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

***Running title*: Panax ginseng, vitamin E, stress, nicotine, reproductive toxicity**

ABSTRACT

Exposure to nicotine (Nic) based product through smoking or other means to douse the effects of societal stress is on the increase among young male adults of reproductive age. Reports have it that either of nicotine or stress alters male reproductive functions. This study assessed the protective role of vitamin E (VE) and *panax ginseng (*PG*)* in alleviating the detrimental effect of Nic + Chronic stress on reproductive functions in 30 male Wistar rats weighing 150-200g. The rats were randomly divided into 5 groups of 6 rats each. Control (0.2ml of castor oil/day as drug vehicle), Nic (1.5mg/kg/day)+ Chronic stress (generator noise 90-120dB or open environment 8am to 4pm daily), Nic + Chronic stress + VE (100mg/kg/day), Nic + Chronic stress+ PG (500mg/kg/day) and Nic + Chronic stress + VE + PG daily. Drugs were administered orally for 28 days, after 7 days of acclimatization, while the stress groups were exposed to stressors from during the acclimatization. Nic + Chronic stress reduces sperm count, motility, rapid progressive forward movement and sperm viability compared to control, but increases percentage of sperm debris, non-motile sperm and slow progressive forward movement compared to control. Testosterone, luteinizing hormone and follicle stimulating hormone levels were reduced in Nic + Chronic stress compared to control. Testicular and epididymal tissues of Nic + Chronic stress rats were seriously impaired compared to control rats. PG and VE recovered the harmful changes in the assessed parameters and tissues compared to the untreated groups. PG and VE supplement appeared to attenuate the adverse effect induced by Nic + Chronic stress on male reproductive parameters.

Keywords: Vitamin E, sperm debris, *Panax ginseng,* Nicotine + Chronic stress

INTRODUCTION

Stress is the feeling of being under too much mental or emotional pressure. Pressure turns into stress when one feel unable to cope [1]. Many of life's demands can cause stress, and when one feel stressed, it can alter the physiological state of health of an individual. Stress is not an illness itself, but it can cause serious illness if it isn't addressed [2]. In coping with stress many adopt an unhealthy coping method one of which is smoking/exposure to nicotine based product. Stress could be as important a risk factor as smoking/exposure to nicotine based product [1]. Our society is a highly stressed one and there is no quick-fix cure for stress. However, there are simple things one can do to change the common life problems that can cause stress or make stress a problem [3].

Stress has been linked to decreased reproductive function particularly in the males [3, 4]. Nicotine use has also been associated with decreased reproductive function [3, 5, 6, 7]. Current statistics have shown that there is increased use of nicotine based products particularly smoking among the young reproductive population [4, 5]. In spite of the precarious economic situations in Nigeria and major parts of the globe there has been an increasing level of exposure to stress. To douse the effects of stress, and sometimes to be able to fit into some social class, young people (particularly males) of reproductive age take to use of nicotine containing products. These combined exposure to stress and nicotine has therefore been on the increase with the consequent possibility of decreased reproductive function [3, 6, 8].

Vitamin E is known to have great importance in reproductive physiology. In rats, it has been shown to help in the maintenance of seminiferous tubule epithelium, and its deficiency lead to degenerative changes with resultant sterility. In 1922, studies with rat models showed that, rats whose diet was devoid of vitamin E became sterile. Vitamin E is

known to be essential in protecting cellular health including sperm and egg [9]. Sinclair, [10] reported that some botanical medicines have ameliorative effect on sperm parameters.

Duke, [11] and Blumenthal, [12] observed that *Panax ginseng*, is a well-known adaptogen and a restorative herbal preparation. *PG* has been used in conditions such as infertility, liver disease, amnesia, colds, menopause, and erectile dysfunction [11, 12, 13]. *Panax ginseng* was reported to be effective in anti-stress and anti-aging activities [14], is also reported to increase libido and sexual satisfaction [15]. Kitts & Hu [16] observed that the anti-inflammatory and antineoplastic effect of ginseng is as a result of its antioxidants properties.

Therefore, it is very possible that vitamin E and or *panax ginseng* used singly or as a combination therapy would reverse the decreased fertility profile in rat models of stress and nicotine induced infertility. The results from this study would provide useful information to health providers on a possible means of reversing stress and nicotine induced infertility.

MATERIALS AND METHODS

Laboratory animals

Thirty (30) male albino Wistar rats weighing 150–220g were used for this study. The animals were purchased from the Department of Zoology, Benue State University, Markudi, Benue State, and housed 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. 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 protocol.

Experimental design and drug administration

The animals were randomly assigned into five groups of (n=6). Group 1 serve as control (normal rat chow and water), Group 2 as nicotine (1.5mg/kg/day) + chronic stress (generator noise 90-120dB or open environment 8am to 4pm daily), Group 3 as nicotine + stress + vitamin E (100mg/kg), Group 4 as nicotine + stress + *panax ginseng* (500mg/kg). Nicotine, vitamin E and *panax ginseng* supplement were purchased from Unipervit Pharmacy, Ikot Omin, Calabar, Nigeria. The different drugs were orally administered, once a day. The control group received normal rat chow with 0.2ml of castor oil orally daily, used as a vehicle. The animals in the stress induced groups were exposed to either of the two forms of stressors aforementioned as used in a recent study [3] 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 frequency of sound. 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, 3, 8]. The animal cages were kept in an open environment where the voice and footstep of people were heard, and which attracts the attentions of passers-by.

The experiment lasted for 4 weeks, 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 10 µl 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 [17].

Determination of epididymal sperm count

Assessment of epididymal sperm count was done using the method described by Wyrobek et al [18]. 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.

Assessment of sperm viability and morphology

Sperm viability was evaluated using the method described by Wyrobek et al. [18]. 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 by method of Wyrobek et al. [18].

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 enzyme-linked immuno-sorbent assay (ELISA) kit method as used by [19].

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. The Microsoft Excel 2010 and SPSS 16.0 softwares were

employed 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

Semen analysis

Table 1: Effect of *Panax Ginseng* and Vitamin E on the Sperm Motility Parameters in Nicotine and Stress Induced Reproductive Toxicity in Male Rats

GROUP	Sperm Motility (percent)	Non Motile Sperm (percent)	RPFM (percent)	SPFM (percent)	RM (percent)
Control	81.25 ± 3.15	17.50 ± 3.23	48.75 ± 3.75	12.00 ± 5.40	10.00 ± 2.04
Nic + Stress	35.76 ± 7.38*	64.25 ± 11.55*	25.00 ± 2.89*	41.12 ± 2.89*	15.00 ±2.89
Nic + Stress + VE	57.34 ± 7.89 *,[a]	39.25 ± 11.71*,[a]	34.68 ± 3.63*,[a]	33.00 ± 2.29*,[a]	5.75 ± 1.49*
Nic + Stress+ PG	52.56 ± 5.91*,[a]	33.75 ± 5.91*,[a]	41.25 ± 6.25*,[a]	33.75 ± 3.15*,[a]	11.25 ± 3.15
Nic + Stress+ PG+ VE	53.75 ± 7.47*,[a]	43.75 ± 11.43*,[a]	38.21± 3.54*,[a]	30.00 ± 4.08*,[a]	8.75 ± 2.39

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

Table 2: The Effect of *Panax Ginseng* and Vitamin E on the Sperm Status in Nicotine and Stress Induced Reproductive Toxicity in Male Rats

GROUP	Sperm Count (x 1 million cells/L)	Sperm Viability (percent)	Serm debris (percent)
Control	69.39 ± 8.79	82.50 ± 1.44	4.75 ± 0.25
Nic + Stress	22.15 ± 5.46*	32.56 ± 4.33*	16.25 ± 0.58
Nic + Stress + VE	40.00 ± 3.78*,[a]	53.56 ± 6.00*,[a]	9.89 ± 1.75
Nic + Stress+ PG	51.12 ± 3.63*,[a]	45.45 ± 7.07*,[a]	6.00 ± 0.00

Nic + Stress+ PG + VE	33.90 ± 7.14*,[a]	61.25 ± 8.26*,[a]	9.50 ± 0.50

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

Semen Characteristics

Sperm motility parameters

Motile sperm was significantly (P<0.05) decreased in nicotine + stress, nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + *panax ginseng* + vitamin E groups when compared to control (Table 1). Motile sperm was significantly (P<0.05) increased in nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + *panax ginseng* + vitamin E groups when compared to nicotine + stress (Fig.1). Rapid progressive forward movement (RPFM) follow similar trend as motile sperm (Fig. 3), while in (Fig. 2) non-motile sperm and (Fig. 4) slow progressive forward movement (SPFM) was significantly (P<0.05) increased in nicotine + stress, nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + *panax ginseng* + vitamin E groups when compared to control. Residual movement was significantly (P<0.05) reduced in stress + nicotine + vitamin E compared to nicotine + stress (Table 1).

