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EVALUATION OF THE EFFICACY OF PANAX GINSENG SUPPLEMENT ON NICOTINE AND CHRONIC STRESS INDUCED REPRODUCTIVE TOXICITY IN MALE ALBINO WISTAR RATS

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ABSTRACT

The current study assessed the efficacy of *panax ginseng* supplement in nicotine and stress induced reproductive toxicity in 48 male albino Wistars rats (150-200g body weight). The rats were grouped into two sets, the first set was control, stress, nicotine and nicotine + stress induced, while the second sets were treated with *panax ginseng*. The result was analysed using One way ANOVA, followed by post hoc multiple comparisons and level of significance set at $p<0.05$. Sperm count, motility, rapid progressive forward movement and sperm viability were significantly reduced ($p<0.05$) in nicotine + stress compared to stress and nicotine groups. Percentage of sperm debris, non-motile sperm and slow progressive forward movement were significantly increased ($p<0.05$) in nicotine + stress compared to stress and nicotine groups. Luteinizing hormone and testosterone levels were significantly reduced ($p<0.05$) in nicotine + stress compared to stress and nicotine. Follicle stimulating hormone levels were significantly reduced ($p<0.05$) in stress and stress + nicotine, but significantly increased ($p<0.05$) in nicotine compared to control. Testicular and epididymal tissues of nicotine + stress rats were seriously impaired compared to stress and nicotine rats. *Panax ginseng* significantly ($p<0.05$) recovered the changes in the assessed sperm characteristics and hormonal parameters, and as well improved the testicular and epididymal tissue alterations observed in the untreated groups. *Panax ginseng* appeared to ameliorate the adverse effect induced by nicotine plus chronic stress on male reproductive parameters.

KEYWORDS: Testicular and epididymal tissues, sperm debris, nicotine + stress.

INTRODUCTION

Some view life as a sequence of stresses, with our responses being minor adaptations. Sometimes adaptation requires major effort and may have responses that result in health problems.[1] Direct or indirect or overt behaviour that accompany stress include increased smoking/ exposure to nicotine based products among others. Some individuals use drugs to calm nerve rather than eliminate those conditions that induce stress in them. [2] The effect of stress is never pleasant. Studies have shown that there is no "safe level of smoking". Even light smoking is associated with reduced male fertility.[3] It has been reported that the highest exposure to nicotine by various means is showed in young male adults (between 20 and 39 years) during their reproductive period.[4] Effects of nicotine on reproduction was reported [1,5,6] that nicotine adversely affected spermatogenesis and epididymis sperms. Studies have it that nicotine affects sperm count, motility and fertilizing potential of sperms.[7,8,9] Environmental stress including noise, a natural teratogenic stress agent are commonly experienced in virtually everywhere in our modern society and has been shown to impact adversely on life function especially reproductive functions with reduction in reproductive rate.[2] Generally, ambient temperature even a little higher, than the normal upper limits that an organism can withstand results in reduced fertility.[10]

A number of studies have been conducted on the use of herbal plants in the treatment of infertility. Ginseng a member of the *Araliaceae* family of plants is one of the most widely used herbal drugs and is reported to have a wide range of therapeutic and pharmacological activities.[11,12,13] The primary types of ginseng are Asian ginseng (*Panax ginseng*), American ginseng (*Panax quinquefolius*), and Siberian ginseng (*Eleutherococcus senticosus*). A recent study on human and laboratory animal showed that *Panax ginseng (PG)* enhance libido and copulatory performance and facilitate erectile dysfunction in males.[14] The root of *PG* is used preferably from plants older than six years of age.[15] Bioactive of *PG* possess antioxidant property, quenching free radicals, protecting low density lipoproteins from oxidation and inhibiting lipid

peroxidation.[16,17] It was observed that *PG*, is a well-known adaptogen and a restorative herbal preparation.[18,19] *PG* was reported to be effective in anti-stress and anti-aging activities,[20] is also reported to increase libido and sexual satisfaction.[21] Reviews suggest *PG* has immuno-modulating activity by affecting the hypothalamic-pituitary-adrenal (HPA) axis.[19,22] According to a World Health Organization review, ginseng saponins "are thought to decrease serum prolactin, thereby increasing libido" in male impotence.[23]

This study aimed to evaluate the efficacy of *PG* supplement in nicotine and stress induced reproductive toxicity in male albino Wistar rats.

MATERIALS AND METHODS

Materials- Digital noise sensor, Nicotine and *PG* was purchased from unipervit pharmacy, ikot Omin, Calabar, Nigeria.

