



EFFECT OF CHRONIC ALTERNATING STRESS ON CORTICOSTERONE, TESTOSTERONE AND JOHNSEN TESTICULAR HISTOLOGY SCORE IN TWO GENERATIONS OF MALE RATS

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ABSTRACT

Background: Stress has become a ubiquitous aspect of modern day-to-day life, adversely affecting all bodily systems, including the reproductive system and its performance. The objective of this study was to compare the effect of chronic alternating stress on two generation of rats by evaluating the corticosterone and testosterone levels and score the cross section of testis of male rats for spermatogenesis.

Methods: One hundred and thirty-six healthy wistar albino rats 11 weeks old were used in this experimental case-control study. They were distributed into case and control parent groups after assaying for baseline parameters. Case parents were given three weeks of chronic alternating stress while the control parents were not given any stress. Following mating within respective groups the offspring were placed into 5 groups each. One group was assayed at 5 weeks without any stress, the other was given 3 weeks of stress and assayed at 8 weeks, the third group was given stress at the age of 5 and 11 weeks, one group was given stress only at 11 weeks of age. There were two control groups which were offsprings that did not receive any stress and were assayed at the ages of 8 weeks and 11 weeks. Blood sera were assayed for corticosterone and testosterone using Enzyme Linked Immunosorbent Assay Technique. In both generations behavioural tests were done and analysed.

Testicular specimens were taken from 4 male rats in each group and slides were made using histological stains and were scored for spermatogenesis.

Results: Case parents exhibited significantly higher stress hormone levels (ACTH: $P = 0.004$; Corticosterone: $P < 0.001$) and increased anxiety in behavioural tests (decreased locomotion, $P < 0.001$) compared to control parents. The rats given early life stress had raised corticosterone levels ($P=0.002$) and decreased testosterone levels shown by significant p-values ($P=0.045$). Histological analysis revealed that early life stressed offspring had lower Johnsen scores for the testes compared to late life stressed offspring however these were because of the age difference and when compared with same aged control offsprings there was no significant difference. **Conclusion:** The early life stressed rats did not fare well as compared to rats given repeated stress in terms of behavioural tests and reproductive hormones however there was no significant difference in spermatogenesis score in testis.

Key words: Chronic Stress Rat Model, Behavioural tests, Corticosterone, Testosterone, Testis, Johnsen score.

INTRODUCTION

The term “stress” was used for the first time in medical literature by Hans Selye who emphasized that stress was the body’s nonspecific response to any demand which gets accumulated as allostatic load.¹ When a person's capacity to handle external stressors is exceeded, allostatic overload occurs. Stress can make an organism prone to a lot of diseases like diabetes, heart diseases, obesity, psychiatric and psychological diseases, and endocrine abnormalities.² Increased number of diseases occurring in early childhood has become a worldwide problem. Modern lifestyle and the increased levels of stress on parents in their career building years which are also childbearing years can cause the appearance of diseases in the child. One such disease is Diabetes Mellitus type 1 which has increased in children under 5 years but especially in children aged 10-14 years and has increased a lot in the past 50 years.³ Environmental factors and stress during neonatal life and early postnatal life are said to be the reason behind this.⁴ Prenatal stress has been proven now to change the activity of the Hypothalamo-Pituitary-Adrenal (HPA) axis. In experiments on animals, prenatal stress has been shown to disrupt the normal surge of testosterone in the developing male.⁴ In male rats, chronic stress can impair testicular function, leading to decreased sperm production and quality. Adult male rats showed a decrease in weight and serum testosterone level after continued chronic stress.⁵ It has been reported that consuming corticosteroids and being exposed to stressors both inhibit the hypothalamic release of gonadotropin Releasing Hormone (GnRH). During restraint stress, plasma levels of Luteinizing Hormone (LH) decreased in rats and ewes, but the response to exogenous GnRH remained.⁶ There is abundant evidence that Corticotropin Releasing Hormone (CRH) inhibits GnRH secretion and, consequently, decreases Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) levels, particularly because it disrupts the GnRH and LH surge.⁷ Hormones of the HPA axis can attenuate the sensitivity of the pituitary gland to GnRH. ACTH injections in rats increased plasma corticosterone levels while decreasing LH, FSH, and testosterone levels.⁸ Despite the possibility of simultaneous alterations in LH levels in the blood, declines in plasma testosterone levels which are independent of gonadotropins are well documented. During immobilization and heat stress, rats' plasma levels of LH did not alter much, but their testosterone levels did. Similar outcomes were seen following extended corticosteroid therapy. Glucocorticoids have been demonstrated to directly inhibit binding capacity of LH on testis and consequently decrease steroidogenesis.⁹ Thus activation of the Hypothalamo-Pituitary-Adrenal Axis during stress has been shown to suppress the Hypothalamo-Pituitary-Gonadal Axis.¹⁰ Modern lifestyle and the increased stress on parents in their career-building years which are also childbearing years are now considered to cause different diseases in the child. We wanted to find out if there is any difference in response to stress in the offsprings of stressed vs non-stressed parents. Our objective was to find the effect of stress by

comparison of Corticosterone levels and Total testosterone level. We also wanted to compare the spermatogenesis score in testicular cross-sections.

METHODOLOGY

This was an experimental case control study which spanned for a duration of two years. Ethical approval was taken from Ethical Committee of Khyber Medical University (Reference No = DIRKMU-EB/HS/000675) and from Peshawar Medical College (Reference No = Prime/IRB/2023-207). We used the animal house and Pathology lab of Peshawar Medical College and histopathology lab of Khyber Medical University laboratory as well as Animal Research Lab at Agriculture University Peshawar. There were 136 healthy Wistar rats of 11 weeks age in the parent generation. Sample size of offsprings was calculated by resource equation which came to be 156 in both case and control offspring group. In each of the twelve group of offsprings number of rats= $156/12=13$ but to cover for attrition we took 14 rats.¹¹ In each group, 12 rats were recorded for behavioural tests due to high variability in results of behavioural tests, 10 were required for blood sampling, 4 for histology. For histology of gonads 4 male testis samples were taken.¹² In the parent generation we included healthy stress free 11 weeks old rats while unhealthy, stressed or pregnant rats were excluded. Open field and Hole board behavioural tests were used to find out stress behaviour in rats.¹³ The parent rats were divided into two groups. One group were the case parents to whom we gave 3 weeks of chronic unpredictable stress and to the other group of control parents we did not give any stress. The genders were kept separate till after the stress duration was over. The case and control parents were allowed mating within their respective groups. The offspring of case and control parents were kept separate from each other and were divided into 6 groups each and were analysed at the age of 5, 8, 11 and 14 weeks. Some of the offsprings were given no stress and assayed at 5 weeks, some were given early life stress and some were given late life stress, some were given both early and late life stress while there were control offsprings in each group who were not given any stress. The stress protocol used was the same for the parents as well as the offsprings. Three stressors were applied, each one on a different day. On day 1-alteration of day-night cycle, on day 2-exposure to cold water immersion test followed by restraint stress on the third day. This cycle was repeated for a total of 21 days.¹⁴ In this way, adaptation to stress did not occur.¹⁵ This paper is part of the research thesis of first author and the stress protocol is detailed in another published article¹⁶ and more articles will be published. Rats were anesthetized using isoflurane/propylene glycol mixture. It was used to anesthetize rats by open drop procedure. Rats remain deeply anesthetized for approximately one minute.¹⁷ During this time we euthanized the rat via exsanguination through intracardiac puncture using a syringe.¹⁸ Blood was centrifuged to separate sera. The supernatant sera were collected, divided into aliquots in Eppendorf test tubes and stored at -80°C . Elisa kits were used for assaying various hormones in sera. In this article I will focus on Corticosterone levels and Testosterone levels. We dissected out four samples of testis per group and placed in a fixative solution which was formalin (10% neutral buffered formalin). Samples were processed according to the standard protocols by Bancroft and Gamble.¹⁹ We used the Hematoxylin and Eosin (H&E stain) and we also used the PAS stain for the same section of the testis. Hematoxylin stains nuclei blue while eosin imparts a pink colour to the cytoplasm and the extracellular matrix.²⁰ We also did special staining of the testicular slides with Periodic Acid Schiff. ²¹Periodic acid–Schiff (PAS) stain is a widely used staining method in histology and pathology. It is mainly employed to detect polysaccharides such as glycogen, glycoproteins and proteoglycans typically found in connective tissue, mucus, the glycocalyx and basal laminae.²² Polysaccharides and mucous substances are stained magenta. The acrosomes of sperms were stained magenta with PAS. The nuclei were stained blue.²¹ Histological examinations of testis was done to check for differences in the offsprings of cases and controls. Small cross sections of testis were embedded in paraffin and stained with Periodic Acid Schiff (PAS) and with Eosin and Hematoxylin. PAS staining was used to study the structure and function of these tubules, as it can highlight basement membranes and other structural components rich in polysaccharides like the acrosomes.²³ Johnsen's scoring was used to grade the seminiferous tubules. According to this system

the seminiferous tubules were given a score from 1 to 10 by the presence of spermatogenic cells. Score of 10 Means there is complete spermatogenesis and presence of many spermatozoa.²⁴

Score 1: No seminiferous epithelium.

Score 2: No germinal cells, Sertoli cells only

Score 3: Spermatogonia only

Score 4: No spermatozoa or spermatids, few spermatocytes

Score 5: No spermatozoa or spermatids, many spermatocytes

Score 6: No spermatozoa, no late spermatids, few early spermatids

Score 7: No spermatozoa, no late spermatids, many early spermatids

Score 8: Less than five spermatozoa per tubule, few late spermatids

Score 9: Slightly impaired spermatogenesis, many late spermatids, disorganized epithelium Score 10: Full spermatogenesis

Besides the spermatogenic staging we also looked for abnormality of Sertoli cells, Leydig cells, basement membrane and germinal epithelium. We skipped the Johnsen score of testis of 5 week old rats as the rats are not sexually mature and Johnsen scoring is not feasible at that age.²⁴ The data was analysed by SPSS software version 25. The data showed a non-normal distribution. Kruskal Wallis test was applied for comparison between the groups and for comparison between two groups Mann Whitney U test was used. $P \leq 0.05$ was taken as a cutoff point for significance.

RESULTS

The offspring and parent groups are explained in Table- 1. 'P1' stand for parent generation and 'F1' stands for first filial generation. 'A' stand for the case parents or the offsprings of case parents while 'B' stands for control parents or offsprings of control parents. In a rat study the offsprings of the same parents like the case parents are considered as the same and the point of assaying of an offspring can be generalised to all the offsprings of the same parents so the early life stressed offspring of the case parent is taken as representative of the early life stress given rats in F1B2 which was the group given stress two times except that the rat in F1B2 after early life stress was not assayed and was assayed after late life stress, at the age of 14 weeks.

Table-1: Description of various experimental groups

P1B	Control group of parents to whom no stress was given
P1A	Case parent group to which stress was given
F1A	5 wks old rat who were the offspring of case parents and from whom preliminary parameters were collected.
F1A1	Offspring of case parents given early life stress from 5 wks to 8 wks and were assayed at 8 wks
F1A2	Offsprings of case parents given stress twice once at 5 wks and the other at 11 wks and they were assayed at 14 wks age
F1A3	Offsprings of case parents given late life stress at 11 wks and were assayed at 14 wks
CtF1A1	Case parent offsprings not given stress and assayed at 8 wks
CtF1A2	Case parent offspring not given stress and assayed at 14 wks
F1B	5 wks old rat who were the offspring of control parents and from whom preliminary parameters were collected
F1B1	Offspring of control parents given early life stress from 5 wks to 8 wks and were assayed at 8 wks
F1B2	Offsprings of control parents given stress twice once at 5 wks and the other at 11 wks and they were assayed at 14 wks age

F1B3	Offsprings of control parents given late life stress at 11 wks and were assayed at 14 wks
CtF1B1	Control parent offsprings not given stress and assayed at 8 wks
CtF1B2	Control parent offspring not given stress and assayed at 14 wks

The corticosterone levels were increased in the case parents P1A when compared with P1B ($p<0.01$) and to its offsprings. F1A1 had significantly raised corticosterone levels as compared to the F1A offspring who were assayed at 5 weeks before starting early life stress ($p=0.03$). F1B1 had significantly raised corticosterone levels as compared to F1B ($p=0.05$), CtF1B1 ($p=0.01$) and Ct F1B2 ($p<0.01$). The control group offsprings had decreased stress levels as shown by significantly decreased corticosterone levels as compared to the offspring given stress. Early life stressed offspring were more stressed as compared to the rest of the offsprings. (Table-2, 3).

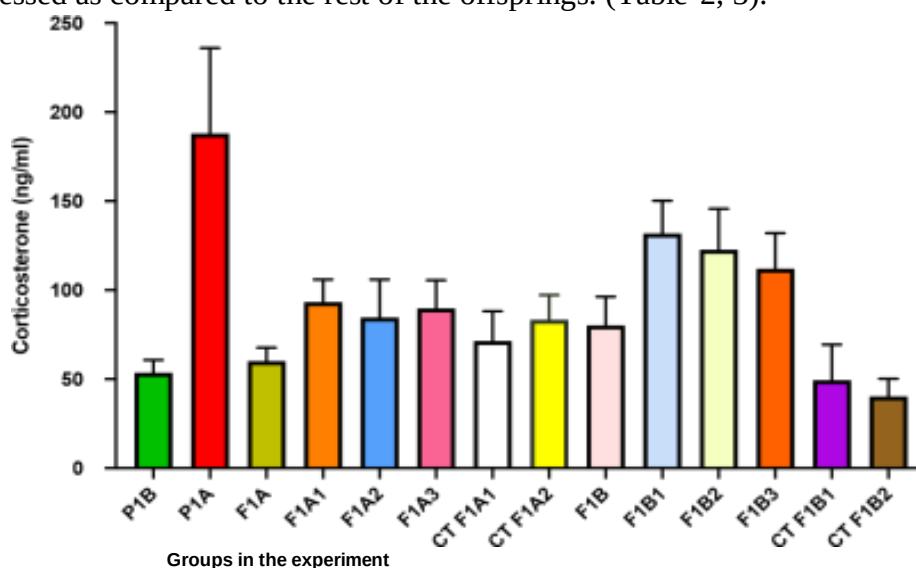


Figure 1: Comparison of Corticosterone Levels in ng/ml (Mean With SEM) Between all The Groups

Table 2: Corticosterone Levels in various Experimental Groups	
Groups	Corticosterone (ng/ml) Mean ± SEM
P1B	53.57±7.13
P1A	187.82±48.24
F1A	60.06±7.66
F1A1	93.16±12.82
F1A2	84.58±21.36
F1A3	89.67±15.93
Ct F1A1	71.33±16.81
Ct F1A2	83.25±13.90
F1B	80.11±16.18
F1B1	131.70±18.47
F1B2	122.57±23.16
F1B3	111.90±20.07
Ct F1B1	49.33±19.98
CtF1B2	40.20±10.08

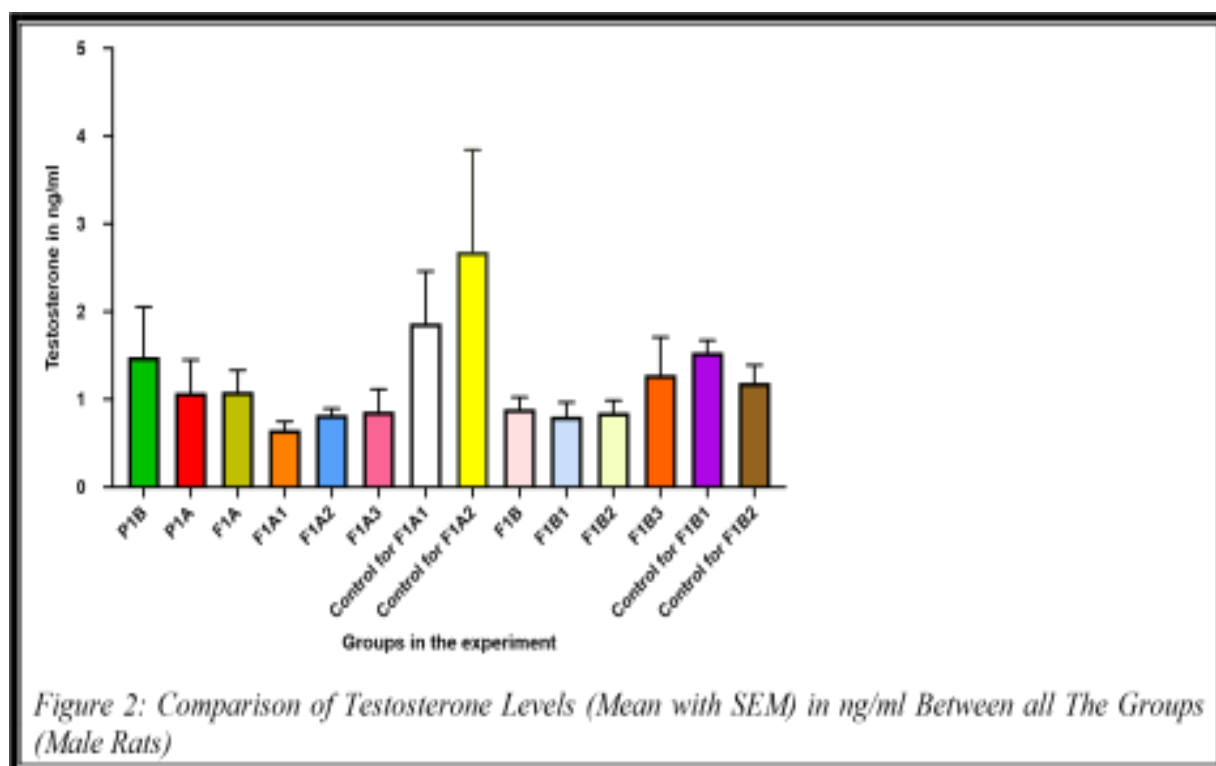
Effect of Chronic Alternating Stress on Corticosterone, Testosterone and Johnsen
Testicular Histology Score in Two Generations of Male Rats

Table 3: Significant Differences (p-value) among Groups in Corticosterone Levels

Groups	P1A	P1B	F1A	F1A1	F1A2	F1A3	CtF1A1	CtF1A2	F1B	F1B1	F1B2	F1B3	CtF1B1	CtF1B2
P1A		0.000	0.001	0.028	0.05	0.016	0.005	0.006	0.019	0.326	0.496	0.089	0.002	0.000
P1B			0.597	0.019	0.705	0.082	0.496	0.082	0.290	0.000	0.082	0.023	0.070	0.199
F1A				0.034	0.880	0.140	0.821	0.130	0.734	0.010	0.112	0.041	0.034	0.112
F1A1					0.597	0.623	0.131	0.734	0.290	0.130	0.257	0.496	0.013	0.003
F1A2						0.650	0.821	0.762	0.705	0.082	0.226	0.364	0.199	0.174
F1A3							0.226	0.880	0.406	0.173	0.326	0.364	0.023	0.016
CtF1A1								0.130	0.880	0.041	0.290	0.151	0.028	0.05
CtF1A2									0.496	0.082	0.326	0.257	0.019	0.013
F1B										0.059	0.290	0.174	0.028	0.070
F1B1											0.820	0.520	0.013	0.002
F1B2												0.571	0.820	0.034
F1B3													0.049	0.016
CtF1B1														0.597
CtF1B2														

Table 4: Significant Differences (p-value) among Groups in Testosterone Levels (Male Rats)

Groups	P1A	P1B	F1A	F1A1	F1A2	F1A3	CtF1A1	CtF1A2	F1B	F1B1	F1B2	F1B3	CtF1B1	CtF1B2
P1A		0.602	0.602	0.361	0.806	0.754	0.117	0.327	0.602	0.806	0.917	0.806	0.142	0.465
P1B			0.917	0.045	0.142	0.251	0.917	0.462	0.465	0.327	0.175	1.000	0.221	0.465
F1A				0.144	0.327	0.754	0.465	0.327	0.917	0.462	0.465	1.000	0.142	0.602
F1A1					0.670	0.715	0.045	0.007	0.273	0.670	0.068	0.136	0.011	0.045
F1A2						0.624	0.086	0.248	0.624	1.000	0.462	0.149	0.021	0.086
F1A3							0.047	0.327	0.754	1.000	0.347	0.462	0.086	0.347
CtF1A1								0.806	0.465	0.142	0.175	0.624	0.624	0.754
CtF1A2									0.221	0.248	0.221	0.386	0.564	0.462
F1B										0.624	0.917	0.462	0.014	0.251
F1B1											0.624	0.386	0.043	0.221
F1B2												0.327	0.027	0.117
F1B3													0.386	0.806
CtF1B1														0.462
CtF1B2														



In male rats alone testosterone was raised in non-stressed parents as compared to F1A1 which are the early life stressed rats of case parents. Among the offsprings of stressed parents, early life and both early and late life stressed rats had decreased testosterone as compared to the control for early life stress which shows that early life stressed rat were affected the most from stress.

Table 5: Significant Differences (p-value) among Groups in Testosterone Levels (Male Rats)

Groups	P1A	P1B	F1A	F1A1	F1A2	F1A3	CtF1A1	CtF1A2	F1B	F1B1	F1B2	F1B3	CtF1B1	CtF1B2
P1A		0.602	0.602	0.361	0.806	0.754	0.117	0.327	0.602	0.806	0.917	0.806	0.142	0.465
P1B			0.917	0.045	0.142	0.251	0.917	0.462	0.465	0.327	0.175	1.000	0.221	0.465
F1A				0.144	0.327	0.754	0.465	0.327	0.917	0.462	0.465	1.000	0.142	0.602
F1A1					0.670	0.715	0.045	0.007	0.273	0.670	0.068	0.136	0.011	0.045
F1A2						0.624	0.086	0.248	0.624	1.000	0.462	0.149	0.021	0.086
F1A3							0.047	0.327	0.754	1.000	0.347	0.462	0.086	0.347
CtF1A1								0.806	0.465	0.142	0.175	0.624	0.624	0.754
CtF1A2									0.221	0.248	0.221	0.386	0.564	0.462
F1B										0.624	0.917	0.462	0.014	0.251
F1B1											0.624	0.386	0.043	0.221
F1B2												0.327	0.027	0.117
F1B3													0.386	0.806
CtF1B1														0.462
CtF1B2														

Table 6: Testosterone Levels in various Experimental Groups

Groups	Testosterone in male rats (ng/ml) mean with SEM
P1B	1.48± 0.57
P1A	1.07 ± 0.37
F1A	1.08 ± 0.24
F1A1	0.64 ± 0.09
F1A2	0.82 ± 0.07
F1A3	0.86 ± 0.25
Ct F1A1	1.86 ± 0.59
Ct F1A2	2.68 ± 1.16
F1B	0.88 ± 0.13
F1B1	0.80 ± 0.16
F1B2	0.84± 0.13
F1B3	1.27 ± 0.42
Ct F1B1	1.53 ± 0.14
Ct F1B2	1.18 ± 0.20

Histological Evaluation of Male Testicular Tissue:

The Male testis were evaluated. We checked the various stages of spermatogenesis using the Johnsen score. The Johnsen score, also known as the Johnsen testicular biopsy score, is a system used to evaluate the histological appearance of seminiferous tubules in the testis. It was developed by Torvald Johnsen in 1970. The score ranges from 1 to 10, with each score reflecting different stages of spermatogenesis observed in the seminiferous tubules.

This scoring system is commonly used in the evaluation of male infertility, helping to diagnose the cause of the issue by assessing the level of sperm development within the testis. The Johnsen score for each tubule can vary so we counted 25 tubules in each section of the testis and took an average.²⁵ The Male Testis was evaluated for histology. According to Creasy et al in 2003 the 5 week rats testis was not evaluated as they had still not reached sexual maturity.²⁶ The F1A1 and F1B1 had significantly decreased Johnsen score as compared to the rest of the groups but it was because of their age. These were of smaller age as compared to the rest of the groups and similarly their controls also had a decreased Johnsen Score. There was no significant difference between the testis of the rats in the different groups in terms of Epithelial Irregularity, Sertoli cells, Leydig cells and Spermatozoa after evaluation with PAS Staining.

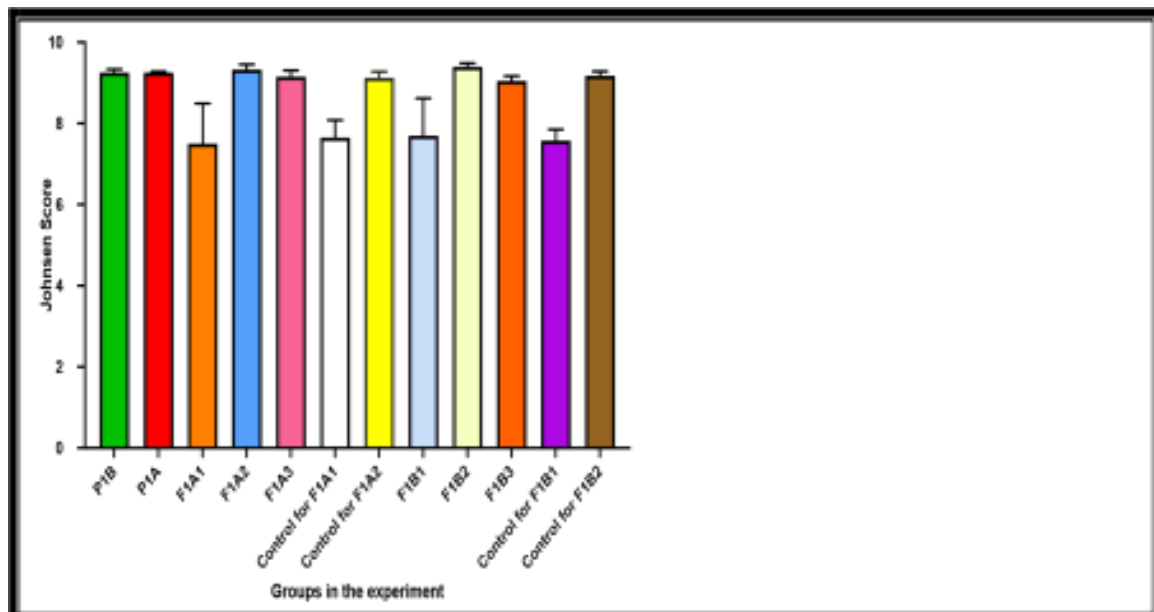
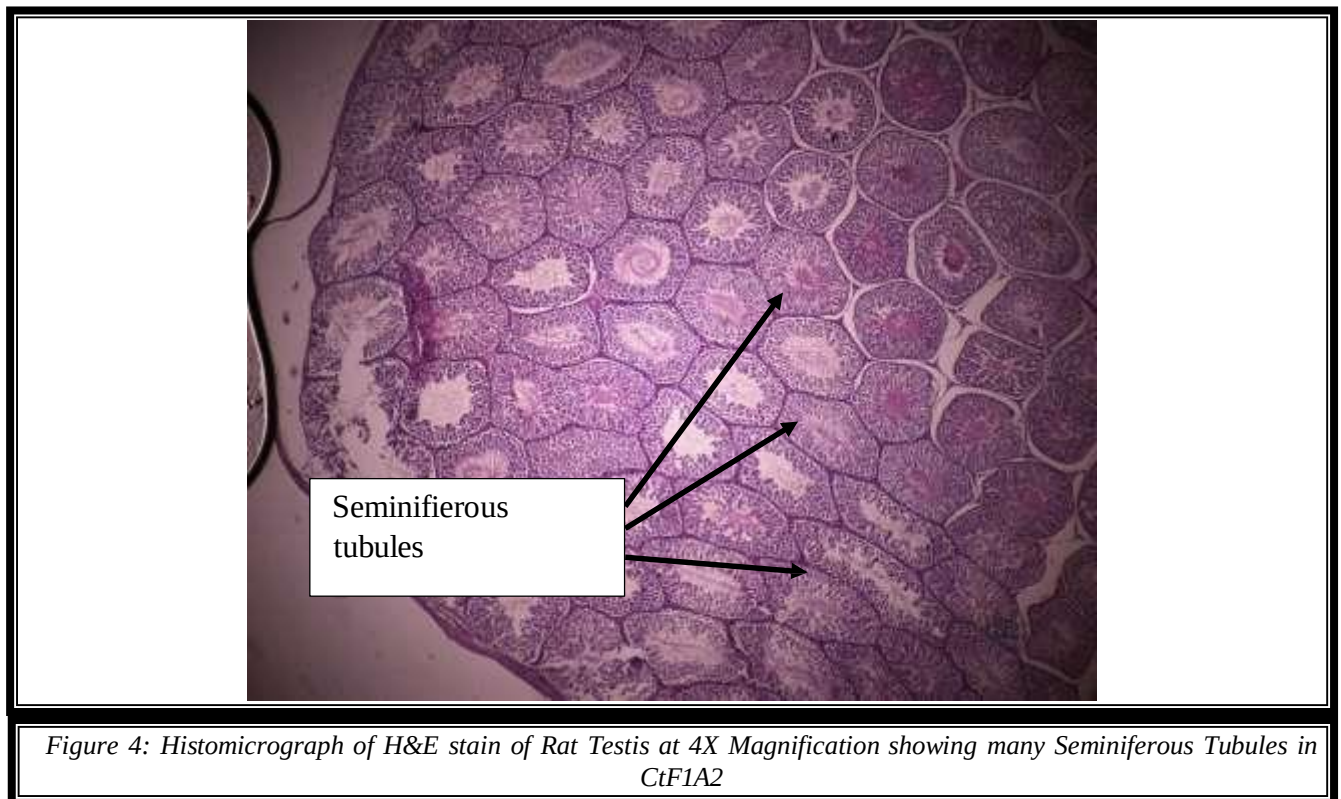


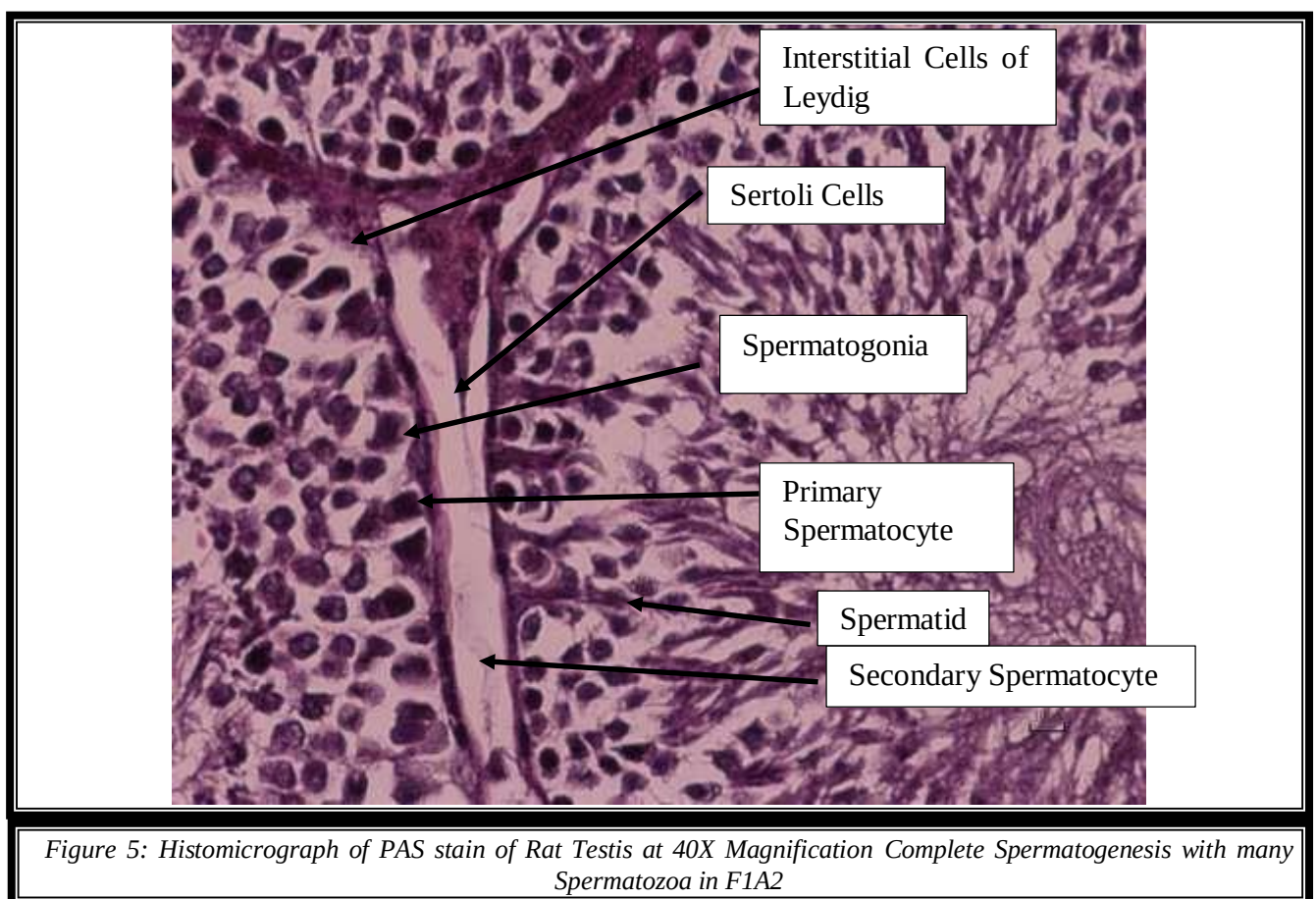
Figure 3: Comparison of Johnsen Score (Mean with SEM) in Testis of Male

Table 7: Johnsen Score of Male Rats In various groups

Groups in the Experiment	Johnsen Score Mean ± SEM
P1B	9.25 ± 0.09
P1A	9.25 ± 0.03
F1A	Not Scored
F1A1	7.50 ± 0.99
F1A2	9.32 ± 0.13
F1A3	9.15 ± 0.16
CtF1A1	7.65 ± 0.43
CtF1A2	9.12 ± 0.15
F1B	Not Scored
F1B1	7.70 ± 0.92
F1B2	9.40 ± 0.08
F1B3	9.05 ± 0.12
CtF1B1	7.58 ± 0.28
CtF1B2	9.18 ± 0.11



Scale Bar at 100um



Scale Bar at 10um



Figure 6: Histomicrograph of PAS stain of Rat Testis at 10X Magnification showing Seminiferous Tubules in Ct F1B2 (Scale bar =100um)

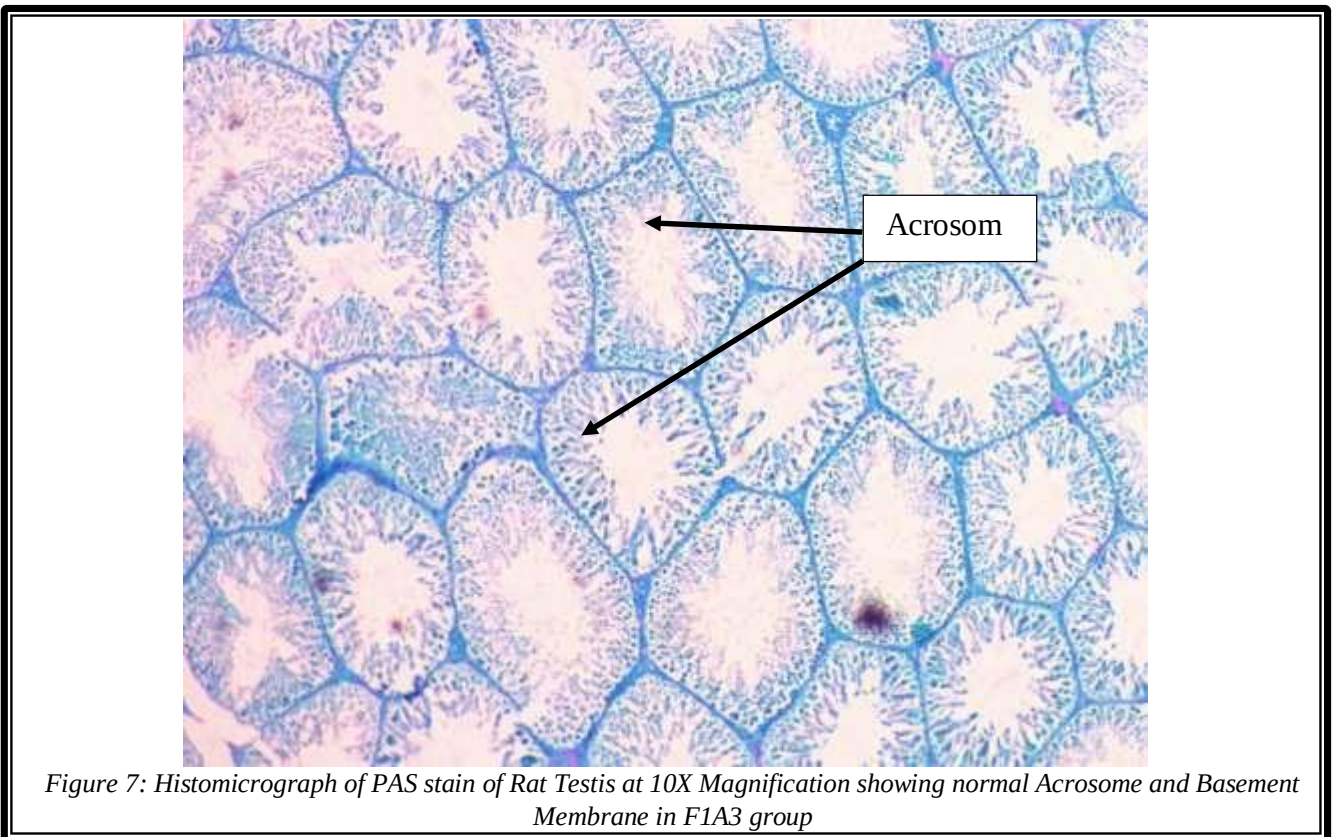


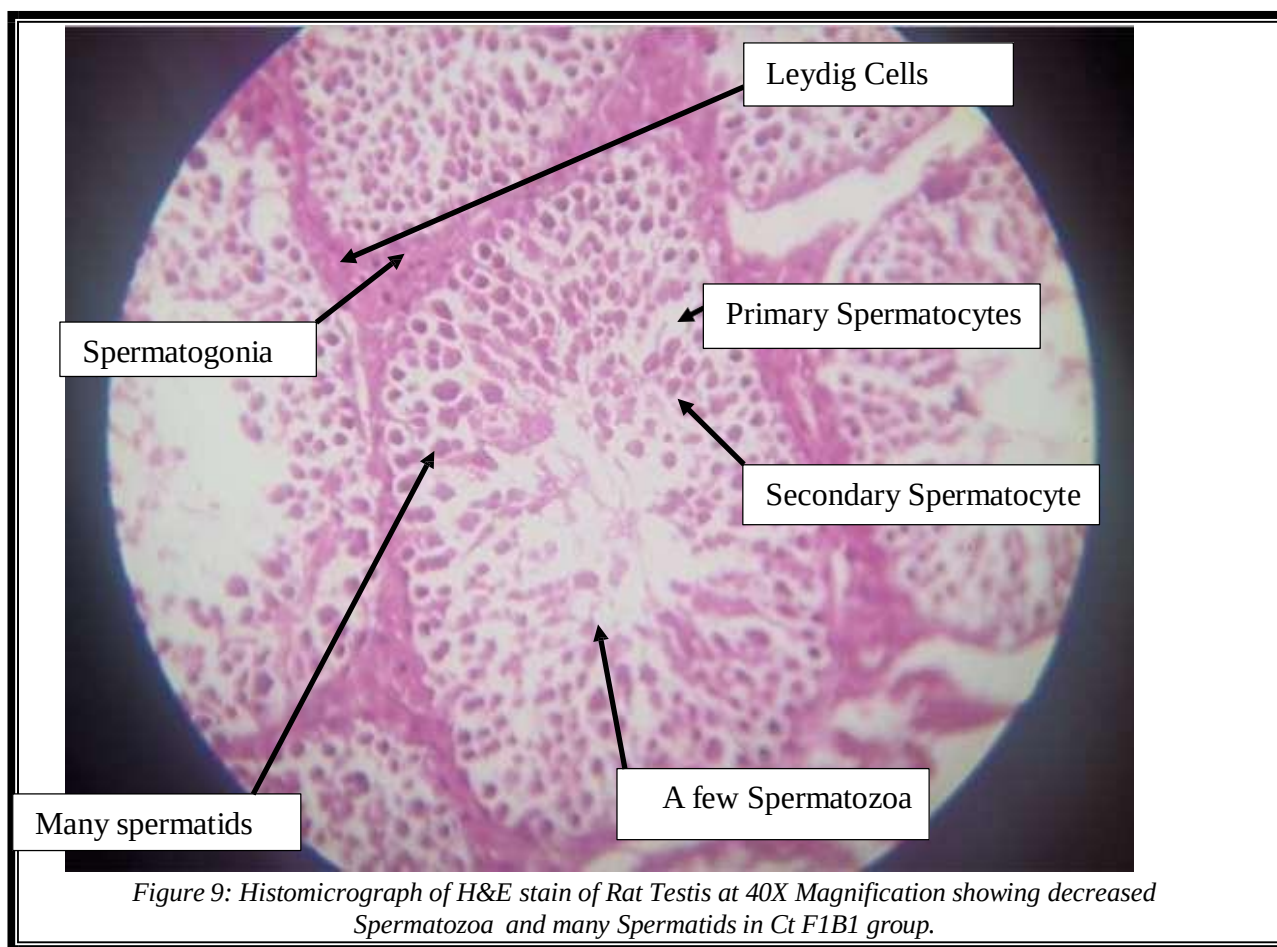
Figure 7: Histomicrograph of PAS stain of Rat Testis at 10X Magnification showing normal Acrosome and Basement Membrane in F1A3 group

The above picture is of Periodic Acid Schiff stain of the semiferous tubules. We can see that the basement membranes are regular and with no thick portions and have taken the magenta stain. The heads of the sperms also take magenta stain because of the presence of polysaccharides and mucins.



Figure 8: Histomicrograph of H&E stain of Rat Testis at 40X Magnification showing decreased Spermatozoa in Ct F1A1

The above pic shows the testis of an 8 week old rat showing a few spermatozoa and this will have a Johnsen score of 8. This is a young rat as the seminiferous tubules' cellularity is not dense and it has not gone through many cycles of spermatogenesis.



The above picture is that of a testis showing a few spermatozoa while there are spermatids also. The layers of the germinal epithelium include the spermatogonia which are the outermost followed by primary spermatocytes and secondary spermatocytes and spermatids while the spermatozoa are present in the center most layer.

DISCUSSION

The lifestyle has dramatically changed in the past few decades and so has the incidence of various diseases. Epidemiological studies provide compelling evidence that stressful early life events such as famine or war may significantly influence the likelihood of developing diseases in future generations. Stress during pregnancy is closely linked to a higher likelihood of neurodevelopmental problems, such as attention deficit-hyperactivity disorder²⁷, schizophrenia²⁸, and autism spectrum disorders.²⁹ Both the stress experienced by the mother and father may be transmitted to their kids either directly during pregnancy or via epigenetic modifications in the germ cells.³⁰

In this research project we compared the effect of stress across two generations of rats and we found different responses that were dependent on the age at exposure and the number of times the stress was given.