Sperm count

Sperm count was significantly (P<0.05) reduced in nicotine + stress, nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + *panax ginseng* + vitamin E groups when compared to control (Table 2). Sperm count was significantly (P<0.05) increased in nicotine + stress, nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + *panax ginseng* + vitamin E groups when compared with nicotine + stress (Fig.6).

Viable sperm cells

Viable sperm cells was significantly (P<0.05) reduced in nicotine + stress, nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + *panax ginseng* + vitamin E groups when compared with control (Table 2). Sperm viability was significantly (P<0.05) increased in nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + *panax ginseng* + vitamin E groups compared to nicotine + stress (Fig.7).

Sperm debris

Sperm debris was significantly (P<0.05) increased in nicotine + stress, nicotine + stress + vitamin E and nicotine + stress + *panax ginseng* + vitamin E groups compared to control, but was not significantly different in nicotine + stress + *panax ginseng* treated group compared to control. Sperm debris was significantly (P<0.05) reduced in nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + *panax ginseng* + vitamin E groups compared to nicotine + stress groups (Fig.8).

Hormonal Profile:

Table 3: Effect of Vitamin E on Testosterone, LH and FSH in Nicotine and Stress Induced Reproductive Toxicity in Male Rats

GROUP	Testosterone (ng/mL)	LH (µ/mL)	FSH (ng/mL)
Control	4.48 ± 0.15	4.73 ± 0.05	1.00 ± 0.05
Nic + Stress	2.14 ± 0.06*	1.70 ± 0.06*	0.20 ± 0.01*
Nic + Stress + VE	3.28 ± 0.04*,[a]	2.85 ± 0.03*,[a]	0.42 ± 0.01*,[a]
Nic + Stress+ PG	3.20 ± 0.13*,[a]	2.63 ± 0.08*,[a]	0.38 ± 0.01*,[a]
Nic + Stress+ PG + VE	3.15 ± 0.06*,[a]	2.58 ± 0.11*,[a]	0.65 ± 0.02*,[a]

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

Testosterone concentration

The testosterone concentration was significantly (P<0.05) reduced in nicotine + stress, nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + vitamin E + *panax ginseng* treated group compared to control (Table 3). The testosterone concentration was significantly (P<0.05) increased in nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and stress + nicotine + vitamin E + *panax ginseng* treated group compared to nicotine + stress group respectively (Fig.9).

Luteinizing hormone concentration

The LH concentration was significantly (P<0.05) reduced in nicotine + stress, nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + vitamin E + *panax ginseng* treated group compared to control (Table 3). The LH concentration was significantly (P<0.05) increased in nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + vitamin E + *panax ginseng* treated group compared to nicotine + stress group respectively (Fig.10).

Follicle stimulating hormone concentration

The FSH concentration was significantly (P<0.05) reduced in nicotine + stress, nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and nicotine + stress + vitamin E + *panax ginseng* treated group compared to control (Table 3). The FSH concentration was significantly (P<0.05) increased in nicotine + stress + vitamin E, nicotine + stress + *panax ginseng* and stress + nicotine + vitamin E + *panax ginseng* treated group compared to nicotine + stress group respectively (Fig.11).

DISCUSSION

Several studies have reported the toxic effects of either of nicotine or stress on different facets of the male reproductive system. However, the toxic effect of both nicotine and stress

on male reproductive system is scarcely reported. Additionally, there are no reports showing whether singly or co-administration of *panax ginseng* and vitamin E can ameliorate the likely effects of both nicotine and stress on male reproductive system.

The changes in sperm motility, count, viability, RPFM, SPFM and sperm debris in nicotine + stress induced rats observed in this study is in agreement with earlier report of [3]. Sperm motility, sperm count, sperm viability and debris are important sperm parameter in determining male fertility. Although some studies [20, 21] described sperm debris as the best indicator, yet others observed that it is not an indicator [22] of male infertility. However, sperm debris of the ejaculated spermatozoa is still commonly employed as a measure of fertility during analysis of the seminal fluid [23]. The decreased in sperm motility, count, viability and RPFM is an indication of worsen infertility. Since spermatogenesis is a complex process involving various stages in the formation of mature spermatozoa, disruption at any stage would result in sperm debris (7, 8).

Low sperm counts are associated with reduced fertility because sperm from ejaculates with low counts often contain many sperm with poor motility and sperm debris. Studies have shown that Vitamin E supplementation improve both sperm quality and quantity [24, 25, 26, 27], while other studies reported that *panax ginseng* enhance male fertility [11, 12, 28, 29]. The beneficial effect of vitamin E is mostly due to its antioxidant potentials which plays a major protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals which are toxic by-products of many metabolic processes in biological membranes [24]. Kitts & Hu [16] mentioned that *ginseng* has powerful antioxidants properties.

In stress + nicotine induced rats the level of testosterone was significantly reduced when compared to either control or treated rats. Decrease level of testosterone is one of the indicators of chemical toxicity in male reproduction [30] as it is essential to maintain spermatogenesis. Low testosterone production adversely affects the quality of ejaculates and subsequent fertility of males.

In stress + nicotine induced rats the hormonal level of FSH and LH was significantly reduced when compared to control.

However, the primary site of FSH action is the epithelium of seminiferous tubule Sertoli cells [31]. Testosterone can bind to cytoplasmic receptors in these Sertoli cells and plays an essential role in spermatogenesis [31].

LH is the primary regulator of testosterone biosynthesis in Leydig cells [32].

Significant increase in serum testosterone and LH levels as well as the normalcy of FSH level were observed in treated rats supplemented with vitamin E and *panax ginseng*. Several studies have shown that vitamin E improve serum levels of testosterone, FSH and LH [9, 33, 34, 35].

It was reported [36, 37] 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. Damage to the testicular tissue particularly, seminiferous tubules may be responsible for the changes in sperm indices in stress + nicotine groups. Exposure to environmental and occupational toxicants may adversely affect reproductive potential of male during sperm development or epididymal storage. 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 the poor cellular morphology in the stress + nicotine treated groups. Several studies [38, 39] had reported degenerative changes in the testicular tissue following chronic exposure to stressors. Nicotine effects had been reported to cause decrease in testicular germ cells in rats [40]. It has also been reported to cause testicular degeneration in rats [41, 42]. Stress + nicotine induced rats could induce specific lesions during the development of spermatozoon, which may be the reason either directly or indirectly harmful to spermatogenesis.

Normal development of sperm plays an essential role in enabling reproductive capacity.

Studies have shown that supplementation of Vitamin E improve both sperm quality and quantity [24, 25, 26, 27, 43]. Vitamin E and *panax ginseng* alleviated the histo-pathological degenerations of the testes observed in stress + nicotine induced rats. Vitamin E is essential in maintaining the physiological integrity of testes, epididymis and accessory glands [25], which has vital role in spermatogenesis and sperm maturation consequently improving sperm quality and quantity.

