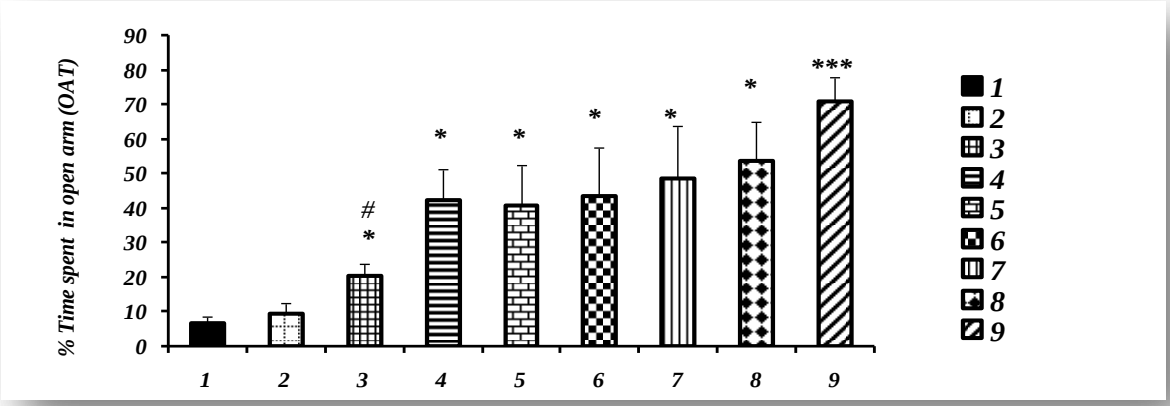

Figure 1(A). 1= Control (saline). 2 = (sham) group, sesame Oil as solvent. 3 = (Progesterone 5 mg/kg), 4 = (Progesterone 10 mg/kg). 5= (Progesterone 30 mg/kg). 6 = (Vitamin C 80 mg/kg), 7 = (Progesterone 5mg/kg + Vitamin C), 8= (Progesterone 10 mg/kg+ Vitamin C), 9 = (Progesterone 30mg/kg + Vitamin C).The effect of progesterone (5, 10 and 30 mg/kg) and vitamin C 80 mg/kg alone and in combination on anxiety like behaviors. In combination therapy rats were injected with vitamin C 30 min before progesterone (5, 10 and 30 mg/kg). Each bar represents mean ± SEM. %OAT (A), OAE (B) or Locomotors activity (LA). * $p<0.05$, *** $p<0.001$ as compared to the 1 (Control) group. # $p<0.05$ as compared progesterone (5 mg/kg, group 3) with vitamin C (80 mg/kg, group 6.)

B) Results showed that there were no significant difference between progesterone (5 and 10 mg/kg) groups with control group ($p>0.05$), there was a significant difference between progesterone 30 mg/kg group with control group ($p<0.05$), on the number of entries into open arms (OAE). According to **Figure 1 (B),** there is a significant difference between vitamin C with control group ($p<0.05$), the number of entries into **OAE**. Pre-treatment of vitamin C with different doses of progesterone significantly increased the number of entries into OAE when compared to the control group ($P<0.01$). In combination therapy, Pre-treatment of vitamin C with progesterone significantly increased the OAE when compared to the control group ($p<0.05$, $p<0.001$). There is a significant difference between progesterone 5 mg/kg with vitamin C ($p<0.05$).