Laboratory animals

Forty eight male albino Wistar rats weighing 150–220g were used for this study. The animals were housed in cages and were kept in well ventilated room in the Department of Physiology Animal house, University of Calabar, Nigeria. Standard animal cages with wood dust as bedding were used in keeping the animals. They were allowed *ad libitum* access to rat chow and clean water, and exposed to 12/12-hr light/dark cycle. The animals were acclimatized for 7 days prior to experimentation. Indeed, the animals were kept in line with laid down principles for animal care as prescribed in Helsinki's 1964 declaration. The animal ethics committee of the University of Calabar graciously approved our study.

After one week of acclimatization the animals were randomly assigned into two sets of basic groups of (n=24). Each set was further divided into four groups (n=6).

In the first set: Group I served as: Normal control, Group II: Stress induced, Group III: Nicotine induced received 1.5mg/kg/day and Group IV: Nicotine + Stress induced received 1.5mg/kg/day of nicotine induced and stress induced for 8 hours. While in the second set, Group V served as: *PG* control and received 500mg/kg/day, Group VI: Nicotine + *PG* received 1.5mg/kg of nicotine induced and treated with 500mg/kg of *PG*, Group VII: Stress+ *PG* was stress induced for 8 hours/day and treated with 500mg/kg of *PG* and Group VIII: Stress+ Nicotine+ *PG* was stress induced for 8 hours/day and 1.5mg/kg/day of nicotine induced and treated with 500mg/kg/day of *panax ginseng*

Exposure to stress

The following stressors were used:

(1) Yamaha ET 950 generator (90-120dB, at 8 hours per day) and digital noise sensor was used to detect the noise levels.
(2) Open environment (8 hours per day).

The animals in the stress induced groups were exposed to either of the two forms of stressor at daily intervals to avoid adaptation. For the generator noise, the animal cages were kept at varying distance from the source and a digital noise sensor was used to detect the level of noise. Rodents are quite not comfortable in an open environment and this constitutes a form of stress. Majority of studies has it that scent materials and vocalization are typically used to simulate predation risks[2,10] The animal cages were kept in an open environment where the voice and footstep of people were heard, and which draws the attentions of some to look at the rats.

Experimental design and drug administration

Herbal factor *PANAX GINSENG* C. A. Meyer manufactured by Natural Factors Canada and consists of soft gel with a gel volume of 1ml, composed of *PG* extract (root) 100mg and 10% ginsenoside in each soft gel. Each soft gel of *PG* was dissolved in 2ml of castor oil to derive a stock solution of (50mg/ml). *PG* was administered orally at a dosage of 500mg/kg/day.

Strew berry liquid nicotine of concentration 6mg/10ml, with a stock solution of 0.6mg/ml was administered at a dosage of 1.5mg/kg/day.

The control group received normal rat chow and water. The experiment lasted for 28 days, after which the animals were sacrificed under chloroform anaesthesia and the testes and epididymis carefully harvested for semen analysis and histopathological examination, blood serum was obtained by cardiac puncture to examine hormonal assay.

Assessment of sperm motility

Sperm motility was assessed by placing 10µl of sperm suspension collected from the left epididymis on a clean pre-warmed slide, covered with a coverslip and examined using a light microscope (Leica DM 750, Switzerland) equipped with a heated stage (37°C), at 100× magnification. [24] Rapid progressive forward movement (RPFM), slow progressive forward movement (SPFM), residual movement (RM) and non-motile sperm were determined.[24]

Determination of epididymal sperm count

Assessment of epididymal sperm count was done using the method described by Wyrobek.[25] The left cauda epididymis from each rat was placed in 2 ml of normal saline, pre-warmed to 37°C. Small incisions were made in the cauda epididymis and spermatozoa were obtained and suspended in saline solution. Two hundred microlitres of the suspension was transferred to both chambers of a Neubauer haemocytometer using a Pasteur pipette by touching the edge of the coverslip and allowing each chamber to be filled by capillary action.