CONCLUSION

The study showed that combined exposure to societal stressors and nicotine worsen male reproductive characteristics in rat compared to exposure of either of societal stressors and nicotine. *Panax ginseng* and vitamin E supplement restored their levels to an optimum rate. The report from this study will be (or prove) invaluable in the study of male infertility.

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Figures

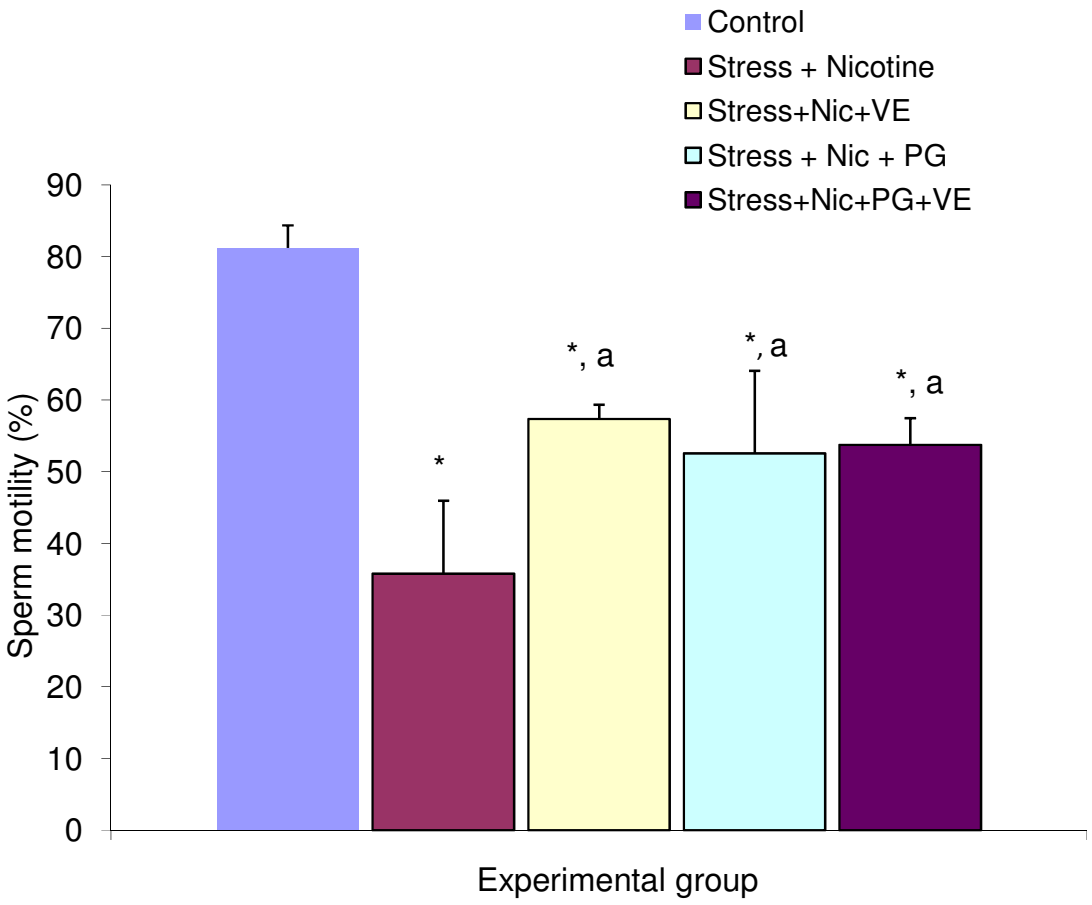


FIGURE 1: Comparison of sperm motility in combined stress + nicotine-induced infertility and Vitamin E + *Panax ginseng* treatment.

Values are expressed as mean ±SEM, n = 6.
* = significantly different from control at $p<0.05$
a = significantly different from stress + nicotine at $p<0.05$

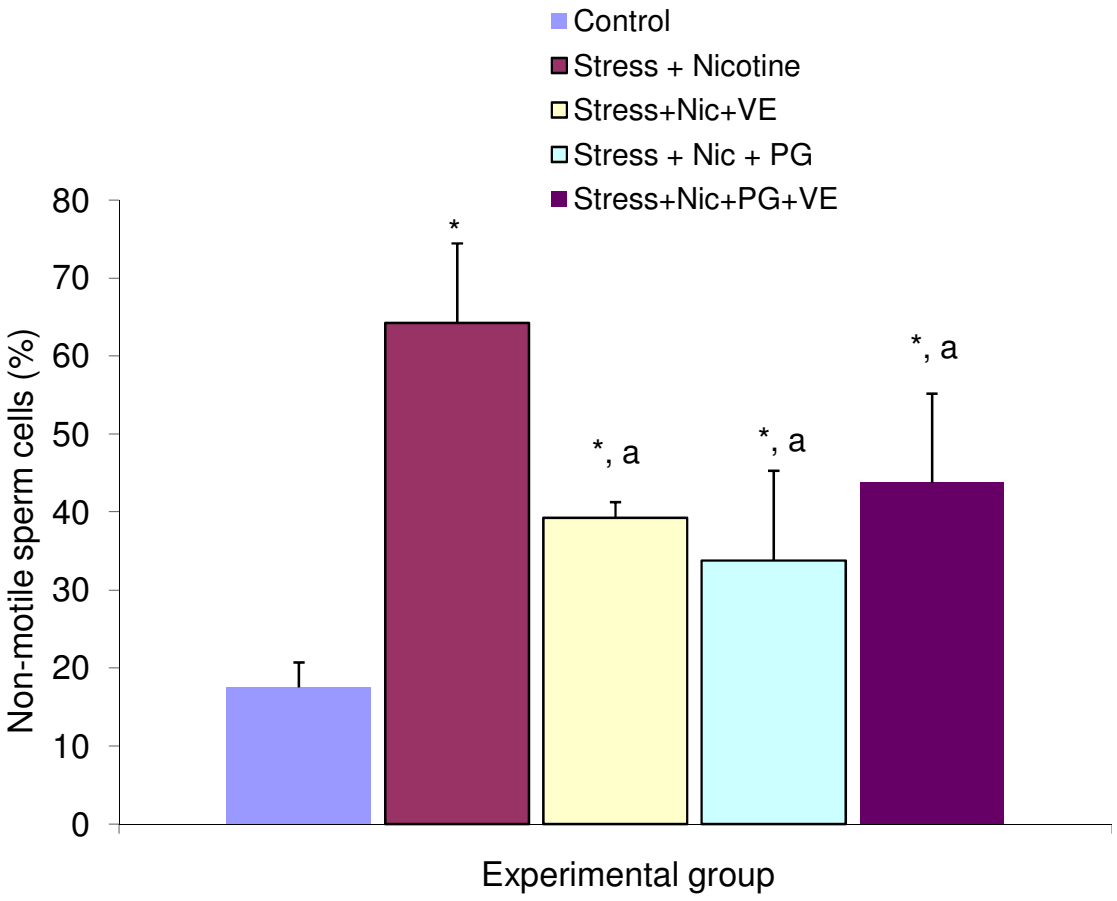


FIGURE 2: Comparison of non-motile sperm cells in combined stress + nicotine-induced infertility and Vit. E + PG treatment.

