AMELIORATIVE EFFECTS OF SELENIUM AND VITAMIN C ON NICOTINE- INDUCED HEMATOTOXICITY, OXIDATIVE DAMAGE, HISTOPATHOLOGICAL CHANGES AND REPRODUCTIVE TOXICITY IN MALE ALBINO RATS

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ABSTRACT

Background: A significant public health issue is the abuse of nicotine through cigarette smoking. Free radical generation and oxidative stress are caused by nicotine. A micronutrient and element found in trace amounts in all living things is selenium. Ascorbic acid, a water-soluble antioxidant with multiple uses, is a form of vitamin C. It plays a significant function in the brain, nervous system, and immunological system, aids in boosting the immune system, and prevents various diseases**. Aim of work:** In the current study adult male albino rats were used as experimental animals to determine the hemo-toxicity, hormonal toxicity, reproductive system toxicity, and oxidative damage caused by nicotine. Selenium and vitamin C were also evaluated for their potential protective benefits. **Material and methods:** This work was performed on 42 adult male albino rats weighing between 140-200 mg. The animals were divided into 7 groups, each containing 6 animals. All groups were treated daily, for 4 weeks. The Study Design was a distributed as: Negative control group, Positive control group, Nicotine group, Selenium group, Vitamin C group, Nicotine with Selenium group and finally Nicotine with Vitamin C group**. Results:** Our findings clearly showed that oral nicotine treatment caused harmful side effects in the testes, including hormonal alterations, hematological damage, and toxic consequences. These negative effects may be related to the development of oxidative stress. Nicotine administration combined with selenium and vitamin C provided protection from the harmful effects of nicotine.

Key words: Nicotine, Selenium, Vitamin C, Oxidative process, and toxicity.

1. INTRODUCTION

Tobacco is the second most used psychoactive substance worldwide ***(Le Foll et al., 2022)***. Tobacco products are very addictive, partly because they contain nicotine, which is reinforcing, but also because they include appealing aromas and tastes named as flavour additives. Flavour additives are such sensory stimuli which enhance attractiveness. It is now considered to be one of the most insidiously addicting substances ***(Tannous et al., 2021).***

One of the most popular licit medications is Nicotine ***(Carstens and Carstens, 2021).*** Because most nicotine users quickly become tolerant of it and experience unusually enduring cravings for it when trying to stop, nicotine is currently regarded as one of the chemicals that causes addiction in the most subtle ways. Free radical generation and oxidative stress are caused by nicotine. Nicotine-induced oxidative stress is a risk for smokers and others who are exposed to cigarette smoke through breathing the air in the same setting ***(Leventhal, et al., 2023).***

A micronutrient element, Selenium is present in living things in trace amounts. Based on the dose and species considered, its qualities range from essentiality to toxicity. Because of these factors, its significance to human health is still up for debate ***(Vinceti et al., 2021).*** Both pro- and antioxidant properties are present. Via the direct oxidation of thiol groups and the indirect production of reactive oxygen species, selenium may negatively impact cellular redox state (ROS) ***(Urbano, et al., 2023).***

An essential dietary antioxidant, Vitamin C considerably reduces the harmful effects of ROS produced in cells. Many biochemical, clinical, and epidemiological investigations have suggested that vitamin C may be helpful in treating chronic illnesses like cancer, cataract, and cardiovascular disease, probably through antioxidant mechanisms ***(Chen et al. ,2020).*** Via its ability to decrease -tocopherol and keep this antioxidant in an active state, vitamin C also helps to enhance spermatogenesis, at least in part. The testes have an abundance of a Glutathione-dependent dehydroascorbate reductase, which keeps vitamin C itself in a reduced condition. Vitamin C deficiency caused the testes to experience oxidative stress, which impairs both spermatogenesis and the generation of testosterone ***(Yoo et al., 2020).***

2.AIM OF THE WORK

The purpose of this study was to examine the harmful effects of nicotine and assess the contribution of Selenium and Vitamin C to prevent hemotoxicity, hormone toxicity, reproductive system toxicity, and oxidative damage in adult male albino rats.

2. MATERIALS AND METHODS

The Research Ethics Committee at the Faculty of Medicine, Benha University (REC-FOMBU), Egypt, gave its approval to the experimental design study with approval number MD 4.10.2020.

3.1 Animals

A 42 healthy adult male albino rats were used in this investigation with an average body weight of between 140 and 200 gm.

Before the experiment began, all the animals at the animal bread house in the Benha Faculty of Veterinary Medicine underwent a week of passive preliminaries (consuming food and water without any drugs) to ensure their physical well-being and to weed out any ill animals. All the animals received the same food (Wheat, Bread & Milk). Medication administration for all animals was planned to start at noon. The animals were given ether anesthesia before being put to death 24 hours following the last treatment.

3.2 Chemicals

All medications, reagents, and chemicals of analytical grade or higher purity utilised in this research study were purchased from Sigma Chemical Co. through EICI and HIMEDIA lab chemicals & biochemicals. ***a. Nicotine:*** We used the chitosan polymer nicotine hydrogen tartrate (NHT) is biodegradable as Nicotine is less stable than NHT. ***b. Selenium (Se):*** Selenium was in the form of sodium selenite ***c. Vitamin C:*** Vitamin C (100%) purity was in the form of L- ascorbic acid available in powdered crystalline solids with white color and molecular weight 176.13 g/mole and specific gravity 1.65.

3.3 Duration of the study

All groups were treated daily, for 4 weeks.

3.4 Grouping and experimental design

**The animals were divided into 7 groups, each of which had six rats:*

1- ***Negative control group:*** A standard protein meal of 18% casein, 70% carbohydrates, 7% fat, 4% salt mixture, and 1% vitamin mixture was fed to the rats.

2- ***Positive control group (Solvent group):*** These rats were injected intraperitoneal by

0.9% normal saline water by dose (2.5 mg/kg body weight) for 4 weeks.

3- ***Nicotine group:*** These rats were fed with normal protein diet and treated with effective dose of nicotine hydrogen tartrate salt (2.5 mg/kg body weight) for 4 weeks. This dose was dissolved in 0.9% saline water with concentration (1:1) and was administrated via the intra-peritoneal route according to ***(Madiseh et al., 2020)***. The selected dose is equivalent to the amount of nicotine passing to the blood of the heavy smoker ***(Aydos et al., 2001)***. Also, the dose corresponds to the intake range of habitual smokers ***(Valenca et al., 2004).***

4- ***Selenium group:*** These rats were fed with normal protein diet and treated with effective dose of sodium selenite (3 mg/kg body weight) for 4 weeks. Selenium was orally dissolved in distilled water with concentration (1:1) according to ***Liu et al., (2012).*** The form and the dose of selenium was based upon the fact that increasing the dose by more than 3 mg/kg body weight was eliminated unabsorbed in the urine. Also, the form of selenium administered affects the extent of the urinary excretion, as it appeared that the other selenium form than sodium selenite is somewhat more eliminated un-absorbed in urine ***(Lippman et al., 2005).***

5- ***Vitamin C group:*** These rats were fed with normal protein diet and treated with effective dose of Vitamin C in the form of L- ascorbic acid. The recommended dose of vitamin C for rats varies depending on several factors, including the rat's age, weight, and overall health. Generally, the safe dose of vitamin C for rats is about 10-200 mg/kg body weight per day. It is also important to note that administering too little vitamin C can lead to inefficient results while high doses of vitamin C may cause gastrointestinal disturbances as diarrhea in rats. So, it was given in a dose of 27 mg/rat/day orally dissolved in distilled water with concentration (1:1) according to ***Autifi et al., (2018)*** study.

6- ***Nicotine and Selenium group:*** These rats were given a regular protein diet along with a treatment of nicotine (2.5 mg/kg body weight) and sodium selenite (3 mg/kg body weight) over the course of four weeks.

7- ***Nicotine and Vitamin C group:*** These rats were given a normal protein diet and treated with an effective dose of nicotine hydrogen tartrate salt (2.5 mg/kg body weight). Next, an effective dose of Vitamin C in the form of L-ascorbic acid was added as a supplement at a dose of 27 mg per rat per day, orally dissolved in distilled water with a concentration of (1:1), for a period of four weeks.

3.5 Parameters of the study:

I. ***Body weight and relative weight of testis:*** The body weights at the start and the end were recorded. After the experiment, the testes of rats were removed, stripped of fatty tissues and blood vessels, blotted, and their weights were recorded.