The epididymal sperm count for each animal was then obtained and recorded.[25]

Assessment of sperm viability and debris

Sperm viability was evaluated using the method described by Wyrobek.[25] Twenty microlitres of 0.05% eosin Y–nigrosin was added to an equal volume of sperm suspension and incubated at room temperature for 2 min. After incubation, all slides were viewed under a light microscope (Leica DM 750) at magnifications of ×100 and ×400. Live spermatozoa were not stained, while dead spermatozoa were stained pink. For each assay, 400 spermatozoa were counted and viability percentages were calculated.[25]

Measurement of serum reproductive hormones

Serum was obtained following the method previously described in preceding sections. The serum was then used for reproductive hormonal assay. Serum Testosterone, Luteinizing hormone and Follicle stimulating hormone concentrations were determined using the ELISA kit method as used by.[26]

Histological Studies

The testis and epididymis of the control and treated rats were carefully removed, cleared of connective tissues and fixed in Boiun's fluid [0.2% picric acid/2% (v/v) formaldehyde in PBS]. Sections were obtained and stained with heamatoxylin and eosin (H & E) stains. The microscopic slides were labeled appropriately. Photomicrographs were done with the help of a light microscope (Leica DM 750, Switzerland) and magnifications of x100 were viewed.

Statistical Analysis

One way analysis of variance (ANOVA), followed by post hoc multiple comparisons was used for the statistical analysis. Results was presented as means ± Standard Errors of means (SEM) and probability levels $p< 0.05$ was accepted as significant.

RESULTS

Table 1: Efficacy of *panax ginseng* on semen parameters of male albino wistar rats induced with nicotine- and -stress

Group	Control	Stress	Nicotine	Nicotine + Stress	*PG*	*PG* + Stress	*PG* + Nicotine	*PG* + Nicotine + Stress
Sperm Motility (%)	81.25 ± 3.15	51.50 ± 8.45*	55.25 ± 2.04*	35.76 ± 7.38*,a, b	86.25 ± 2.39	63.45 ± 16.58*,a	65.21 ± 3.75*,b	52.56 ± 5.9*,c
RPFM (%)	48.75 ± 3.75	33.75 ±2.68*	32.50 ±4.79*	25.00 ± 2.89*,a, b	65.00 ± 3.54*	46.33 ± 8.78a	49.21 ± 3.23b	41.25 ± 6.25c
SPFM (%)	12.00 ± 5.40	32.46 ±1.25*	29.83 ± 1.25*	41.12 ± 2.89*,a, b	10.00 ± 2.50	16.67 ± 5.20a	20.00 ± 2.89b	33.75 ± 3.15*,c
RM (%)	10.00 ± 2.04	12.50 ±3.23	8.75 ± 2.39	15.00 2.89	6.25 ± 1.25	11.50 ± 4.33	11.25 ± 1.25	11.25 ± 3.15
Non Motile Sperm (%)	17.50 ± 3.23	45.00 ± 10.21*	30.00 ± 2.04*	64.25 ± 11.55*,a,b	13.70 ± 19.51*	32.85 ± 14.93*,a	20.18 ± 3.75b	33.75 ± 5.91*,c
Sperm Count (x 1 million cells/L)	69.30 ± 8.79	36.81 ± 9.64*	32.10 ± 6.87*	22.15 ± 5.46*,a, b	65.87 ± 5.14	55.35 ± 2.55*	57.61 ± 1.69*,b	51.12 ± 3.63*,c
Sperm Viability (%)	82.50 ± 1.44	46.76 ± 5.22*	44.21 ± 4.08*	32.56 ± 4.33*, a, b	73.01 ± 12.08	52.50 ± 11.64*,a	55.00 ± 6.77*,b	45.45 ± 7.07*,c
Sperm debris (%)	4.75 ± 0.25	8.50 ± 0.50*	7.65 ± 0.25*	16.25 ± 0.58*,a, b	4.89 ± 0.25	5.00 ± 0.41a	5.25 ± 0.50b	6.00 ± 0.00*,c

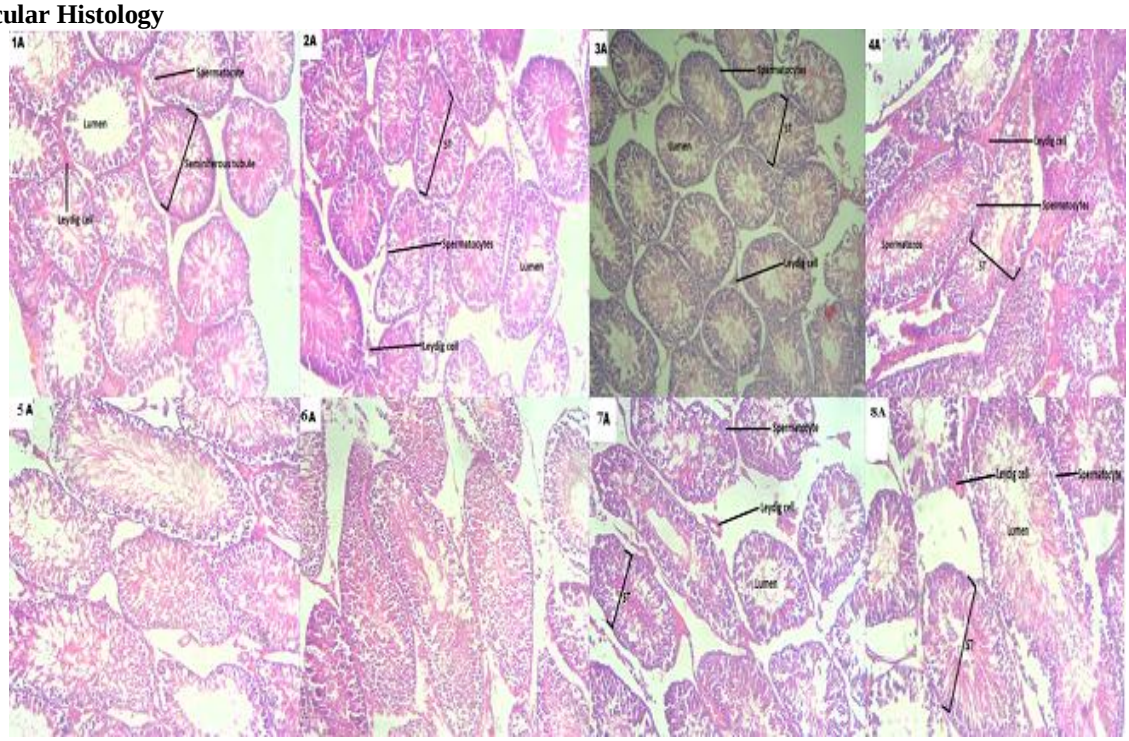
Data are expressed in Mean ± SEM of 6 rats, *, a, b, c are Mean significant difference relative to control, stress, nicotine and nicotine + stress induced groups respectively at P<0.05

Table 2: Efficacy of *panax ginseng* on testosterone, Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) of male albino wistar rats induced with nicotine- and –stress.

GROUP	Testosterone (ng/mL)	LH (IU/mL)	FSH(ng/mL)
Control	4.48 ± 0.15	4.73 ± 0.05	1.00 ± 0.05
Stress	3.13 ± 0.14*	2.13 ± 0.10*	0.22 ± 0.01*
Nicotine	2.95 ± 0.10*	2.15 ± 0.03*	2.02 ± 0.59*
Nicotine + Stress	2.14 ± 0.06*,a, b	1.70 ± 0.06*,a, b	0.20 ± 0.01*
Panax ginseng	4.65 ± 0.19	3.95 ± 0.17	0.86 ± 0.03
Panax ginseng+ Stress	3.72 ± 0.13*,a	2.48 ± 0.17*,a	0.42 ± 0.04*,a
Panax ginseng + Nicotine	3.53 ± 0.17*,b	3.23 ± 0.07*,b	0.65 ± 0.06b
Panax ginseng + Nicotine + Stress	3.20 ± 0.13*,c	2.63 ± 0.08*,c	0.38 ± 0.01*,c

Data are expressed in Mean ± SEM of 6 rats, *, a, b, c are Mean significant difference relative to control, stress, nicotine and nicotine + stress induced groups respectively at P<0.05.

Testicular Histology

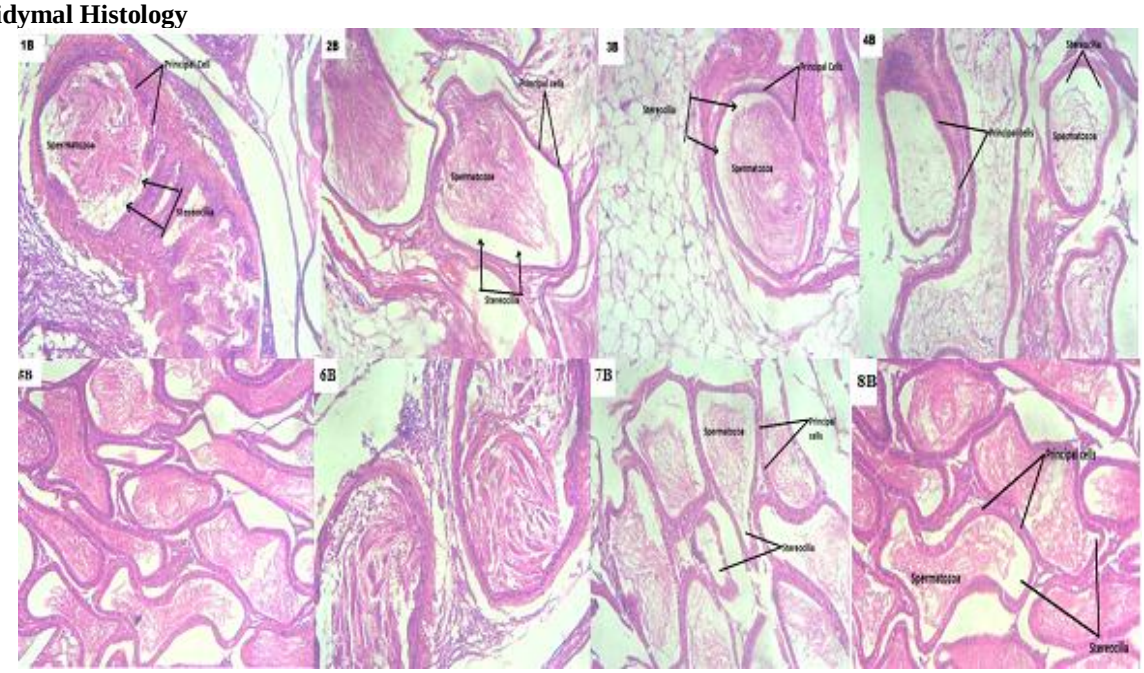


Magnification: x100

Figure 1: Photomicrograph showing H and E stained testes.

Key: Photomicrograph of the testes showing:
(1A) Control with normal testes histo-architecture (2A) Stress induced with ST poorly populated with spermatozoa. (3A) Nicotine induced with various ST showing noticeable reduction of sperm cells. (4A) Stress and nicotine induced with poorly cellular morphology. **(**5A**)** *Panax ginseng* control with normal appearance of testicular tissue (6A) Stress induced and *panax ginseng* treated with no visible distortion of the general tissue structure**.** (7A) Nicotine induced and treated with *panax ginseng* showing normal distribution of the various ST with adequate spermatozoa. (8A) Stress and nicotine induced treated with *panax ginseng* showing some ST having adequate spermatocytes and spermatozoa while others having inadequate spermatocytes and spermatozoa.

Epididymal Histology



Magnification: x100

Figure 2: **Photomicrograph showing H and E stained epididymis.**

Key: Photomicrograph of epididymis showing:
(1B)Control with normal appearance of the general tissue structure. (2B) Stress induced with histological architecture less populated with spermatozoa. (3B) Nicotine induced with noticeable inflammation of the principal cells. (4B) Stress + nicotine induced with noticeable inflammation of the principal cells. **(**5B) *Panax ginseng* control with normal distribution of spermatozoa
(6B) Stress induced and treated with *panax ginseng* showing normal appearance of the various cell components. (7B) Nicotine induced and treated with *panax ginseng* showing no visible inflammation. (8B) Stress and nicotine with *panax ginseng* treated group heavily populated with spermatozoa.

DISCUSSION

The alteration in sperm motility, count, viability, debris, RPFM and SPFM in stressed rats observed in this study is in accordance with earlier report of.[27,6,8,28,29,9] Viau[30] reported that the negative effect of stressors on sperm indices may be due to activation of the hypothalamic-pituitary- adrenal (HPA) axis, with hyper-secretion of glucocorticoid. There is also a relationship between stress and reproduction, and between HPA and hypothalamic- pituitary- gonadal (HPG) axis as a cause of male infertility.[30] Following treatment with *PG* the semen parameters were improved. Hong *et al*[21] reported that *panax ginseng* improved sperm parameters following immobilization stress.

The alteration in sperm motility, count, viability, debris, RPFM and SPFM in nicotine induced rats observed in this study is in accordance with earlier reports.[31,32,33,34,9] Pook *et* al[29] and Seema[33] observed that reduction in sperm indices may be due to action of nicotine in generating ROS and lipid peroxidation in the testes, these nicotine effect had been reported to cause decrease spermatogenesis in rats.[32,35] Treatment with *PG* ameliorated the negative changes observed in nicotine induced rats.

In stress + nicotine induced rats sperm motility, count, viability, debris, RPFM and SPFM was markedly impaired compared to either of stress or nicotine induced rats. Increase in number of sperm debris results in impaired motility as normal intact sperm morphology is prerequisite for linear progressive motility. The decrease observed in sperm motility, count, viability and RPFM in stress + nicotine induced compared to stress and nicotine induced respectively is an indication of worsen reproductive function. Following treatment with *PG*, the result showed a positive trend towards recovery.

From the assay of these hormones, the results show that either of stress and nicotine each caused a derangement in the normal male reproductive hormonal levels with these derangement worsened with a combination of stress and nicotine and therefore possibly induced infertility in the male rats. Treatment with *PG* showed recovery in the reproductive hormone level.

The reduction in testosterone level in the nicotine group is in accordance with that of Sarasin[36] who reported decreased plasma level of testosterone compared to control. Charpenet *et al*[37] found stress-induced decreased basal testosterone production.

In the contrary, Armario *et al*[38] and Pollard *et al*[39] reported an elevated plasma level of testosterone in response to different stressors in male rats. While Stahl et al[40] and Maric *et al*[41] revealed that stress did not affect plasma levels of testosterone in male rats.

The alterations in FSH and LH levels in rats exposed to stress are in accordance with that shown by Orr & Mann[41] and Srivastava *et al* [42] who reported that immobilization stress reduced the levels of these hormones

The reduction in the FSH and LH in the stress group compared to control in this study is contrary to that of

Aydos *et al*[6] and Jorsaraei *et al*[29] who reported increased FSH and LH level in noise stressed (90-130dB and 100dB respectively) male rats. Heidary *et al*[43] reported that the intermittent scrotal hyperthermia stress resulted in raised plasma FSH and LH. It is assumed that the elevated FSH level in the nicotine group may be secondary to decreased inhibin B negative feedback, which in turn induces a compensatory Sertoli cell response, thereby resetting the pituitary-testicular axis.

Kim & Park[16] and Kiefer & Pantuso[22] reported that panax *ginseng* extract showed an increase in serum testosterone, FSH and LH levels.

Significant histological distortions were observed in photomicrographs of the testes and epididymis of rats induced with stress, nicotine and stress + nicotine compared with control. Damage to the testicular tissue (particularly, the seminiferous tubules) may be responsible for the decreased sperm count in the treated groups. Exposure to environmental and occupational toxicants may adversely affect male reproductive potential during sperm development or epididymal storage. Sperm count decreases because of disruption of seminiferous tubules or acute infection in testis. Moreover, environmental stressors decrease the activity of enzymes that protect against oxidative damage in testicular tissue of rats. Reduction in the plasma testosterone level may be back to disorganization of Leydig's cells.

Furthermore, the alteration in the levels of FSH and LH may be responsible for the decreased spermatozoa density in the testes and epididymis which further confirmed the testicular damage, since mature spermatozoa are stored in the epididymis pending

ejaculation. Previous studies had reported degenerative changes in the testicular tissue following chronic stress.[44, 45]

CONCLUSION

The study showed significant alteration in reproductive parameters which are used in determining male fertility. This alteration was worsened in nicotine + stress induced rats. *Panax ginseng* appeared to ameliorate the adverse effect induced by nicotine plus chronic stress on male reproductive parameters.