Values are expressed as mean ±SEM, n = 6.
* = significantly different from control at $p<0.05$
a = significantly different from stress +

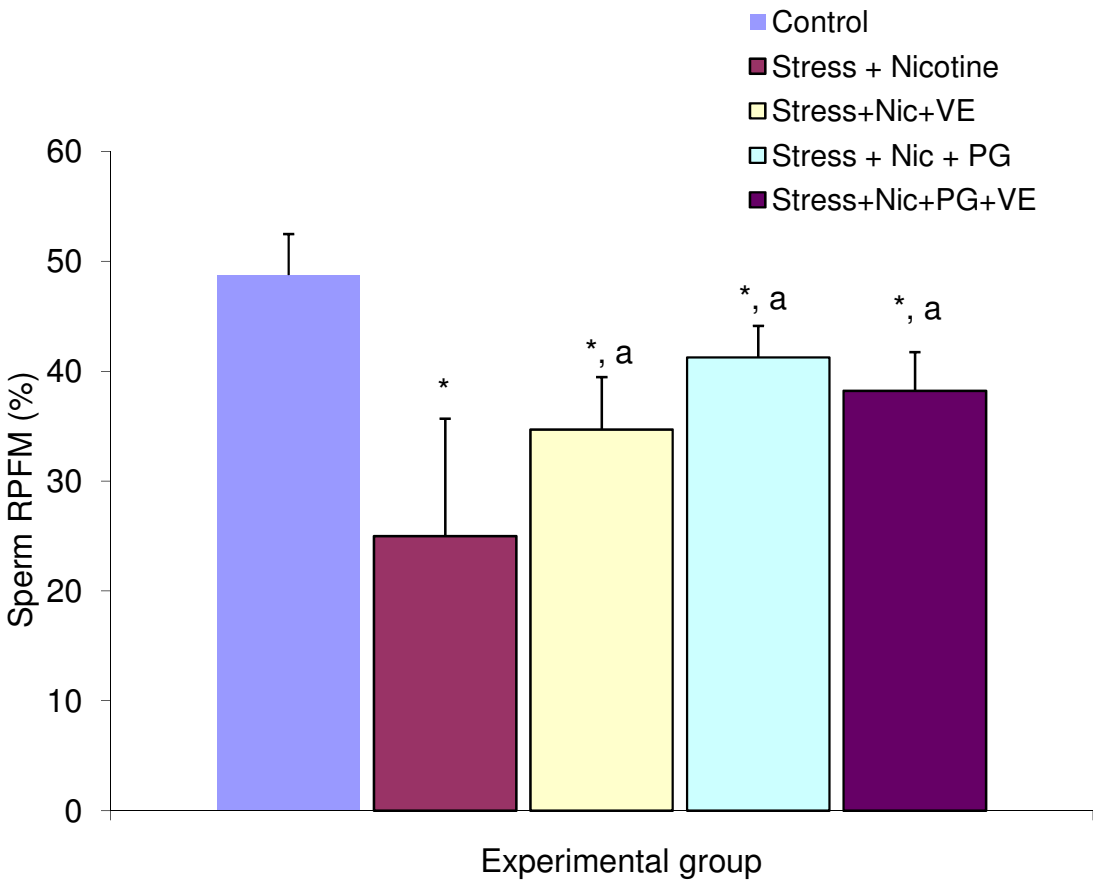


FIGURE 3: Comparison of sperm cells with rapid progressive forward movement in combined stress + nicotine-induced infertility and Vit. E + PG treatment.

Values are expressed as mean ±SEM, n = 6.
* = significantly different from control at $p<0.05$;
a = significantly different from nicotine + stress at $p<0.05$

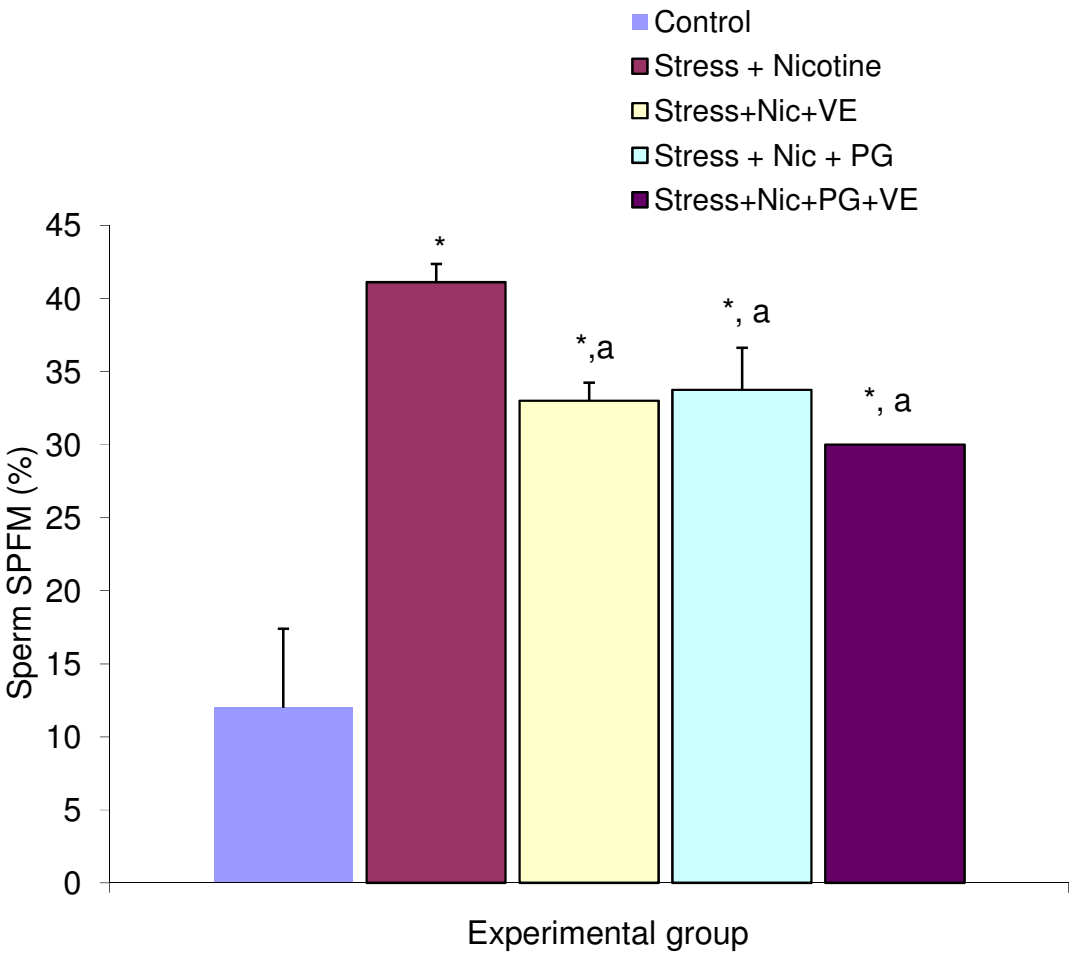


FIGURE 4: Comparison of sperm cells with slow progressive forward movement in chronic stress + nicotine-induced infertility and Vit. E + PG treatment.
Values are expressed as mean ±SEM, n = 6.
* = significantly different from control at P<0.05;
a = signific