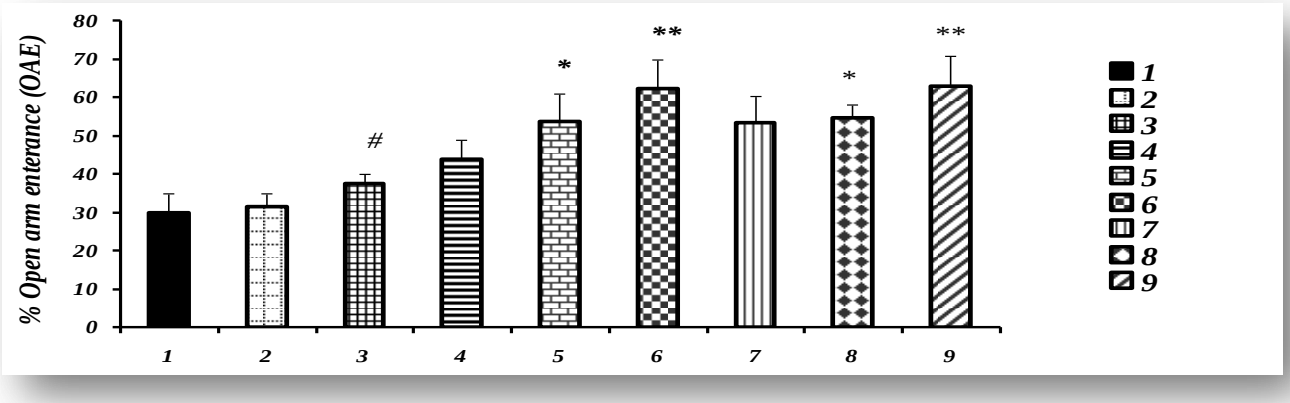

Figure 1 (B). The effect of progesterone (5, 10 and 30 mg/kg) and vitamin C alone and in combination on anxiety like behaviors. In combination therapy rats were injected with vitamin C 30 min before progesterone (5, 10 and 30 mg/kg). Each bar represents mean ± SEM. % OAE (B) * $p<0.05$, ** $p<0.01$ as compared to the 1 (Control) group. # $p<0.05$ as compared progesterone (5 mg/kg, group 3) with progesterone (30 mg/kg + vitamin C, group 9)

C) As shown in **Figure 1(C),** administration of different doses of progesterone and vitamin C alone and in combination therapy did not change the locomotors activity of animals.

2) Effects of Progesterone and vitamin C alone and in combination on oxidative stress markers in prefrontal cortex:

A) -Lipid peroxidation assay

As shown in **Figure 2 (A),** there were significant differences in malondialdehyde (MDA) level between different doses of progesterone groups (5, 10 and 30 mg/kg) with control group. Progesterone (5 mg/kg) increased the level of MDA ($p<0.001$), Progesterone (10 and 30 mg/kg) reduced the level of MDA ($p<0.05$). In comparation of control group with vitamin C group, significant decrease in MDA level was observed ($p<0.001$). Pretreatment of vitamin C with different doses of progesterone significantly reduced the level of MDA in the doses of progesterone 10 and 30 mg/kg ($p<0.01$ and $p<0.001$ respectively).