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REFERENCES

1. Di Chiara, G. Role of dopamine in the behavioural actions of nicotine related to addition. European Journal of Pharmacology, 2009; 393: 293-314.
2. Rajabzadeh, A., Sagha, M., Gholami, MR Hemmati, R. Honey and vitamin E restore the plasma level of gonadal hormones and improve the fertilization capacity in noise stressed rats. Journal of Medical Biological Science, 2015; 2(2): 64-68.
3. Ramlau-Hansen, AM., Thulstrup, AS., Aggerholm, MS., Jensen, GT & Bonde, JP. Is smoking a risk factor for decreased semen quality? A cross-sectional analysis, 2007; 22(1): 188–196.
4. Oyeyipo, WA, Shittu, ST., Kolawole, TA, Ubaneche, P. & Akinola, AO. Protective effect of vitamin E on nicotine induced reproductive toxicity in male rats. Nigerian Journal of basic and applied sciences, 2015; 23(1): 7-13.
5. Harvey, DM. Nicotine serves as an effective reinforce of intravenous drug taking behaviours in human cigarette smoking. Phycho pharmacology, 2007; 175: 134-142.
6. Aydos, K., Guven, MC, Can, B. & Ergun, A. Nicotine toxicology to the ultra-structure of the testis in rats. British Journal of Urology International, 2006; 88: 622-626.
7. Wong, WY, Thomas, CM, Merkus, HM, Zielhuis, GA, Doesburg, WH, Steegers- Theunissen, RP. Cigarette smoking and the risk of male factor subfertility: minor association between cotinine in seminal plasma and semen morphology, 2005; 74(5): 930-5.
8. Dhawan, K. & Sharma, A. Prevention of chronic alcohol and nicotine-induced azospermia, sterility and decreased libido, by a novel tri-substituted benzoflavone moiety from Passiflora incarnate Linneaus in healthy male rats. Life Sciences, 2007; 71: 3059-3069.
9. Oyeyipo, IP, Raji, Y., Emikpe, BO. & Bolarinwa, AF. Effects of nicotine on sperm characteristics and fertility Profile in Adult Male Rats: A Possible Role of Cessation. Journal of Reproductive Infertility, 2011; 12(3): 201-207.
10. Yates, DT., Montoya, AF, Yates, LJ, Warners, CA, Otis AR, Lankford, LM. & Ross, T T. Effects of dietary vitamin E on daily intake, serum testosterone and epididymal sperm quality in sprague-Dawley rats subjected to heat stress. Journal of Veterinary Medicine of Animal Health, 2009; 2(2): 11-12.
11. Bladt, S., Wagner H, Woo WS. Fingerprint analysis and standardisation of *Eleutherococcus senticosus* (*Acanthopanax*) extract. Planta Med, 1990; 56: 78.
12. Chang, TH., Jih, MH. & Wu, TC. Relationship of sperm antibodies in women and men to human in vitro fertilization, cleavage, and pregnancy rate. American Journal Reproductive Immunology, 1993; 30(2–3): 108–12.
13. Chuang, WC, Wu, HK. Sheu, SJ., Chiou, SH, Chang, HC., Chen, YP. A comparative study on commercial samples of ginseng radix. Planta Med, 1995; 61: 459-465.
14. Tamler R, Mechanick JI. Dietary supplements and nutraceuticals in the management of andrologic disorders. Endocrinol Metab Clin North Am, 2007; 36(2): 533-552
15. McElhaney JE, Goel V, Toane B, Hooten J, Shan JJ. Efficacy of COLD-fx in the prevention of respiratory symptoms in community-dwelling adults: a randomized, double-blinded, placebo controlled trial. Journal of Alternative Complementary Medicine, 2006; 12(2): 153–7.
16. Kim, SH. & Park, SH. Effects of Panax ginseng extract on lipid metabolism in humans. Pharmacological Research, 2005; 48: 511–513.
17. Jung, CH., Seog, HM, Choi, IW, Choi, HD. & Cho, HY. Effects of wild ginseng leaves on lipid peroxidation levels and antioxidant enzyme activities in streptozotocin diabetic rats. Journal of Ethno-pharmacology, 2005; 98: 245–250.
18. Duke J. The Green Pharmacy Herbal Handbook: You're Comprehensive Reference to the Best Herbs for Healing. Emmaus, PA: Rodale, 2010; 115-116.
19. Blumenthal M, Goldberg A, Brinkmann J. *HerbalMedicine:* Expanded Commission E Monographs.Austin, TX: Integrative Medicine Communications, 2006; 170-177.
20. Kim, M. K., Lee, J. W., Lee, K. Y. & Yang D. Microbial conversion of major ginsenoside Rb1 to pharmaceutically active minor ginsenoside Rd, Journal of Microbiology, 2006; 43(5): 456-462.
21. Hong, B., Ji, Y. H., Hong, J. H., Nam, K. Y. & Ahn, T. Y. A double-blind crossover study evaluating the efficacy of Korean red ginseng in patients with erectile dysfunction: a preliminary report. Journal of Urology, 2012; 168(5): 2070-2073.
22. Kiefer D, Pantuso T. *Panax ginseng*. Am Fam Physician, 2003; 68: 1539-1542.
23. World Health Organization, "Radix Ginseng" in WHO Monographs on Selected Medicinal Plants, Geneva: World Health Organization, 1999; 168-182

