Original Article

Effect of chronic unpredictable stressors on reproductive parameters in male wistar rats: role of ascorbic acid


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ABSTRACT

AIM: Chronic unpredictable stress (CUS) may be more applicable as a model of post-traumatic stress disorder because of the lack of predictability, which could permit habituation. The present study, was aimed to investigate the effect of chronic unpredictable stressors on reproductive parameters and their possible relation with testicular ascorbic acid level.

MATERIALS AND METHOD: Adult male Wistar rats were divided into two groups as non-stressed group (n = 10) and stressed group (n = 10). The stressed groups were exposed to 10 days of CUS. The epididymal sperm count, sperm abnormalities, testicular weight, testicular lipid peroxidation and ascorbic acid level of the testis were estimated. All experimental procedures and animal maintenance confirmed to the strict guidelines of Institutional Ethics Committee.

RESULTS: The results were analyzed statistically by using student’s t-test. P < 0.05 was considered as significant. Exposure to CUS showed a significant (P < 0.001) decrease in the weight of the reproductive organs, sperm count, and ascorbic acid level. Further, significant increase (P < 0.001) was observed in testicular lipid peroxidation level and incidence of sperm abnormality.

CONCLUSION: The present data suggest that CUS has deleterious effect on spermatogenesis. Further, stress induced oxidative damage might be mediated through its effect on reducing ascorbic acid level.

1. Introduction

In the recent years concern has aroused for the decrease in the fertility in man. One of the goals when evaluating an infertile man is to identify reversible conditions that are responsible for infertility. Chronic stress has been a potential risk factor for reproductive function [1,2]. In males, physical and psychological stressors might inhibit reproductive function mainly through the suppression of hypothalamus-pituitary-gonadal (HPG) axis and activation of hypothalamus-pituitary-adrenal (HPA) axis [3]. Several reports have suggested a stress related decline in semen quality, sperm concentration, morphology and percentage of motility [4,5]. This leads to a close correlation between the sperm concentration and fertility potential of males.

The Vitamin C is one of the most commonly available and accessible molecules and is a part of food commonly consumed by most [6]. The ascorbic acid is a known antioxidant present in the testis with the precise role of protecting the latter from the oxidative damage [7]. Deficiencies of vitamins C have been lead to a state of oxidative stress in the testes that disrupts both spermatogenesis and the production of testosterone [8,9]. In recent years, Supplementation with Vitamin C has also been shown to increase total sperm output and sperm concentration. Lipid peroxidation has been considered as a serious consequence of free radical toxicity leading to profound changes in the membrane structure and function that may cause even cell death [10,11]. Malondialdehyde (MDA) is one of the end products of lipid peroxidation and extent of lipid peroxidation is measured by estimating MDA levels most frequently. Increased serum level of MDA has been reported in cardiovascular, neurological and other diseases [10, 11]. Chronic unpredictable stress (CUS), one of the most clinically relevant stress paradigms in rodents, mimics a number of behavioral characteristics observed in patients with

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was conducted in the filtrate as per the standard method in
suspension was filtered through a nylon mesh. The sperm count
carefully separated from the testis.

In this study, a different model of chronic unpredictable stress
was chosen in order to avoid habituation to the stressor and to
maintain robust the stress response. The purpose of the present
study was to determine whether exposure to chronic unpredictable
stressors applied to adult male rats induces changes in reproductive
parameters and its possible relation with testicular ascorbic acid level.

2. MATERIALS AND METHODS

Adult male rats of Wistar strain weighing between 140 - 220 g
was taken in this study. All the rats were given standard rat chow
and tap water ad libitum and were housed at 25 ± 2°C on a 12-hour
dark/light cycle. All experimental procedures and animal
maintenance confirmed to the strict guidelines of Institutional
Ethics Committee and that of Federal laws for the use of animals in
the experiment. The animals were divided into two groups as non
stressed group (n = 10) and stressed group (n = 10).

Experimental stress procedure [14]: Rats assigned to the
chronic stress group were exposed to the following CUS protocol:
Day one - 11:00 a.m. 50 min forced swimming stress (The rats were
forced to swim in the plastic tub, circular in shape with a height of
60cm and a diameter of 40cm (water temperature; 28°C; water
level kept at 30 см from the bottom), and 12:00 p.m. 60 min cage
tilting (the rats are placed in a polycarbonate cage and tilted at an
angle of 45°); Day two - 1:00 p.m. 4 h wet bedding (400 ml tap water
in home cage), and 6:00 p.m. lights on overnight; Day three - 12:00
p.m. 2 h overcrowding stress, and 3:00 p.m. 60 min restraint stress
(6 x 2 x 1.6 cm Plastic tube restrainer); Day four - 6:00 p.m. 50 min cage
tilting, and food and water deprivation overnight (15 h); Day five -
3:00 p.m. 1 hour overcrowding stress (pooling the rats from 3 cages
into 1 (size; 24 x 39 x 23 cms), and 4:00 p.m. isolation housing
overnight (17 h); Day six - 11:00 a.m. 4 h wet bedding, and 3:00 p.m.
2 h forced swimming stress; Day seven - 1:00 p.m. 30 min overcrowding
stress, and 6:00 p.m. 1 h lights on; Day eight - 10:00 a.m. 20 min cage tilting, and 3:00 p.m. 60 min restraint stress; Day
nine - 10:00 a.m. 4 h wet bedding, and 6:00 p.m. food and water
depression; Day ten - 6:00 p.m. isolation housing and lights on
overnight. Immediately after the last stressor, Animals were
sacrificed. The laparotomy was performed and the reproductive
organs, sperm count, and ascorbic acid level were weighed. The epididymis was carefully separated from the testis.