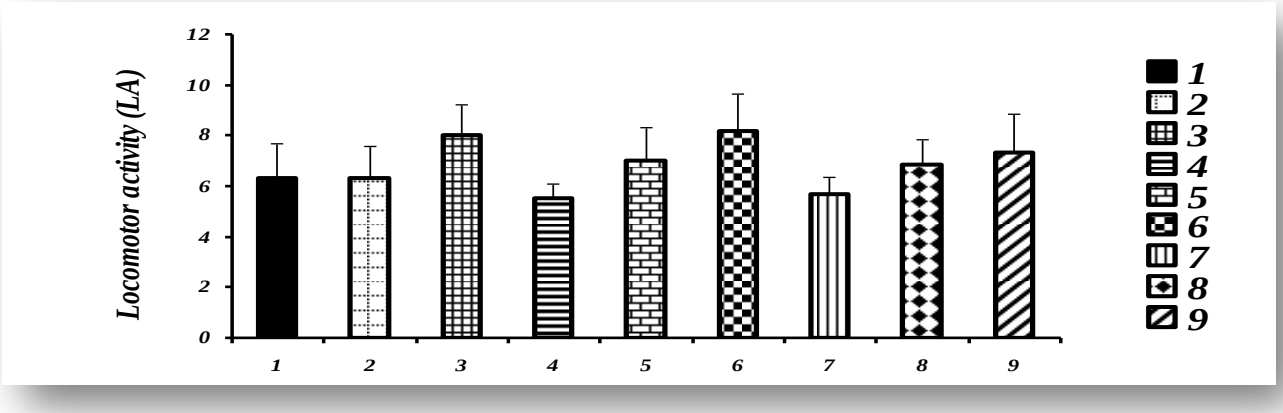

Figure 1(C). The effect of progesterone (5, 10 and 30 mg/kg) and vitamin C alone and in combination on anxiety like behaviors. In combination therapy rats were injected with vitamin C 30 min before progesterone (5, 10 and 30 mg/kg). Each bar represents mean ± SEM. Locomotor activity (LA). There were no significant differences between groups.

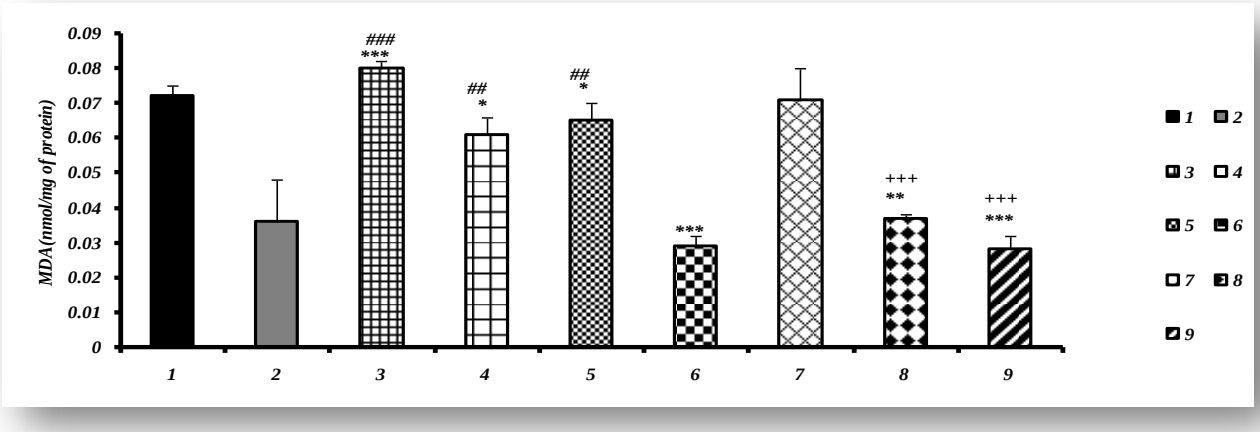

Figure 2 (A). 1= Control (saline). 2 = (sham) group, sesame Oil as solvent. 3 = (Progesterone 5 mg/kg), 4 = (Progesterone 10 mg/kg). 5= (Progesterone 30 mg/kg). 6 = (Vitamin C 80 mg/kg), 7 = (Progesterone 5mg/kg + Vitamin C), 8= (Progesterone 10 mg/kg+ Vitamin C), 9 = (Progesterone 30mg/kg + Vitamin C). Effect of progesterone (5, 10 and 30 mg/kg) and vitamin C alone and in combination on the MDA level in prefrontal cortex of rats. Bars represent mean ± SEM (n=7).* $p<0.05$ compared the groups of 4 (Progesterone 10 mg/kg), 5 (Progesterone 30 mg/kg) with the group of 1 (Control). ** $p<0.01$ compared the group of 8 (Progesterone 10 mg/kg+ Vitamin C) with the group of 1(Control). *** $p<0.001$ compared the groups of 3 (Progesterone 5 mg/kg), 6 (Vitamin C) and 9 (Progesterone 30 mg/kg+ Vitamin C) with the group of 1 (Control). ## $p<0.01$ compared the groups of 4 (Progesterone 10 mg/kg), 5 (Progesterone 30 mg/kg) with the group of 6 (Vitamin C). ### $p<0.001$ compared the group of 3 (Progesterone 5 mg/kg) with the group of 6 (Vitamin C). +++ $p<0.001$ compared the groups of 8 (Progesterone 10 mg/kg+ Vitamin C) and 9 (Progesterone 30 mg/kg+ Vitamin C) with the group of 3 (Progesterone 5 mg/kg).