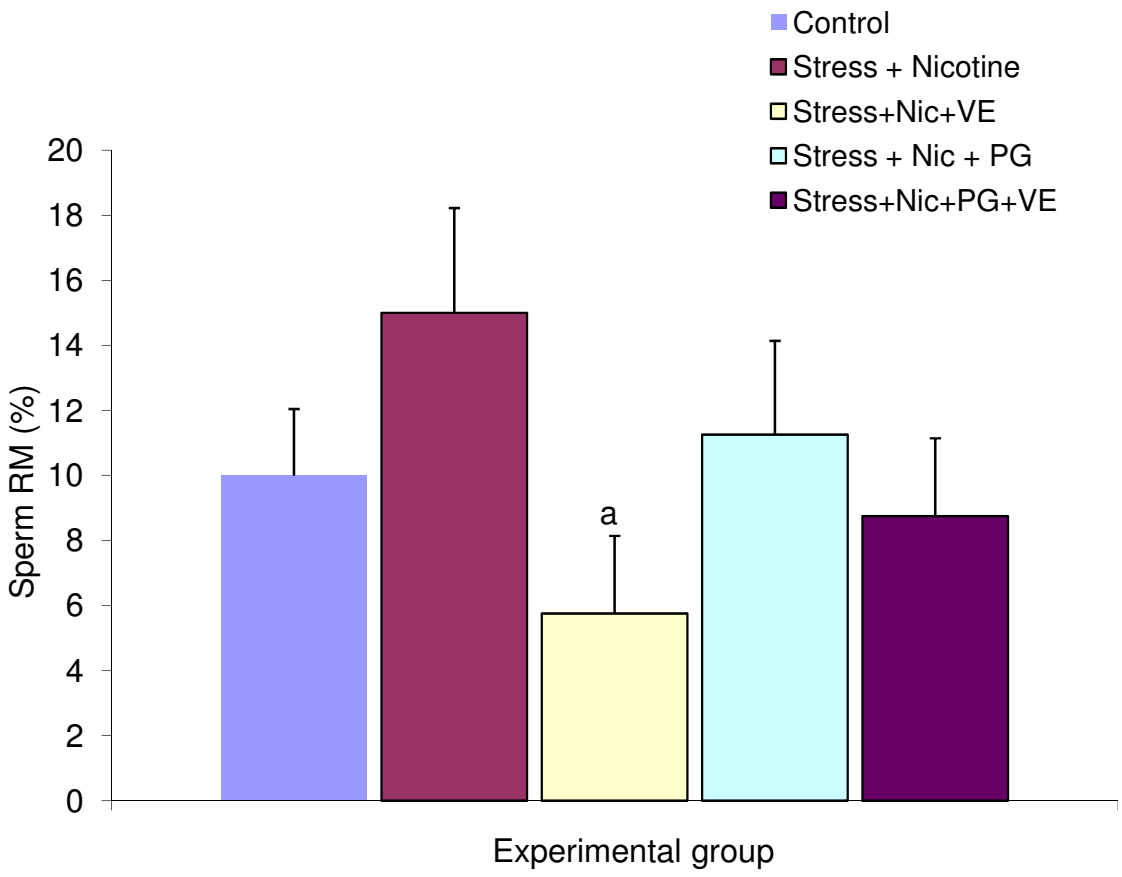


FIGURE 5: Comparison of sperm cells with residual movement in combined stress + nicotine-induced infertility and Vitamin E + PG treatment.

Values are expressed as mean ±SEM, n = 6.
a = significantly different from stress + nicotine at $p<0.05$

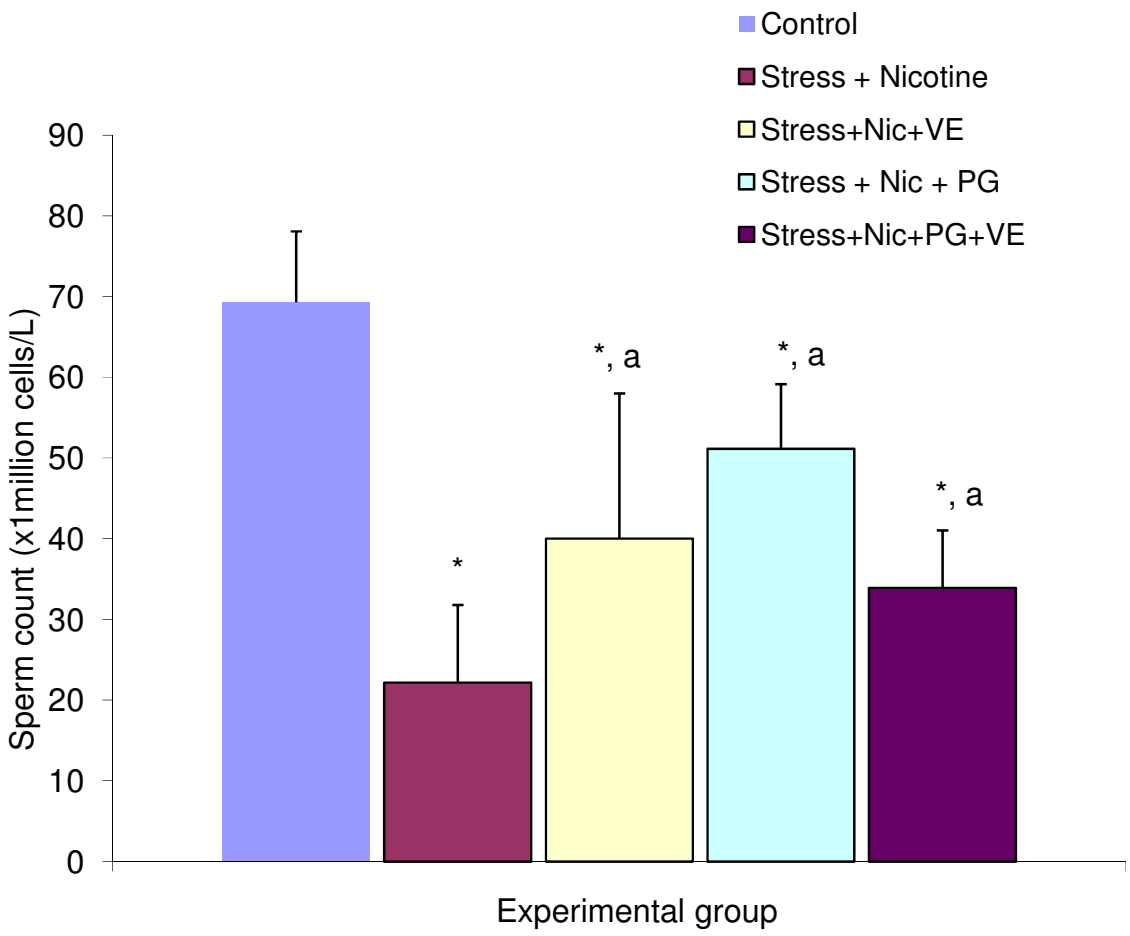


FIGURE 6: Comparison of sperm count in combined stress + nicotine-induced infertility and Vitamin E + *Panax ginseng* treatment.

Values are expressed as mean ±SEM, n = 6.
* = significantly different from control at $p<0.05$
a = significantly different from stress + nicotine at $p<0.05$

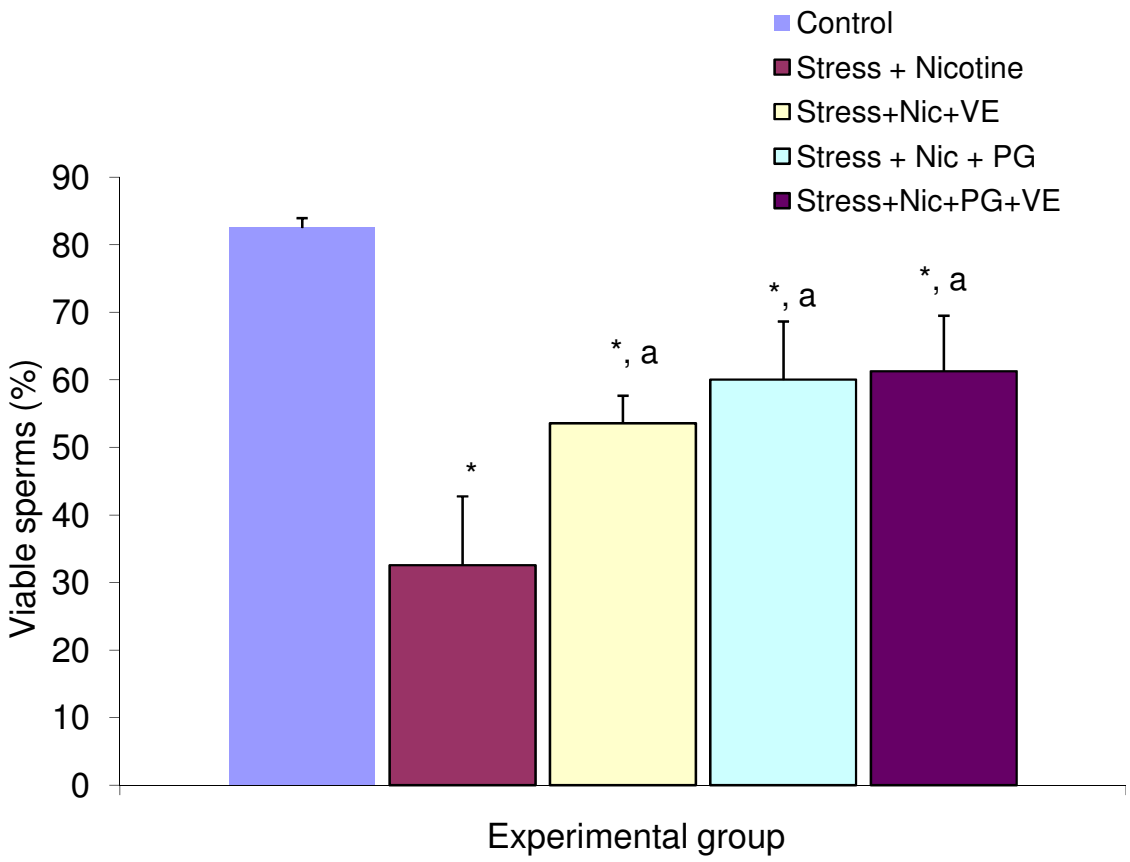


FIGURE 7: Comparison of viable sperm cells in combined stress + nicotine-induced infertility and Vitamin E + *Panax ginseng* treatment.
Values are expressed as mean ±SEM, n = 6.
* = significantly different from control at $p<0.05$
a = significantly different from stress + nicotine at $p<0.05$

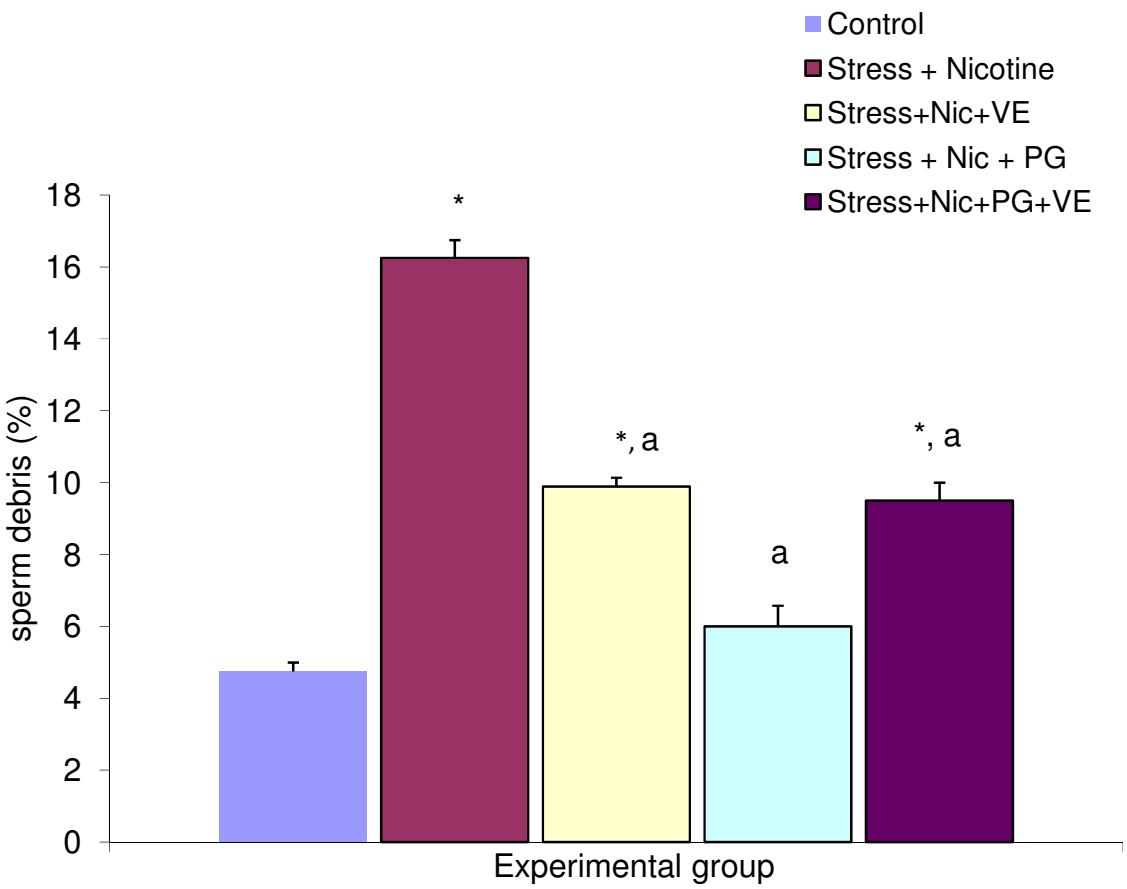


FIGURE 8: Comparison of sperm cells with total defect in combined stress + nicotine-induced infertility and Vitamin E + *panax ginseng* treatment.

Values are expressed as mean ±SEM, n = 6.
* = significantly different from control at $p<0.05$
a = significantly different from stress + nicotine at $p<0.05$

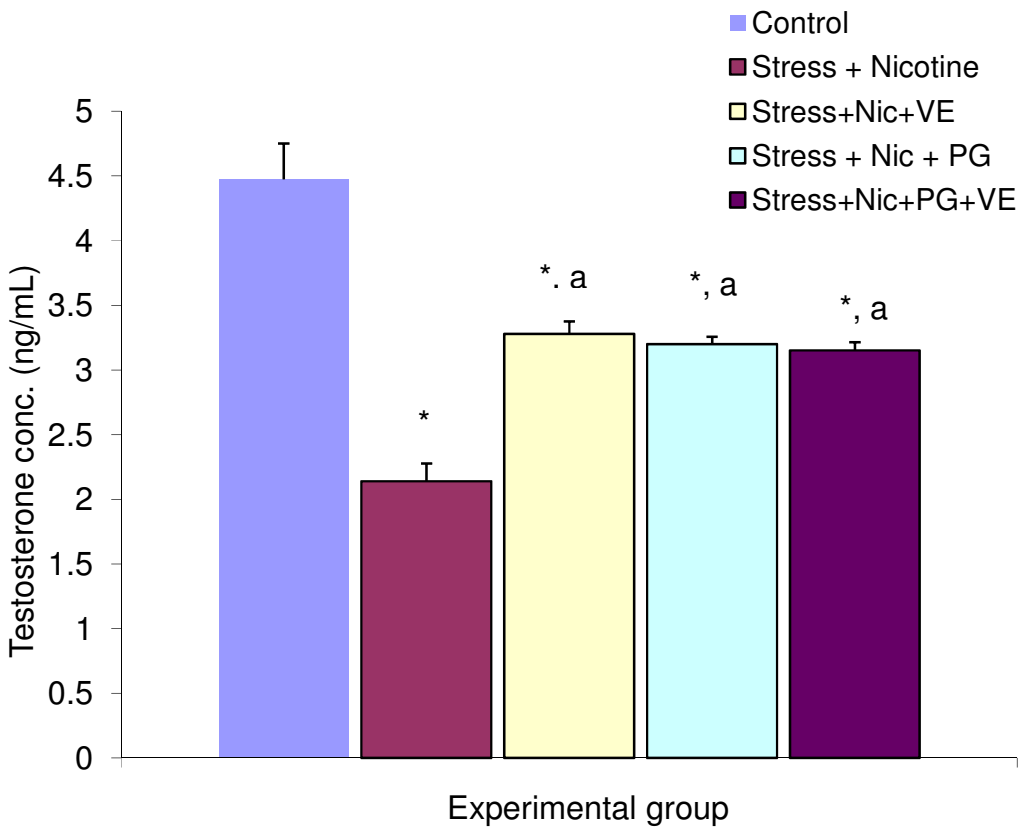


FIGURE 9: Comparison of testosterone concentration in combined stress + nicotine-induced infertility and Vitamin E + *panax ginseng* treatment.

Values are expressed as mean ±SEM, n = 6.
* = significantly different from control at $p<0.05$
a = significantly different from stress + nicotine at $p<0.05$

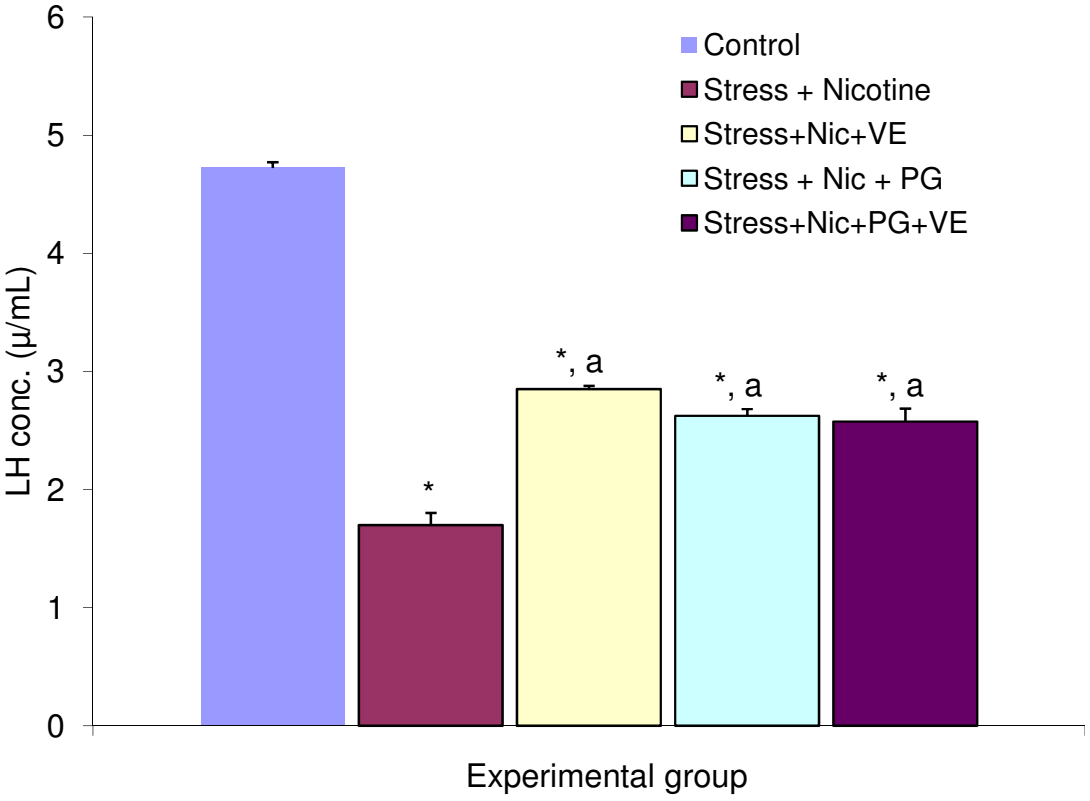


FIGURE 10: Comparison of luteinizing hormone concentration in combined stress + nicotine-induced infertility and Vitamin E + *panax ginseng* treatment.

Values are expressed as mean ±SEM, n = 6.
* = significantly different from control at $p<0.05$
a = significantly different from stress + nicotine at $p<0.05$

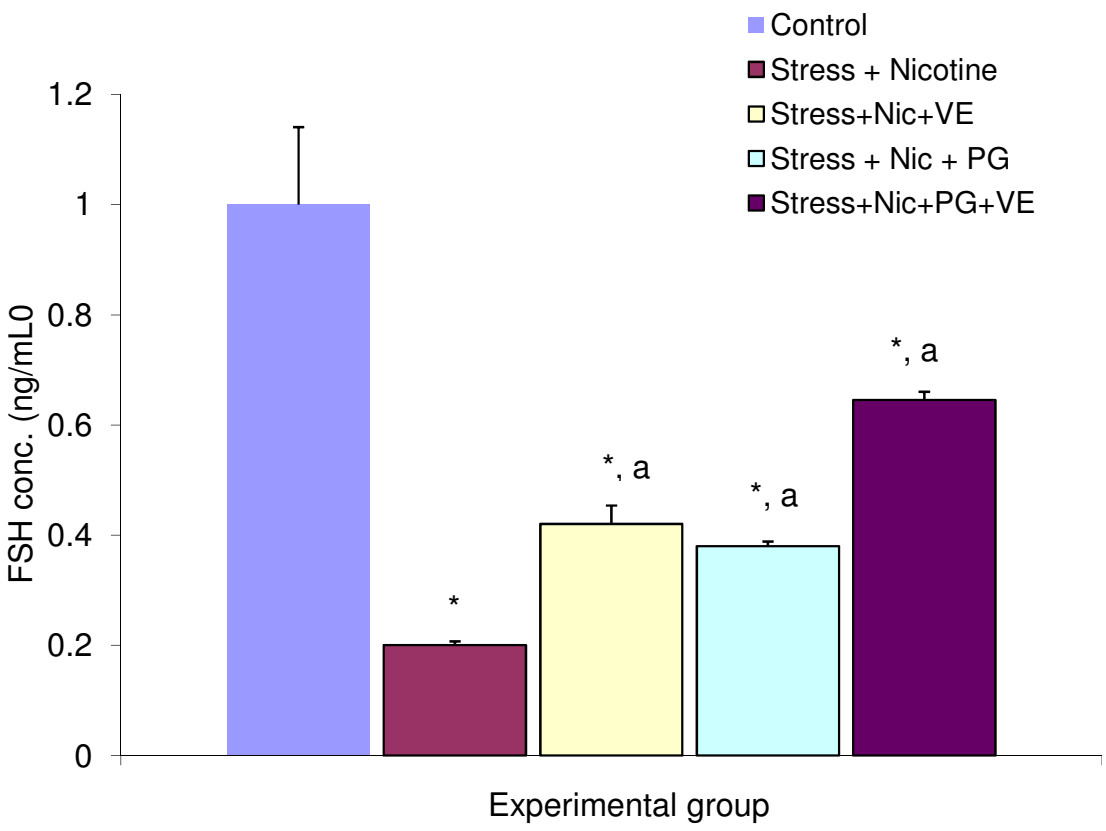


FIGURE 11: Comparison of follicle stimulating hormone concentration in combined stress + nicotine-induced infertility and Vitamin E + *panax ginseng* treatment.

Values are expressed as mean ±SEM, n = 6.
* = significantly different from control at $p<0.05$
a = significantly different from stress + nicotine at $p<0.05$

Testicular Histology

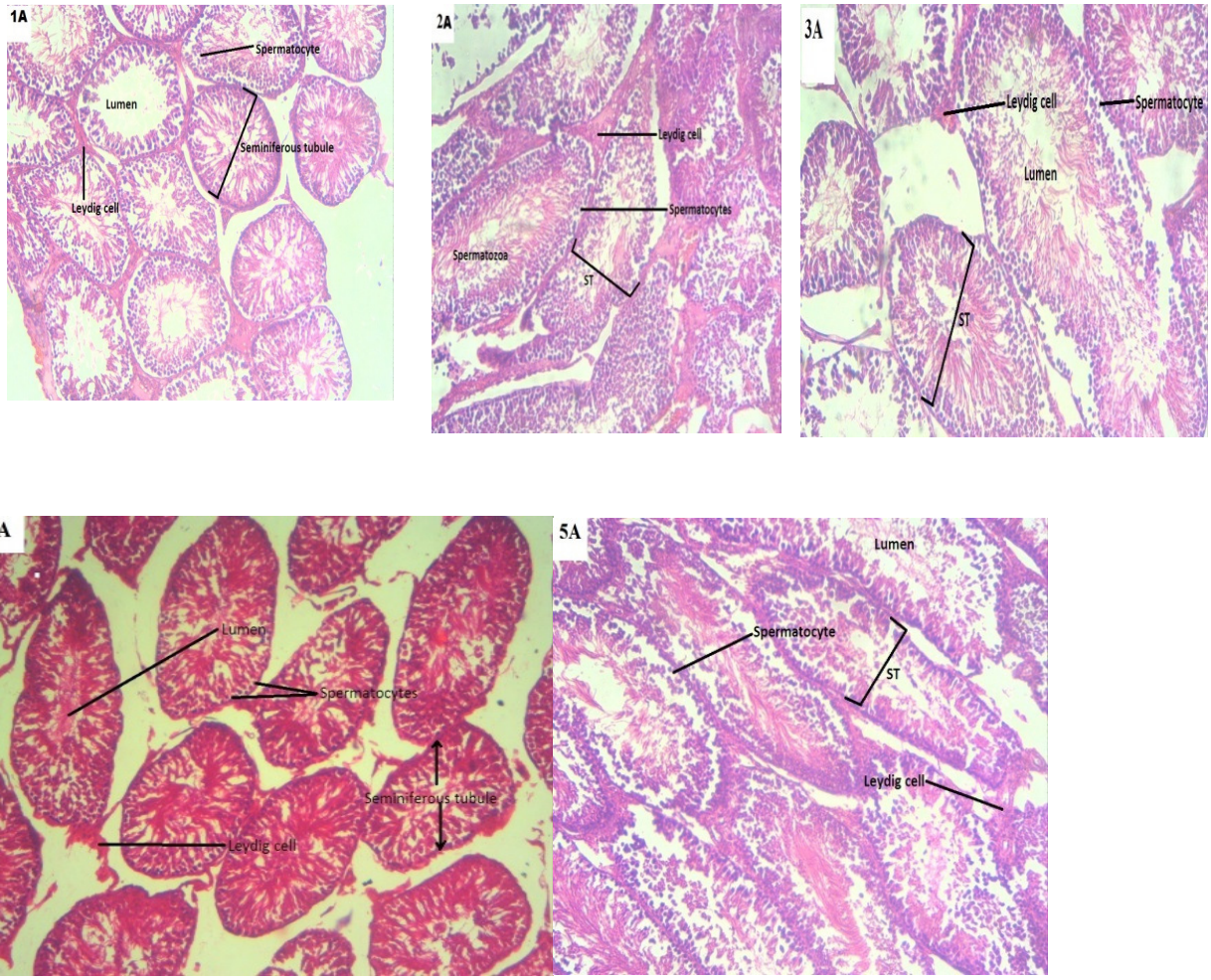


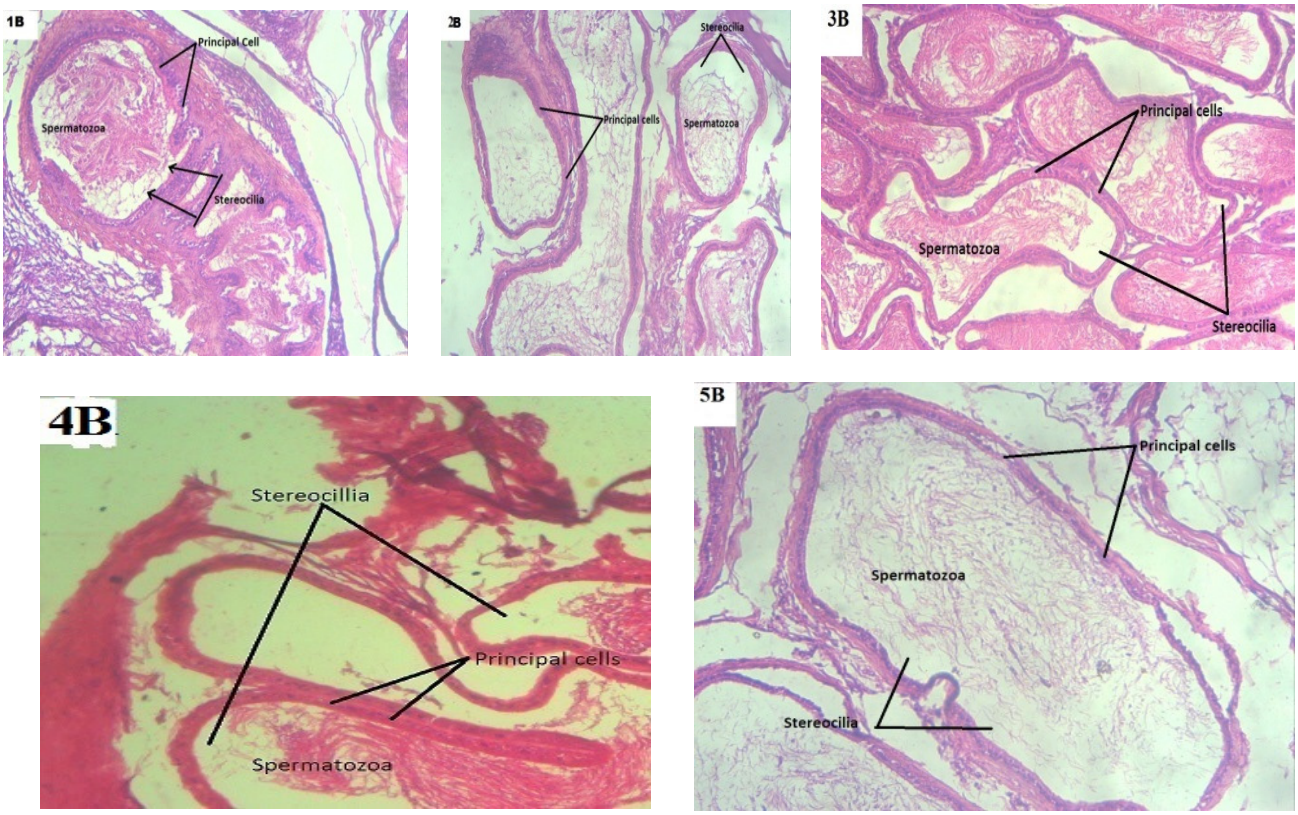
PLATE 3: Photomicrograph showing H and E stained testes of
Key:
(1A) Control group showing normal testes histo-architecture

(2A) Nicotine + stress group showing poorly cellular morphology.

(3A) Nicotine + stress **+** *panax ginseng* treated group showing some ST having adequate spermatocytes and spermatozoa while others having inadequate spermatocytes and spermatozoa. (4A) Nicotine + stress + vitamin E treated group showing numerous ST with adequate spermatocytes and spermatozoa with no visible distortion.

(5A) Nicotine + stress+ vitamin E + *panax ginseng* group showing slight reduction in the population of both spermatocyte and spermatozoa in the lumen

Epididymal Histology



Magnification: x100

PLATE 4: **Photomicrograph showing H and E stained epididymis**

Key:

(1B) Control group showing normal appearance of the general tissue structure

(2B) Stress and nicotine group showing noticeable inflammation of the principal cells.

(3B) Stress and nicotine with *panax ginseng* treated group showing heavy populated spermatozoa.

(4B) Stress and nicotine with vitamin E treated group showing no noticeable inflammation.

(5B) nicotine + stress + vitamin E + *panax ginseng* group showing no visible distortion of the general tissue structure.