24. Atashfaraz, E., Farokhi, F., & Najafi, G. Protective effect of ethyl pyruvate on epididymal sperm characteristics, oxidative stress and testosterone level in methotrexate treated mice. Journal of Reproduction and Infertility, 2013; 14: 190–196.
25. Wyrobek, AJ, Gordon, LA., Burkhart, JG., Francis, MW., Kapp, RW., Letz, G., Whorton, MD. An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals: A report of the US Environmental Protection Agency Gene-Tox Program. Mutation Research/Reviews in Genetic Toxicology, 1983; 115(1): 1–72.
26. Khaki, A., Fathiazad, F., Nouri, M., Khaki, AA, Khamenehi, HJ. & Hamadeh, M. Evaluation of androgenic activity of Allium cepa on spermatogenesis in the rat. Folial Morphology (Warsz), 2009; 68(1): 45-51.
27. Giblin PT, Poland ML, Moghissi KS, Ager JW, Olson JM. Effects of stress and characteristic adaptability on semen quality in healthy men. Fertil Steril, 1988; 49: 127-32.
28. Pook, M., Tuschen-Caffier, B. & Krause, W. Is infertility a risk factor for impaired male fertility? Human Reproduction, 2004; 19: 954-958.
29. Jorsaraei, S.G.A., Shibahara, H., Hirano, Y., Shiraishi, Y., Khalatbari, A., Pasha, Y.Y. & Suzuki, M. The in-vitro effects of nicotine, cotinine and leptin on sperm parameters analyzed by CASA system. Iranian Journal of Reproductive Medicine, 2008; 6 (3): 157-165.
30. Viau V, Meaney M. J. The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. J Neurosci, 2012; 16: 1866–1876.
31. Reddy, A., Sood, A., Rust, P.F., Busby, J.E., Varn, E. & Mathur, R.S. The effect of nicotine on invitro sperm motion characteristics. J Assist Repord Genet, 1995; 12: 217-223.
32. Ushijima C, Kumasako Y, Kihaile PE, Hirotsuru K, Utsunomiya T. Analysis of chromosomal abnormalities in human spermatozoa using multi-colour fluorescence in-situ hybridization. Hum Reprod, 2000; 15: 1107-1111.
33. Seeman TE, Singer B, Wilkinson CW, McEwen B. Gender differences in age-related changes in HPA axis reactivity. Psycho-neuroendocrinology, 2007; 26: 225–240.
34. Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D. Cadmium, Lead, and Other Metals in Relation to Semen Quality: Human Evidence for Molybdenum as a Male Reproductive Toxicant, 2008; 116: 1473-1479.
35. Jana, K.; Jana, N.; De, DK. & Guha, SK. Ethanol induces mouse spermatogenic cell apoptosis in vivo through over-expression of Fas/Fas-L, p53, and caspase-3 along with cytochrome c translocation and glutathione depletion. Molecular Reproduction & Development, 2010; 77(9): 820-833.
36. Sarasin, A., Schlumpf, M., Muller, M., Fleischmann, I., Lauber, ME. & Lichtensteiger, W. Adrenal-mediated rather than direct effects of nicotine as a basis of altered sex steroid synthesis in fetal and neonatal rat. Reproductive Toxicology, 2003; 17: 153–162.
37. Charpenet G., Tache Y., Forest MG., Haour F., Saez JM., Bernier M., Ducharme JR., Collu R. Effects of chronic intermittent immobilization stress on rat testicular androgenic function. Endocrinology, 1981; 109: 1254-1258.
38. Armario A and Castellanos JM. A comparison of corticoadrenal and gonadal responses to acute immobilization stress in rats and mice. Physiol Behav, 1984; 32: 517-519.
39. Pollard I, Bassett JR, and Joss JM. Plasma testosterone levels and delta-5-313-hydroxysteroid dehydrogenase activity in the testis of the rat following prolonged exposure to stress. J Reprod Fert, 1980; 59: 101-106.
40. Stahl F, Glitz F, Dörner G. Plasma testosterone levels in rats under various conditions. Exp Clin Endocrinol, 1984; 84: 277-284.
41. Orr TE, Mann DR. Role of glucocorticoid in the stress-induced suppression of testicular steroidogenesis in adult male rats. Horm Behav, 1992; 26: 350–363.
42. Srivastava RK., Taylor, MF, Mann DR. Effect of immobilization stress on plasma luteinizing hormones, testosterone and corticosterone concentration and on 3β-hydroxysteriod dehydrogenase activity in the testis of adults rats. Proc. Soc. Exp. Biol. Med., 1993; 204: 231-235.
43. Heidary, F., Ahmadi, R. & Lotfi, A. The Effects of Cigarette or Hookah Smoking on Serum Levels of LH, FSH and Testosterone in Male Rats. International Conference on Medical, Biological and Pharmaceutical Sciences, 2012; 17-18.
44. Ahmed, MA, & Kurkar, A. Effects of opioid treatment on testcular functons in adult male rats: The role of nitric oxide and oxidatve stress. Clinical and Experimental Pharmacology and Physiology, 2014; 41: 317–323.
45. Azari, O., Emadi, L., Kheirandish, R., Shafei, B. H., Esmaili, N. M. R., & Faroghi, F. The effects of long-term administraton of tramadol on epididymal sperm quality and testcular tssue in mice. Iranian Journal of Veterinary Surgery, *2014; 9(1): 23–30.*