B) -Superoxide dismutase (SOD) assay

As shown in **Figure 2 (B),** there were no significant differences between groups of progesterone with different doses (5, 10 and 30 mg / kg) in SOD activity in comparation with control group, ($p>0.05$). In comparation of control group with vitamin C group, significant decrease in SOD activity was observed, ($p<0.001$). Pre-treatment of vitamin C with different doses of progesterone significantly reduced SOD activity in the doses of progesterone (5, 10 and 30 mg/kg) in comparation with control group, ($p<0.001$). There are significant differences in SOD activity between different doses of progesterone groups (5, 10 and 30 mg/kg) with vitamin C ($p<0.001$, $p<0.01$, respectively). There were significant differences in SOD activity between different doses of progesterone groups (5, 10 and 30 mg/kg) with pre-treatment of vitamin C + different doses of progesterone (5, 10 and 30 mg/kg), ($p<0.001$).

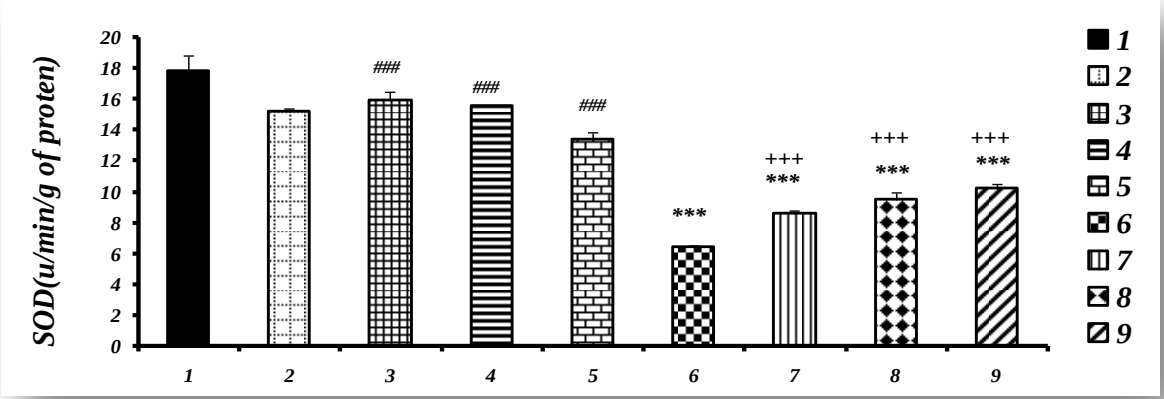

Figure 2 (B). Effect of progesterone (5, 10 and 30 mg/kg) and vitamin C alone and in combination on the SOD activity in prefrontal cortex of rats. Bars represent mean ± SEM (n=7). *** $p<0.001$ compared to the control group. ### $p<0.001$ as compared the groups of 3, 4 and 5 with the group of 6 (vitamin C). +++ $p<0.001$ compared the groups of 7, 8 and 9 with the groups of 3, 4 and 5.

C) -Catalase (CAT) assay

As shown in **Figure 2 (C),** there are significant differences in CAT activity between different doses of progesterone groups (5, 10 and 30 mg/kg) with control group. Progesterone (5, 10 and 30 mg/kg) reduced the level CAT activity, ($p<0.001$ and $p<0.05$ respectively). There are significant differences in CAT activity between different doses of progesterone groups (10 and 30 mg/kg) with pretreatment of vitamin C + progesterone 10 mg/kg (8 group), ($p<0.05$).

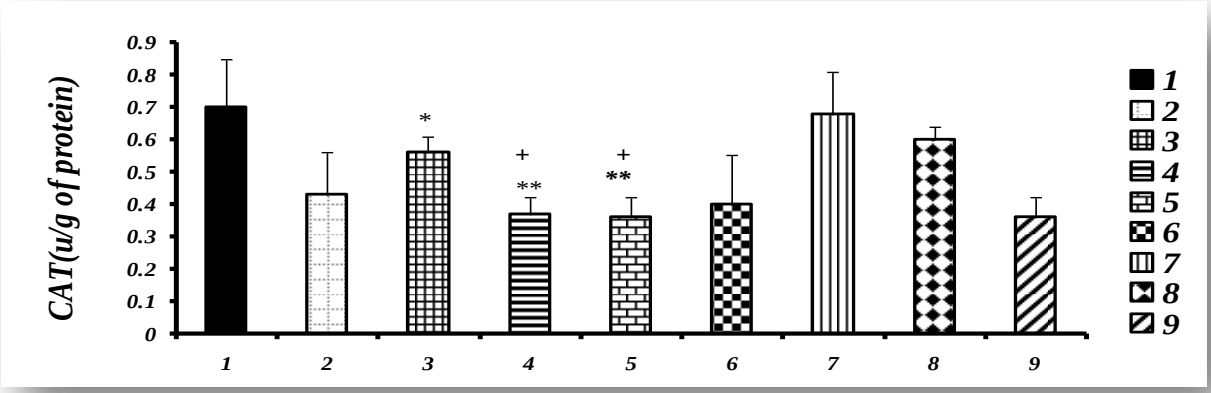

Figure 2 (C). Effect of progesterone (5, 10 and 30 mg/kg) and vitamin C alone and in combination on the catalase activity in prefrontal cortex of rats. Bars represent mean ± SEM (n=7). * $p<0.05$, ** $p<0.01$, as compared to the control group. + $p<0.05$ compared the groups of 4 and 5 with the group of 8 group.

D)- peroxidase (POD) assays

As shown in **Figure 2(D)**, administration of different doses of progesterone and vitamin C alone and in combination therapy did not change the activity of POD.

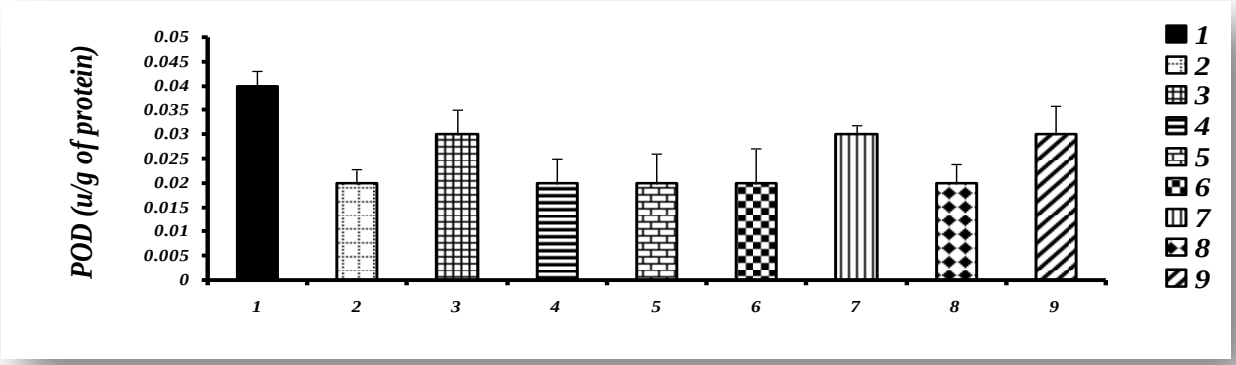

Figure 2 (D). Effect of progesterone (5, 10 and 30 mg/kg) and vitamin C alone and in combination on the Guaiacol peroxidase activity in prefrontal cortex of rats. Bars represent mean ± SEM (n=7). There were no significant differences between groups.

DISCUSSION

There are contradictory studies about the effect of progesterone on anxiety like behaviors (5, 6**)**. In the present study, the effect of progesterone and vitamin C alone and in combination was evaluated on anxiety using EPM in male rats. Progesterone in lower dose (5 mg/kg) increased anxiety. Period of intraperitoneal injection was 5 days. Gulinello et al reported that exposure to progesterone 5 mg /kg for 48 hours had an anxiogenic effect which is in line with the present study (22). Intraperitoneal injection of Progesterone in doses (10 and 30 mg/kg) in our study decreased anxiety significantly. Our results are compatible with the results of other studies. Claudia Gomez et al reported that Progesterone was found to produce the characteristic anxiolytic-type effects at doses of 10 mg/kg which is in line with the present study. It clearly demonstrate that, at some doses, progesterone (10 and 30 mg/kg) possesses specific anxiolytic properties (23). In contrast, Nicola Jayne Starkey et al reported that progesterone (2.5, 7.5 and 15) in male and female gerbils had no change in anxiety-related behaviors in EPM test (24). Administration of different doses of progesterone and vitamin C alone and in combination did not change the locomotors activity of animals, which is in line with the previous study (Gomez C et al. 2001), whereas Cheryl A et al, shown that administration of progesterone increased locomotors activity of animals (25).

Progesterone's effects on anxiety may occur through interactions with estrogen-induced intracellular progestin receptors (PRs) (26) or through actions of its metabolites at other substrates (27). It is important to note that progesterone is metabolized to allopregnanolone (3α-OH-5α-pregnan-20-one) and pregnanolone (3α-OH-5β- pregnan-20-one), both of which act as agonists on the-amino butyric acid A (GABA-A) receptor complex in the brain (28). The GABA transmitter system is inhibitory system in the CNS. GABA binds to the GABA-A receptor, the influx of chloride ions increases, hyperpolarizing the postsynaptic membrane and making the postsynaptic cell less prone to excitation. Allopregnanolone is a GABAA receptor positive modulator and enhances the effect of GABA on the receptor (29, 30).

In our study intraperitoneal injection of vitamin C (80 mg/kg) alone and in combination therapy significantly decreased anxiety. Period of intraperitoneal injection was 5 days. It has been reported that vitamin C has anxiolytic effect, and is thus consistent with several earlier findings which is in line with the present study (31). Robert N et al reported that vitamin C was found to produce the characteristic anxiolytic-type effects at doses of (75 and 100 mg/kg), which is in line with the present study (32). It has

reported the beneficial and statistically significant effect of vitamin C (1000 mg/day) supplementation for six weeks in palliating anxiety levels in diabetic patients (33). Gautam M et al reported that antioxidants (vitamin A, C, E) supplement therapy as an adjuvant therapy is useful in patients with stress-induced psychiatric disorders (34)

Ascorbate may act as a neuromodulator of a number of transmitters and their associated behaviors (35) and including the inhibition of NMDA receptor activity (36) This could lead to decreased anxiety since NMDA antagonism has been shown to be anxiolytic in rats and mice in a similar fashion to benzodiazepines (37). Vitamin C acts as the co-factor for the enzyme dopamine-beta-hydroxylase to convert dopamine into nor-epinephrine, which plays an important role in the regulation of mood (38). Patients with generalized anxiety disorder (GAD) had significantly lower levels of vitamins A, C, and E in comparison to healthy controls. After supplementing these deficient vitamins in the diets of the subjects, a significant reduction in anxiety and depressive scores was observed (39). Ascorbic acid has three biological functions, each dependent on its role as a reducing agent. It is required for biosynthesis of collagen, for biosynthesis of steroids and peptide hormones, and for prevention or reduction of the oxidation of bimolecular (40). A causal relationship has been found between cellular oxidative stress regulation of anxiety and emotional stress (41). A growing body of reports has indicated that free radicals are involved in the etiopathogenesis of some neuropsychiatric disorders (42). Free radicals have relatively short half-lives, and thus the determination of their levels is difficult. Therefore, they can be evaluated indirectly by measurement of some antioxidant enzyme levels such as SOD, CAT, Guayacol peroxidasa (POD), by products of lipid peroxidation such as **MDA** (43).