Stress Hormone Levels Corticosterone Levels

P1A had significantly increased corticosterone levels as compared to P1B. Among the offspring of case parents F1A1 had significantly increased corticosterone level as compared to F1A showing that early life stressed offsprings fared worst against stress. Among the offspring of control parents F1B1 was the most stressed as shown by increased corticosterone levels followed by F1B2 and F1B3, while

both the controls had significantly decreased corticosterone levels. Thus, here the early life stressed offspring had the worst results as shown by Figure 1, Table 2 and Table 3. Our research was similar to another study which took 6-7 week rats and subjected them to chronic unpredictable mild stress and reported raised corticosterone levels.³¹ The corticosterone is secreted from adrenal cortex in response to adrenocorticotrophic hormone released from anterior pituitary gland. Under stressful situations, the secretion of corticosterone is increased. The secretion of corticosterone is highly regulated by its own negative-feedback mechanism.³² Another study by Zhang et al., also reported increased corticosterone levels in rats subjected to chronic unpredictable mild stress in rats.³³ In contrast to our finding of significantly raised corticosterone in younger age group exposed to early life stress, Toth et al., did not observe any significant difference between the effects of chronic mild stress (CMS) on circadian corticosterone levels in the different age groups suggesting the development of resilience.³⁴

Testosterone Levels

Among the group of offsprings given early life stress testosterone was the least in F1A1 and F1B1 which showed that early life stress was not good for reproductive function. In F1A3 and F1B3 the testosterone was comparatively increased showing that late life stress effects can be coped with better. The controls had increased testosterone levels. Refer to Table 4, Table 5, Table 6 and Figure 2. In a study it was reported that the levels of serum testosterone changed with age in rats. Testosterone levels begin to increase after 25 days of age which is 3.5 weeks, and it reaches its peak at 60 to 110 days which is 8.5 weeks to 15 weeks of age.³⁵

The testosterone levels were in the lower range in all the groups. Testosterone levels were low in stressed rats. In a study by Buwalda et al., there was a reduction in testosterone level in rats of social defeat and testosterone supplementation prevented the social stress induced drop in plasma testosterone levels.³⁶ Xiaofan et al., also reported that 21 days of chronic stress decreased the testosterone levels in male rats.⁵

Effect of Stress on Testis

The male testis was evaluated for histology. The 5 week rats testis was not evaluated as they had still not reached sexual maturity which occurs at 8th week of life in rats.²⁶ Johnsen score was used to evaluate the rest of the testis. The F1A1 and F1B1 had decreased Johnsen score as compared to the rest of the groups but it was because of their age. Refer to Table 7 and Figure 3. These were of smaller age as compared to the rest of the groups. There was no significant difference between the testis of the rats in the different groups in terms of epithelial irregularity, sertoli cells, leydig cells and spermatozoa. PAS staining showed normal basement membrane regularity and normal acrosomes on the sperm heads and normal sertoli and leydig cells as shown by Figure 5, Figure 6 and Figure 7. Since the age of 8 weeks is peripubertal and spermatogenesis has not fully started thus the Johnsen score was decreased in the F1A1 and F1B1 group of rats as they were the offsprings euthanized at 8 weeks of age.³⁷ Similarly, the two controls who were euthanized at 8 weeks also had decreased Johnsen scores as shown by Figure 8 and Figure 9.

Contrary to our stress study which found no difference in the Johnsen score another study reported that Johnsen score decreased after 4 weeks of chronic stress. The sperm concentration and motility decreased, and also the number of sperm with abnormal morphology increased.³⁸ However the rats in their experiment were given high fat diet for 12 weeks and then given 4 weeks of stress. In our experiment the rats were not obese and the stress protocol was of 3 weeks which may be the reason why histopathological changes were not seen in our study. Another study also reported that the Johnsen score decreased in rats given the drug cyclophosphamide.³⁹ However they used a chemotherapeutic drug to cause testicular damage. Another study that gave stress in the form of electromagnetic radiations to rats for 30 minutes five days a week for 4 weeks found no difference in the Johnsen score of the seminiferous tubules however testosterone levels were decreased in the case group. These findings were similar to our study.⁴⁰

A study carried out on the effect of drugs on rat testis also reported decreased Johnsen score. The

researchers developed a depression model of CUMS stress in which rats were exposed to chronic unpredictable mild stress for 4 weeks continuously. After 4 weeks the testicular tissues were examined and Johnsen score was decreased in the stressed rats.⁴¹ This study was of 4 weeks duration while our study was of three weeks duration which might be the reason why there was no difference in Johnsen Score. Contrary to our study there was another study of prenatal stress which stated that Johnsen score increased in the stressed rats and this acceleration might expedite the onset of reproductive maturity as a strategic response to an expected adverse environment resulting from prenatal stress.⁴²

In our study, there was a decrease in testosterone levels in the early life stressed offsprings however the Johnsen score of these rats were no different from the Johnsen score of their control rats. Thus, the hormones levels of testosterone were although decreased but they were able to carry out normal spermatogenesis.

CONCLUSION

The hormonal assays show that the early life stressed offspring had elevated levels of corticosterone and decreased levels of testosterone. These hormonal imbalances are less pronounced in the offspring subjected to late life stress, especially those from control parents exposed to both early and late life stress. This suggests that exposure to manageable levels of stress during developmental stages can enhance resilience in adulthood. Histological analysis shows no significant differences between groups in terms of Johnsen Score for males of the same age. These findings highlight the potential benefits of controlled stress exposure during growth for developing resilience in later life.

Recommendations

Future research should focus on elucidating the molecular mechanisms underlying the observed changes in this study. Understanding these mechanisms could provide critical insights into developing strategies for better stress management.

Limitations of the Study

One limitation of this study is that it did not investigate the molecular mechanisms underlying the stress responses observed.

Authors' Contribution

Dr. Madiha Khattak - Conception, acquisition, analysis of data and drafting manuscript

Dr. Muhammad Omar Malik - Designing research project and acquisition of data

Dr. Robina Usman - Conception and design of research project, proof reading.

Mr. Muhammad Luqman - Data Acquisition and Proof Reading

Dr. Ammara Khattak - Data entry, Proof reading and writeup.

Dr. Dur E Shehwar Ali - Proof reading and writeup.

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