International Journal of Medical Research & Health Sciences

www.ijmrhs.com Volume 1 Issue 1 Oct-Dec 2012 Coden: IJMRHS

Received: 1th Aug 2012 Revised: 4th Sep 2012 Accepted: 20thSep 2012

Original research article

An experimental study on effect of antioxidant vitamin E in stress and alcohol induced changes in male fertility in albino rats

*Sanghishetti Vijay Prasad[1], Ghongane BB[2], Nayak BB[1], Raul AR[1], Vijay Kumar AN[1],Mutalik MM[1],Kapure NL[1]

[1]Department of Pharmacology, Padmashree Dr Vitthalrao Vikhe Pail Foundation's Medical College, Ahmednagar, Maharashtra

[2]Department of Pharmacology, Govt. Medical College Miraj, Maharashtra.

*Corresponding author: Email:vijayfarmac@gmail.com

Abstract

Introduction: Physical and Psychological stresses are believed to reduce sexual functions, resulting from neurotransmission changes in various erectile response pathways and reduced blood flow in genital organs. Intake of alcohol depends on numerous genetic and environmental factors. Stress has long been thought to influence the alcohol drinking in humans. Forced swimming in laboratory animals has been widely used as a model of stress to study the physiological changes and the capacity of the organism in response to stress. **Aim:** 1. To assess the effect of forced swimming stress on seminal fluid profile, Serum testosterone level, Testicular lipid peroxidation levels. 2. To assess the effect of Vitamin E on stress induced changes. **Method:** Adult male albino rats weighing 200 – 220 g, aged 12-15 weeks were used in this study. The animals were randomly divided into four groups of 6 animals each. Group1 (control) received distilled water, Group 2 (Forced Swimming Stress) received distilled water Group 3: subjected to Forced Swimming stress with 20% alcohol intake p.o. Group 4: Swimming stress with 20% alcohol treated with Vitamin E (200mg/kg/day orally). The following parameters were studied in all groups 1. Body weight. 2. Sperm count Motility and Life /death Ratio (SLDR). 3. Serum Testosterone 4. Testicular Malondialdehyde level (MDA). **Results:** Forced swimming stress caused loss in body wt, reduction in sperm count, motility and SLDR in sperm analysis, reduction in serum testosterone and increase testicular MDA levels compared to control. All the changes were statistically significant. When alcohol was added along with forced swimming it caused further loss in body wt, sperm count, motility and SLDR, serum testosterone level and slightly further increase in MDA levels. These observations were also statistically significant. In case of group IV in which Vitamin E was administered long with stress with alcohol it showed a trend of reversal phenomenon of stress and alcohol induced change (Group III) exhibited by gain in body wt, increase sperm count, motility and SLDR, serum testosterone and decrease in MDA level. All the results were statically significant except serum testosterone. **Conclusion:** Force swimming indicates that swimming is an effective model for producing stress in albino rats. The stress induced changes were further accentuated by addition of ethyl alcohol. However Vit E treatment reverses the effect of stress and alcohol.

Keywords: Forced swimming stress, Alcohol, Vitamin E, Male fertility

Introduction:

Stress undoubtedly has become an integral part of human life. Stressful conditions have a derogative effect on normal physiological functions leading of variety disease states.[1] Lifestyle diseases like hypertension, Diabetes Mellitus, behavioral disorders, etc have been implicated as one of the many ill-effects of chronic.[2] Experimental models are required to better understand the progressions of the disease and elaborate new therapy.[1]

Various forms of physical and psychological stress are believed to reduce sexual functions. Several studies have examined the relationship between stress and sexual behavior in male rats and primates.[3] These reports shows that chronic psychological and physical stresses induce erectile dysfunction, possibly resulting from neurotransmission changes in various erectile response pathways and a reduced blood flow in male genital organs.[4] Based on these findings, it is suspected that stress can affect sexual functions in both men and women.[5]

Alcohol intake depends on numerous genetic and environmental factors. Studying the link between alcohol consumption and stress is further our understanding of reproductive system. Stress has long been thought to influence the alcohol drinking. Numerous studies have also reported the harmful effects on male fertility by affecting sperm count and quality.[6]

Oxidative stress due to physical as well as psychological forms of stress has become the focus of interest as a potential cause of male infertility. Normally, equilibrium exists between reactive oxygen species (ROS) production and antioxidant scavenging activity in the male reproductive organs. However, strenuous exercise and forced swimming can lead to acute production of ROS causing tissue damage. [7]

Testicular membranes are rich in polyunsaturated fatty acids and thus susceptible to peroxidation injury in accordance, antioxidant enzyme activity has been shown to decrease in experimental cryptochidism, resulting in increased lipid peroxidation.[8] Increased lipid peroxidation in the testis contributes to the suggested vulnerability of this organ to oxidative stress.[9,10] Under stress conditions, mature spermatozoa produces small amount of ROS, which are needed for capacitation acrosome reaction and fertilization. However excessive amount of ROS produced by leukocytes and immature spermatozoa can cause damage to the normal spermatozoa by inducing lipid peroxidation and DNA damage.[7,10] Swimming in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism in response to stress.[2]

Swimming has got a number of advantages over other types of exercise such as treadmill running. The amount of work done during the swimming exercise is far greater than that during the treadmill running of identical time duration.[11] Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human diseases of stress origin. Vitamin E is one of most effective antioxidants in animals.[12] Vitamin E has been shown to improve sperm motility and enhances semen quality and fertility.[13] In recent years, Vitamin E supplements have been widely used in rat diets for enhancing production and reproductive performance.

Supplementation with Vitamin E has also been shown to increase sperm concentration. Apart from the protective effects on membrane integrity that safeguarded cellular functions.[12]

Objective: 1. To assess the effect of forced swimming stress on seminal fluid profile, Serum testosterone level, Testicular lipid peroxidation levels. 2.To assess the effect of Vitamin E on stress induced changes .

MATERIALS AND METHODS

Animal: Adult male albino rats weighing 200 – 220 g and aged 12-15 weeks were obtained from authorized animal breading centre Rural Medical College, Loni, Ahmednagar. The animals were kept in wire bottomed cages in a room under standard

condition of illumination with a 12 – h light-dark cycle at 25 ± 1° C. They were provided with tap water and balanced diet *ad libitum*. The study was approved by the Institutional Animal Ethics Committee (IAEC) and it followed the Committee for Purpose Controlling and Supervision on Experimental Animals (CPCSEA) rules on animal protection.

Experimental design:

Twenty four rats of average body weight 200-220g were used for this study. The rats were randomly divided into four groups of 6 rats each.

Group1: Control Group received distilled water

Group 2: Swimming Stress received distilled water

Group 3: Swimming stress with 20% alcohol intake *ad libitum* **Group 4**: Stress with20% alcohol intake treated with Vitamin E (200mg/kg/day orally)

Stress procedure: Rats were exposed to forced swimming stress daily between 09.00AM to 10.00AM until 60 days.[14] It was modified according to previous researchers Nayanatara et al., was used for this experiment.[15]

Rats were to swim in plastic tanks (length 100cm, width 40cm, depth 60cm) containing tap water maintained at a temperature 36 ± 2° c. The water depth, 35cm was set so that the rats could not rest by supporting the tail on the bottom of tank. The animals were allowed to swim till exhausted (failure to rise to the surface of the water to breathe within 7sec).[15] At this moment, the animals were removed from the tank and dried with a towel and returned to their cages.

Drug preparation and administration: Alcohol preparation and administration: Rats were given ad libitum access to water with 20% ethanol in distilled water along with food. Alcohol solutions were prepared from 99.9% ethanol diluted with distilled water.[6] Spilling and evaporations were minimized by the use of special bottle caps.

Vitamin E preparation and administration:

Vitamin E procured from authentic distributors, manufactured by Apex laboratories Chennai company manufactured on 5/11, Vitamin E administered 200mg/kg/day by oral route. [12,16]

Collection and preparation of samples:

Blood samples: Twenty four hours after the last treatment, blood samples were collected via orbital plexus under the anesthesia (Thiopentone IV). Serum was obtained by centrifugation at 3000rpm for 20 minutes. Serum was used for Testosterone assay.[17]

Semen samples: Left testis and Cauda epididymis dissected out under anesthesia. Cauda epididymis was immersed in 1ml of Phosphate Buffer Saline (PBS) and homogenized by using manual homogenizer. The aliquot was used for seminal analysis. [11]

Tissue preparation: Testis taken as described above. The testicular tissue was transferred into 10% w/v of Phosphate buffer (pH 7.4). The tissue was homogenized using a manual homogenizer. The broken cells debris were removed by centrifugation 11,000g for 10 min by using remi centrifuge (-4^0 c). The obtained supernatant were divided into aliquots and stored in -80^0 c. The level of Lipid peroxidation (MDA) was estimated.[18]

Methods:

1. Body weight: At the beginning and end of the experimental period, the body weight of each individual rat was measured.[19]

2. Sperm functions analysis:

2.1 Sperm count: The epididymis was minced in 1ml of PBS (pH 7.2) to obtain a suspension. The suspension was filtered through nylon mesh. The sperm count was conducted in the filtrate as per standard method Neubauer's chamber. Briefly, an aliquot from the suspension (up to 0.5) was taken in leukocyte hemocytometer and diluted with PBS up to the mark 11. The suspension was well mixed and charged in to Neubauer's counting chamber. The total sperm count in 8 squares (except the central erythrocyte area) of 1 mm^2 each was determined and multiplied by $5x\ 10^4$ to express the number of spermatozoa/ epididymis. [11]

2.2 Sperm motility: The epididymal sperm content was obtained by maceration of the tail of epididymis in 1ml of PBS (pH 7.2).[20] An aliquot of this solution was on slide and percentage of motility was evaluated visually at magnification of X 400.

Motility estimates were performed from the three different fields in each sample. The mean of the three estimations was used as the final motility score. [21]

2.3 Sperm Live and Dead Ratio (SLDR): A drop of epididymal content of each rat was mixed with an equal drop of 1% eosin stain prepared. Thin films were made by spreading the stained content onto clean slides and quickly dried. Viable sperm remains colorless. One hundred sperm cells per rat were scored for determining the viability percent.[22]

3. Biochemical analysis:

3.1 Hormonal assay:

Serum samples were used for determination of testosterone was performed by Radio immune assay method, manufactured by Mindray.[17]

3.2 Lipid peroxidation (LPO) assay:

Malondialdehyde (MDA) level was estimated in the testicular homogenate according to the methods of Burge Aust et al., .Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature 95^0 c for 10 min to form thiobarbituric acid reactive substance (TBARs). The absorbance of the resultant pink product was measured Spectrometrically at 534 nm manufactured by Systronics (India) Ltd, Gujarat.[22]

4. Statistical analysis : Data obtained from all groups was analyzed by using t- test, Group II was compared with Group I, Group III was compared with Group II and group IV was compared with Group III in all parameters. $P<0.05$ had taken as statistically significant.

5. Results: Forced swimming stress (group II) caused loss in body wt ($P < 0.0003$), reduction in sperm count ($P < 0.0001$), Motility ($P < 0.0001$), Life death ratio ($P < 0.0001$), MDA ($P < 0.0001$) and testosterone ($P < 0.005$). All the changes were highly statistically significant. When alcohol was added along with forced swimming it caused further loss in body wt ($P < 0.04$), further reduction in Sperm count ($P < 0.02$), Motility ($P < 0.01$), Life death ratio ($P < 0.04$), slightly further increase in MDA ($P < 0.04$) and testosterone ($P < 0.04$), MDA levels. These observations were also statistically significant. In case of group IV in which Vitamin E was administered along with stress with alcohol it showed a trend of reversal phenomenon of stress and alcohol induced change (Group III) exhibited by gain in body wt ($P < 0.029$), improved Sperm count ($P < 0.0001$), Motility ($P < 0.0001$), Life death ratio ($P < 0.0001$), decreased testicular MDA ($P < 0.0001$) and testosterone ($P < 0.3$). All the results were statically significant except serum testosterone.

Table.1: Effect of stress, stress with alcohol intake and Vitamin E on Body wt

Parameter	Group I	Group II	Group III	Group IV
Body weight on 1st day (g)	214±2.48	213±1.41	213±2.88	211±2.88
Body weight on 60th day (g)	212.5±2.4	191.8±2.9	183.3±2.4	196.5±4.7
Loss(-)/ gain(+) (g)	-1.17±1.62	-20±4.60*	-30±2.84*	-15±5.44*

Data are presented as mean ± SEM. * Significant

Table.2: Effect of stress, stress with alcohol intake and Vitamin E on sperm parameters

Parameter	Group I	Group II	Group III	Group IV
Sperm count (10^6)/ epididymis	71.3±0.9	61.8±1.0*	57.3±1.3*	69.6±0.9*
Motility (%)	82.5±1.6	63.0±1.3*	56.8±1.7*	74.0±1.8*
SLDR (%)	4.4±0.2	1.86±0.1*	1.4±0.1*	2.9±0.2*

Table.3: Effect of stress, stress with alcohol intake and Vitamin E on testosterone and MDA levels.

Parameter	Group I	Group II	Group III	Group IV
Testosterone (ng/ml)	2.0±0.05	1.73±0.07*	1.43±0.09*	1.56±0.10
MDA (µmoles/g tissue)	9.2±0.2	13.0±0.3*	13.9±0.06*	8.4±0.2*

6. Discussion: Effect on body weight: Swimming and alcohol intake significantly decreased the whole body weight as compared to control group. This effect is accordance to other reports in chronic exercise. The decreased weight could be due to decreased food intake in the rats under the influence of stress. Corticotrophin Releasing Hormone (CRH) is commonly released during the stress and might be a factor that suppressed food appetite in the repeated swimming stress.[2]

Sperm count, motility and viability: Marked reduction in sperm count, motile and viability in stress group when compared with the corresponding control group of animals. [11]On another hand, administration of Vitamin E improves sperm count, motility and viability.

Hormonal study: CRH alsoa negative regulator of LH action on the leydig cells. These inhibition effects subsequently attenuate Testosterone production by the leydig cells. Apart from the central dysfunction of HPG axis alternately the most likely mechanism decreased testosterone biosynthesis are perhaps testicular micro trauma or temperature increase.[8]

Biomarkers of Testicular enzymes: MDA levels were increased in stress group. This probably reflects the increased in lipid oxidation due to either increased production of free radicals or decreased antioxidant defense mechanism or both. Supplementation with Vitamin E decreased MDA levels. [23]

7. Conclusion: Various physiological changes produced force swimming indicates that swimming is an effective model for producing stress in albino rats. The stress induced changes were further accentuated by addition of ethyl alcohol. However Vit E treatment reverses the effect of stress and alcohol. From this study it can be concluded that stress and alcohol intake induced physiological changes may be due to increase lipid peroxidation levels for which the antioxidant effect Vitamin E is able reverse the stress induced changes for which Vitamin E can be prescribed to various diseases suspected to be having stress origin like male infertility, hypertension, DM, Psycho somatic disorder etc.

8. ACKNOWLEDGEMENTS Author is thankful to the Head, Prof. Dr. Nayak B B and the members of the Department of Pharmacology for their kind cooperation and supports. We grateful to the Principal and Management of PDVVPF's Medical College for allowing us to carry out the study and support.

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