The present study focused on antioxidant defense mechanisms and examined the amount of MDA formation, which is an end product of membrane fatty acid peroxidation (44) and the activity level of CAT and SOD, which is the enzyme that scavenges superoxide anions (45).

In the present study, progesterone (5 mg/kg) increased the level of MDA and reduced the level CAT activity. Progesterone (10 and 30 mg/kg) reduced the level of MDA and CAT activity. There were no significant differences between groups of progesterone with different doses (5, 10 and 30 mg / kg) in SOD and guayacol peroxidasa activity.

Exogenous administration of progesterone exerts significant antioxidant and renoprotective effect. The progesterone-treated rats observed significant antioxidant effect as witnessed by decrease in lipid peroxidation, superoxide anion generation (SAG), along with increase in GSH and CAT activity. This increase in GSH and CAT activity is important as depleted endogenous antioxidant accounts for more damage rather than increased production of Reactive oxygen species (ROS) (46). Progesterone can modulate the activity of SOD in the tissues through its receptors (47).

In the present study, vitamin C (80 mg/kg) reduced the level of MDA and SOD activity and there was no significant difference in CAT and POD activity.

It has reported that an inverse correlation between serum MDA level and vitamin C has been found in chronic smokers compared to non-smokers (48). Bakhtiari Sedighe et al reported that vitamin C does not increase SOD activity significantly (49).

Vitamin C in combination with different doses of progesterone (10 and 30 mg/kg) reduced the level of MDA, SOD activity and there were no significant difference in CAT and POD activity.

It has been reported patients with social phobia (SP) have significantly higher antioxidant enzyme (SOD, CAT) activity and MDA levels compared with those in controls (50). In another study it was suggested that patients with major depression, had elevated antioxidant enzyme levels and lipid peroxidation (51). In previous studies, inconsistent results were found in patients with psychiatric disorder. It has been reported that decreased activities of SOD and GSH-Px in patients with major depression and bipolar disorder (50). There are reports in the literature that found no difference in SOD or GSH-Px activity between mood disorder patients and healthy controls (52). Also, increased levels of plasma SOD and GSH-Px from patients with mood disorder have been reported (53). These results are contradictory, with lower, elevated or normal activities in mood disorders and discordance in these parameters may be due to several factors such as differences in the

measurement of the methods, differences in medication and stage of the patients.

Based on our search there aren't studies about the interaction of vitamin C and progesterone on anxiety, but there are some other studies which have been investigated the relation of progesterone and vitamin C. Vitamin C together with progesterone and/or its metabolites are involved in the protection against pentylenetetrazol (PTZ)-induced seizures in immature rats (54). In previous studies, it has been demonstrated that exogenous administration of progesterone exerts significant antioxidant and renoprotective effect. Moreover in other reports the involvement of progesterone receptors in ascorbic acid mediated protection against ischemia reperfusion- induced acute kidney injury was investigated. (46).

CONCLUSION

We concluded that progesterone's effect on anxiety is dependent on its doses. Low dose of progesterone has anxiogenic effect but in moderate or high doses has anxiolytic effect. Certainly more studies are needed for final decision. Vitamin C as an antioxidant can improve the disturbances in oxidative stress parameters in the prefrontal cortex of rats which are treated with progesterone.

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