Sperm count: The epididymis was minced in 1ml of phosphate
buffered saline (pH 7.2) to obtain a suspension [15]. The
suspension was filtered through a nylon mesh. The sperm count
was conducted in the filtrate as per the standard method in
Neubauer's chamber. Briefly, an aliquot from the suspension (up to
0.5) was taken in leukocyte hemocytometer and diluted with
phosphate buffered saline up to the mark 11. The suspension was
well-mixed and charged into Neubauer's counting chamber. The
total sperm count in 8 squares (except the central erythrocyte area)
of 1mm² each was determined and multiplied by 5 x 10⁴ to express
the number of spermatozoa /epididymis.

Sperm morphology test: For the evaluation of the sperm
morphology the filtrate obtained was stained with 1% eosin Y or
periodic acid-Schiff’s reaction and morphological defects were
analyzed as explained elsewhere [16]. Briefly, the sperms in the
smears were visualized under oil immersion objectives and any
abnormalities of either heads or tails were noted. The microcephaly,
which was also a type of head abnormality, and cephalo-caudal
junction defects (CC), which were a type of tail defects have been
classified separately. Two hundred sperms were screened for each
animal and total abnormality was expressed as incidence/200
sperm/animal.

Ascorbic acid level in the testis: The right testis was removed
and placed in phosphate buffered saline (pH 7.2) and the tunica
albugenia was removed. Following this, the testis was homogenized
in the same solution and the homogenate was used for the
estimation of ascorbic acid level by 2,4-dinitrophenyl hydrazine
method, calorimetrically[17]. Briefly, the ascorbic acid in the
homogenate is oxidized by Cu²⁺ to form dihydro-ascorbic acid,
which reacts with acidic 4-dinitrophenyl hydrazine to form a red
hydrazone, which is measured at 520 nm.

Lipid Peroxidation: The testicular tissue (1g) was transferred
to a homogenizer containing cold 10ml of 10mM cold potassium
phosphate buffer (pH 7.4). The tissue was homogenized using a
manual homogenizer. The unbroken cells and cell debris were
removed by centrifugation at 3000 rpm for 10 minutes by using
Remi C 24 refrigerated centrifuge (-4°C). The obtained supernatant
was used for the estimation of lipid peroxidation level. The lipid
peroxidation was estimated according to the method of Kartha and
Krishnamurthy[18]. This assay is based upon the reaction of TBA
with malondialdehyde (MDA), one of the aldehyde products of lipid
peroxidation. Values were expressed as nanogram of MDA/gm
tissue (taking molar extinction coefficient of MDA as 1.56 x 10⁵)

3. RESULTS:

The data was expressed as Mean ± SD. The differences between
groups were compared for statistical significance by student t test
with the level of significance set at P < 0.05. Exposure to CUS showed
a significant (P < 0.001) decrease in the weight of the reproductive
organs, sperm count, and ascorbic acid level. Further, significant
increase (P < 0.001) was observed in testicular lipid peroxidation
level and incidence of sperm abnormality.
** P<0.001 ; Non Stressed group versus Stressed group

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In human life, prevalence of Stress has deleterious impacts on brain and body physiology[19, 20]. Exposure to stress leads to the tremendous amount of free radicals[10]. The present study indicates that chronic unpredictable stress significantly increased...
the oxidative damage biomarker of lipid peroxidation in the testis. Stress induced increased in reactive oxygen species might have led to the significant decline in the sperm count and ultimately testicular weight loss. Reactive oxygen radicals are detrimental to the testicular functions and therefore, are regularly being scavenged by a variety of endogenous antioxidants and quenchers including vitamins, enzymes, tripeptides and ascorbic acid could be one of them [21]. Ascorbic acid functions as one of the most important free radical scavenger trapping free radicals protecting bio membrane from oxidative damage [22]. Further, the role of ascorbic acid has long been established as an agent to play a crucial role in the differentiation process of the spermatogonial cells to sperm [23]. In the present context, ascorbic acid level in the CUS exposed rat testes have been declined significantly possibly indicating its role as a potential scavenger of reactive oxygen species. The insufficiency of the ascorbic acid incurred in the CUS exposed rats might have led to decrease transformation of sperm, thereby resulting in a significant decline in the sperm count. Further, increase in the percentage of sperm abnormalities in the stress induced rats coupled with increased lipid peroxidation level in the testicular tissue, emphasizes the possibility of gene alteration in germ cells induced by reactive oxygen species generated during chronic stress exposure.

CONCLUSION:

The present study suggest that at least one of the more possible mechanism in the stress induced toxic effect on the male reproductive system might be mediated through its effect on reducing ascorbic acid level and generating ROS. The assessment of the different types of stress and the different antioxidants during the exposure to chronic unpredictable stress will be an interesting step-forward to identify the mechanisms involved.

References:


