

# Could Co-Administration of Panax Ginseng and Vitamin E be More Effective in Reversing Nicotine- and Chronic Stress- induced Reproductive Toxicity in Male Albino Wistar Rats?

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## ABSTRACT

Exposure to the nicotine-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 (Vit E) and *panax ginseng* (PG) in alleviating the detrimental effect of nicotine+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), nicotine (1.5mg/kg/day)+ Chronic stress (generator noise 90-120dB open environment 8am to 4pm daily), nicotine+ Chronic stress + Vit E (100mg/kg/day), nicotine+ Chronic stress+ PG (500mg/kg/day) and nicotine+ Chronic stress + Vit E + PG daily. Drugs were administered orally for 28 days, after 7 days of acclimatisation, while the stress groups were exposed to stressors from during the acclimatisation. Nicotine+ 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 nicotine+ Chronic stress compared to control. Testicular and epididymal tissues of nicotine+ Chronic stress rats were seriously impaired compared to control rats. PG and Vit E recovered the harmful changes in the assessed parameters and tissues compared to the untreated groups. PG and Vit E supplement appeared to attenuate the adverse effect induced by nicotine+ Chronic stress on male reproductive parameters.

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

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## 1. INTRODUCTION

Stress is the feeling of being under too much mental or emotional pressure. Pressure turns into stress when one feels unable to cope [1]. Many of life's demands can cause stress, and when one feels 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 is not addressed [2]. In coping with stress many adopt an unhealthy coping method one of which is smoking/exposure to the 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. This combined exposure to stress and nicotine has therefore been on the increase with the consequent possibility of decreased reproductive function [3, 6, 8].

Vit 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 leads to degenerative changes with resultant sterility. In 1922, studies with rat models showed that rats whose diet was devoid of Vit E became sterile. Vit E is known to be essential in protecting cellular health including sperm and egg [9]. Sinclair, [10] reported that some botanical medicines have an 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]. PG was reported to be effective in anti-stress and anti-ageing 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 vit E and or PG 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.

## 2. MATERIALS AND METHODS

### 2.1 Laboratory Animals

Thirty (30) male albino Wistar rats weighing 150–200g 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-hour 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 with protocol No. 017PY20216.

### 2.2 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 + vit E (100mg/kg), Group 4 as nicotine + stress + PG(500mg/kg). Nicotine, vit E and PG supplement were purchased from Unipervit Pharmacy, Ikot Omin, Calabar, Nigeria. The different drugs were orally administered, once a day. The control group received 0.2ml of castor oil orally which was used as a vehicle other drugs daily. 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. Majority of studies has it that scent materials and vocalisation 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. 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.

## 2.3 Assessment of Sperm Motility

Sperm motility was assessed by using 10 µl of sperm suspension collected from the left epididymis and examined using a light microscope (Leica DM 750, Switzerland) at 37°C temperature as described by Atashfaraz *et al.* [17].

## 2.4 Determination of Epididymal Sperm Count and Sperm Viability and Morphology

Assessment of epididymal sperm count was done using the method described by Wyrobek *et al.* [18]. Sperm viability was also evaluated using the method described by Wyrobek *et al.* [18].

## 2.5 Measurement of Serum Reproductive Hormones

Serum obtained following the method previously described in preceding sections was used for the reproductive hormonal assay. Serum testosterone, luteinizing hormone and follicle stimulating hormone concentrations were determined using the ELISA kit method as used by Khaki *et al.* [19].

## 2.6 Histological Studies

The testis and epididymis of the control and treated rats were removed and fixed in Bouin's fluid [0.2% picric acid/2% (v/v) formaldehyde in PBS]. Sections were obtained and stained with haematoxylin and eosin (H & E) stains. Photomicrographs were done using a light microscope (Leica DM 750, Switzerland) at magnifications of x100.

## 2.7 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 software were employed for the statistical analysis. Results were presented as means ± Standard Errors of means (SEM) and probability levels  $p < 0.05$  was accepted as significant.

## 3. RESULTS

### 3.1 Semen analysis

#### 3.1.1 Semen Characteristics

##### 3.1.1.1 Sperm motility parameters

Motile sperms were significantly ( $P < 0.05$ ) decreased in nicotine + stress, nicotine + stress + vit E, nicotine + stress + PG and nicotine + stress + PG + vit E groups when compared to control (Table 1). Motile sperms were significantly ( $P < 0.05$ ) increased in nicotine + stress + vit E, nicotine + stress + PG and nicotine + stress + PG + vit E groups when compared to nicotine + stress. Rapid progressive forward movement (RPFM) follow similar trend as motile sperm, while in non-motile sperm and slow progressive forward movement (SPFM) was significantly ( $P < 0.05$ ) increased in nicotine + stress, nicotine + stress + vit E, nicotine + stress + PG and nicotine + stress + PG + vit E groups when compared to control. The residual movement was significantly ( $P < 0.05$ ) reduced in stress + nicotine + vit E compared to nicotine + stress (Table 1).

### 3.2 Sperm Count

Sperm count was significantly ( $P < 0.05$ ) reduced in nicotine + stress, nicotine + stress + vit E, nicotine + stress + PG and nicotine + stress + PG + vit E groups when compared to control. Sperm count was significantly ( $P < 0.05$ ) increased in nicotine + stress, nicotine + stress + vit E, nicotine + stress + PG and nicotine + stress + PG + vit E groups when compared with nicotine + stress (Table 2).

### 3.3 Viable Sperm Cells

Viable sperm cells were significantly ( $P < 0.05$ ) reduced in nicotine + stress, nicotine + stress + vit E, nicotine + stress + PG and nicotine + stress + PG + vit E groups when compared with control (Table 2). Sperm viability was significantly ( $P < 0.05$ ) increased in nicotine + stress + vit E,

nicotine + stress +PG and nicotine +stress +PG + vit E groups compared to nicotine +stress.

### 3.4 Sperm Debris

Sperm debris was significantly ( $P<0.05$ ) increased in nicotine + stress, nicotine + stress + vit E and nicotine + stress + PG + vit E groups compared to control, but was not significantly different in nicotine + stress + PG treated group compared to control. Sperm debris was significantly ( $P<0.05$ ) reduced in nicotine + stress + vit E, nicotine + stress + PG and nicotine + stress + PG + vit E groups compared to nicotine + stress groups.

### 3.5 Hormonal Profile

#### 3.5.1 Testosterone concentration

The testosterone concentration was significantly ( $P<0.05$ ) reduced in nicotine + stress, nicotine + stress + vit E, nicotine + stress + PG and nicotine +stress + vit E + PGtreated group compared to control (Table 3). The testosterone concentration was significantly ( $P<0.05$ ) increased in nicotine + stress + vit E, nicotine + stress + PGand stress + nicotine + vit E + PGtreated group compared to nicotine + stress group respectively.

#### 3.5.2 Luteinizing hormone concentration

The LH concentration was significantly ( $P<0.05$ ) reduced in nicotine + stress, nicotine + stress + vit E, nicotine + stress + PGand nicotine +stress + vit E + PGtreated group compared to control (Table 3). The LH concentration was significantly ( $P<0.05$ ) increased in nicotine + stress + vit E, nicotine + stress + PGand nicotine +stress +vit E + PGtreated group compared to nicotine + stress group respectively.

#### 3.5.3 Follicle stimulating hormone concentration

The FSH concentration was significantly ( $P<0.05$ ) reduced in nicotine + stress, nicotine + stress + vit E, nicotine + stress + PG and nicotine +stress + vit E + PGtreated group compared to control. The FSH concentration was significantly ( $P<0.05$ ) increased in nicotine + stress + vit E, nicotine + stress + PGand stress + nicotine + vit E + PGtreated group compared to nicotine + stress group respectively (Table 3).

### 3.6 Testicular Histology

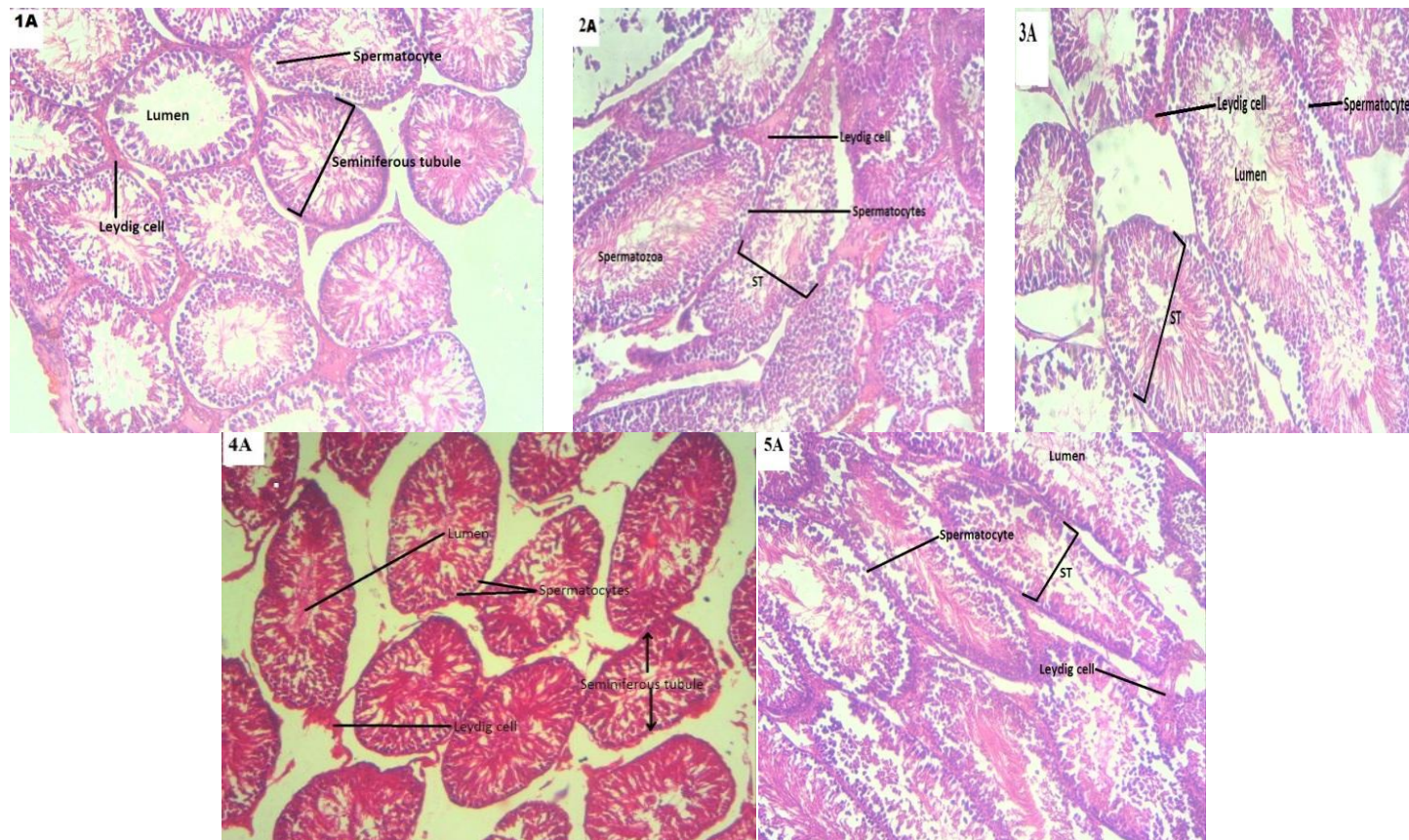
Plate A shows photomicrographs of the testis following the different treatments of *Panax ginseng* and vitamin E.

### 3.7 Epididymal Histology

Plate B shows the photomicrographs of the epididymis. It shows Stress and nicotine group with noticeable inflammation of the principal cells, while treatment with panax ginseng presents heavy populated spermatozoa.

## 4. DISCUSSION

Several studies have reported the toxic effects of either nicotine or stress on different facets of the male reproductive system. However, the toxic effect of both nicotine and stress on the male reproductive system is scarcely reported. Additionally, there are no reports showing whether singly or co-administration of PG and Vit E can ameliorate the likely effects of both nicotine and stress on the male reproductive system.



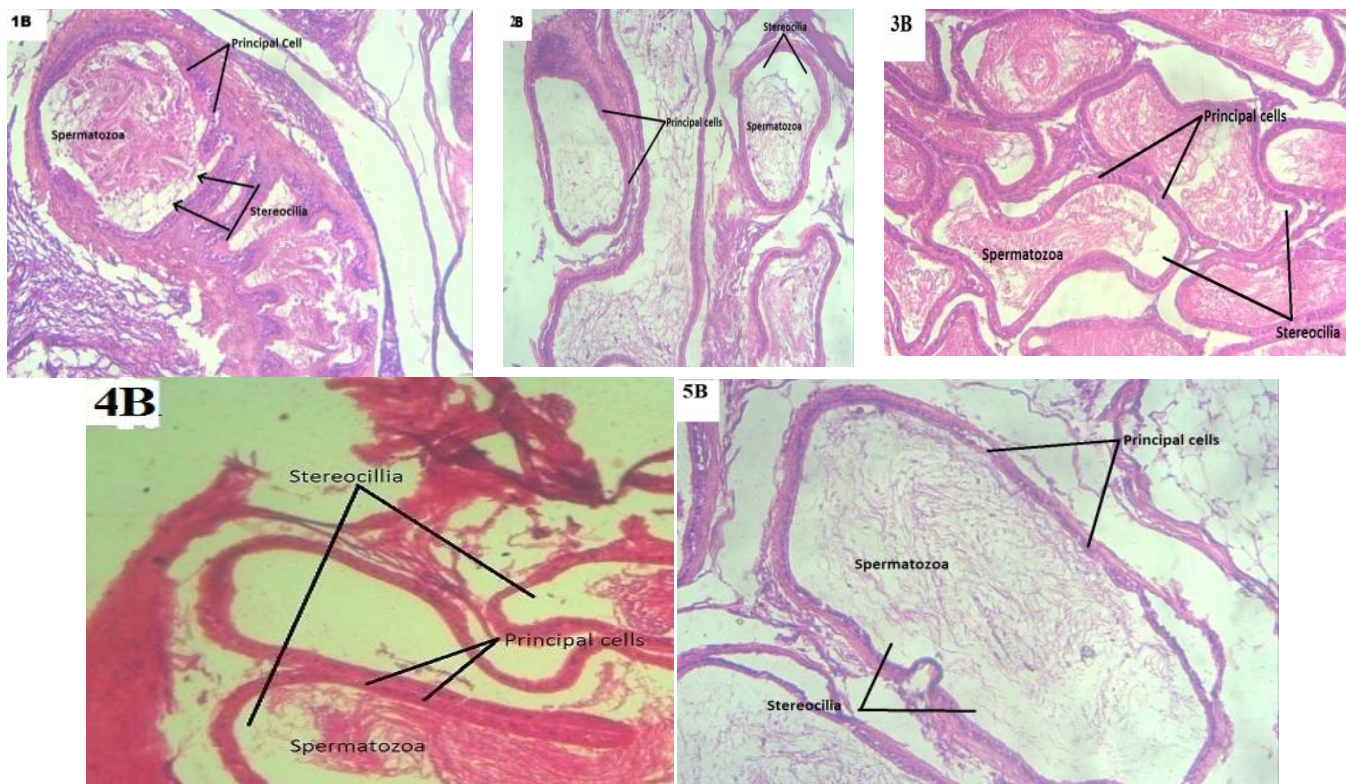
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#### Plate A. Photomicrograph showing H and E stained testes of

*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 a slight reduction in the population of both spermatocyte and spermatozoa in the lumen*





Magnification: x100

#### Plate B. 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.

**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
Nicotine + Stress	35.76 ± 7.38*	64.25 ± 11.55*	25.00 ± 2.89*	41.12 ± 2.89*	15.00 ± 2.89
Nicotine + Stress + Vit E	57.34 ± 7.89*, <sup>a</sup>	39.25 ± 11.71*, <sup>a</sup>	34.68 ± 3.63*, <sup>a</sup>	33.00 ± 2.29*, <sup>a</sup>	5.75 ± 1.49*
Nicotine + Stress+ PG	52.56 ± 5.91*, <sup>a</sup>	33.75 ± 5.91*, <sup>a</sup>	41.25 ± 6.25*, <sup>a</sup>	33.75 ± 3.15*, <sup>a</sup>	11.25 ± 3.15
Nicotine + Stress+ PG+ Vit E	53.75 ± 7.47*, <sup>a</sup>	43.75 ± 11.43*, <sup>a</sup>	38.21 ± 3.54*, <sup>a</sup>	30.00 ± 4.08*, <sup>a</sup>	8.75 ± 2.39

Data expressed as Mean ± SEM (n= 6 per treatment). \*- significantly different from control at  $p < 0.05$ ; <sup>a</sup> – significantly different from nicotine + stress 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)	Sperm debris (percent)
Control	69.39 ± 8.79	82.50 ± 1.44	4.75 ± 0.25
Nicotine + Stress	22.15 ± 5.46*	32.56 ± 4.33*	16.25 ± 0.58
Nic + Stress + Vit E	40.00 ± 3.78*, <sup>a</sup>	53.56 ± 6.00*, <sup>a</sup>	9.89 ± 1.75
Nicotine + Stress+ PG	51.12 ± 3.63*, <sup>a</sup>	45.45 ± 7.07*, <sup>a</sup>	6.00 ± 0.00
Nicotine + Stress+ PG + Vit E	33.90 ± 7.14*, <sup>a</sup>	61.25 ± 8.26*, <sup>a</sup>	9.50 ± 0.50

Data expressed as Mean ± SEM (n= 6 per treatment). \*- significantly different from control at  $p < 0.05$ ; <sup>a</sup> – significantly different from nicotine + stress at  $P < 0.05$

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
Nicotine + Stress	2.14 ± 0.06*	1.70 ± 0.06*	0.20 ± 0.01*
Nicotine + Stress + Vit E	3.28 ± 0.04*, <sup>a</sup>	2.85 ± 0.03*, <sup>a</sup>	0.42 ± 0.01*, <sup>a</sup>
Nicotine + Stress+ PG	3.20 ± 0.13*, <sup>a</sup>	2.63 ± 0.08*, <sup>a</sup>	0.38 ± 0.01*, <sup>a</sup>
Nicotine + Stress+ PG+ Vit E	3.15 ± 0.06*, <sup>a</sup>	2.58 ± 0.11*, <sup>a</sup>	0.65 ± 0.02*, <sup>a,b,c</sup>

Data expressed as Mean ± SEM (n= 6 per treatment). \*- significantly different from control at  $p < 0.05$ ; <sup>a</sup> – significantly different from nicotine + stress at  $P < 0.05$ ; <sup>b</sup> – significantly different from nicotine+stress+vit E; <sup>c</sup> – significantly different from Nicotine + stress +PG at  $p < 0.05$ .

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 Uko *et al.* [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 of male infertility [22]. 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 worsening 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 contains much sperms with poor motility and sperm debris. Some studies have shown that vit E supplementation improves both sperm quality and quantity [24, 25, 26, 27], while other studies reported that *PG* 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.

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]. A significant increase in serum testosterone and LH levels, as well as the

normalcy of FSH level, were observed in treated rats supplemented with Vit E and *PG*. Several studies have shown that vit E improve serum levels of testosterone, FSH and LH [9, 33, 34, 35]. This research is also in agreement with earlier reports that *PG* extract showed an increase in serum testosterone, FSH and LH levels [36, 37].

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 disorganisation of Leydig's cells. Furthermore, the alteration in the levels of FSH and LH may be responsible for 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 a 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 vit E improves both sperm quality and quantity [24, 25, 26, 27, 43]. Vit E and *PG* alleviated the histopathological degenerations of the testes observed in stress + nicotine induced rats. Vit E is essential in maintaining the physiological integrity of testes, epididymis and accessory glands [25], which has a vital role in spermatogenesis and sperm maturation consequently improving sperm quality and quantity. Our results in the study confirmed this reports.

When the treatment with only *PG* was compared with only vitamin E, there did not seem to be any difference between the efficacy of either of them in reversing the damage done by stress and nicotine on the reproductive parameters. The combination of *PG* and vitamin E did not seem to be significantly better than the individual treatments even though there was a trend towards a better performance. This trend was



clearly observed in the concentration of FSH which was higher in the combined treatment of vitamin E and PG when compared to either of PG and vitamin E.

## 5. CONCLUSION

The study showed that combined exposure to societal stressors and nicotine worsen male reproductive characteristics in rat compared to the exposure of either of societal stressors and nicotine. *Panax ginseng* and vitamin E supplement restored their levels to an optimum rate. As a combination, these drugs may be more efficient in preventing damage to the reproductive system due to exposure to stress and nicotine.

## ETHICAL APPROVAL

The animal ethics committee of the University of Calabar graciously approved our study protocol with protocol No. 017PY20216.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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