II. ***Reproductive abnormality (Semen analysis):***

- **a. Sperm motility:** The approach described by was used to explore the development of sperm motility ***(Mosbah, et al., 2015).***
- **b. Sperm livability:** Sperm viability was evaluated, and the percentage was computed in accordance with ***(Oyeyemi et al., 2011).***
- **c. Sperm count and concentration:** This was done using the method described by ***(Bearden and Fuquay, 1984).***
- **d. Sperm abnormalities**: This was recorded according to ***Evans and Maxwell (1987).***

III. ***Biochemical study for hormonal analysis*:** 5 ml syringes were used to draw blood samples from their hearts for estimate (FSH, LH and testosterone). Using enzyme-linked immuno-sorbent assay (ELISA) kits, the serum and organs' hormonal concentrations will be evaluated ***(Picard et al., 2008).*** and

will be determined using VIDAS equipment (BIOMERIEUX Company, France). The obtained sera were kept in storage so that ELISA kits could be used to measure the hormone levels.

IV. ***Hematological analysis:*** Hematocytometry and spectrophotometry will be used to manually measure the number of red and white blood cells, hematocrit, and hemoglobin levels.

V. ***Biochemical study for oxidative stress markers:*** Superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde are examples of antioxidant enzymes (MDA). The parameters of oxidative stress will be examined using Spectro-nanodrop. measurement of the levels of SOD, MDA, CAT, and GSH in testicular tissue.

VI. ***Histopathological study by light microscope:***

Feldman and Wolfe (2014) state that a specimen from each animal's right testis will be taken and placed into aqueous Bouin's solution. Tissues were dehydrated using a graduated ethanol series, cleaned in xylene, and then embedded in paraffin after being fixed for 48 hours. A rotatory microtome was used to cut sections of paraffin blocks that were 5 um thick. They were then attached to a microscope slide, stained with Hematoxylin and Eosin stain and inspected under a light microscope.

Statistical analysis:

The data had been gathered, processed, and examined using SPSS [Statistical bundle for social science] version 20. The mean and standard deviation were added for quantitative data, and recurrence and dissemination were added for subjective data. The 0.05 threshold for significance was used in this study. In statistical comparisons, the mean values between the treatment groups and the control group were compared using one-way analysis of variance (ANOVA).

4.RESULTS

4.1. Body weight and relative weight of testis:

In all the analyzed groups as the negative control and the positive control, a statistically significant rise was seen at the end of the fourth week (Final Body Weight). The mean value of epididymal weight at the end of the four weeks between the studied groups as negative control and positive control, Selenium, Vitamin C, Nicotine with Selenium, and Nicotine with vitamin C all showed increase in body weight while Nicotine group decreased in body weights. However, the mean value of epididymal weight showed no significant difference between the studied groups. **as in table (1).**

4.2 Reproductive abnormality (semen analysis)

Comparing between the semen analysis of the Nicotine, Nicotine with Selenium and Nicotine with Vitamin C groups regarding **sperm count (S.C.C)** there is statistically significant difference between Nicotine with Vitamin C group show the highest concentrations then Nicotine with Selenium group followed by the Nicotine group. For **motility** there is statistically significant difference between Nicotine with Vitamin C group which has the highest concentrations then Nicotine with Selenium group followed by the Nicotine group. As for **Livability** there is statistically significant difference between Nicotine with Vitamin C group which has the highest concentrations then Nicotine

group followed by the Nicotine with Selenium group. **Normal cells** show statistically significant difference between Nicotine with Vitamin C group which has the highest concentrations then Nicotine group followed by the Nicotine with Selenium group. **Head abnormality** show statistically significant difference between the Nicotine with Selenium group which has the highest concentrations followed by Nicotine group then Nicotine with Vitamin C group. **Tail abnormality** show no statistically significant difference between the Nicotine with Selenium group which has the highest concentrations followed by Nicotine group then Nicotine with Vitamin C group **as shown in table (2) and figure (1).**

4.3 Hormonal analysis

Regarding **Follicular stimulating hormone (FSH)** there is no statistically significant difference between the studied groups as the least concentration were in the Nicotine with Selenium group followed by Nicotine with Vitamin C group while the Nicotine group had the highest concentrations Regarding. **Luteinizing hormone (LH)** Nicotine with Vitamin C group showed the least concentration followed by Nicotine with Selenium group while Nicotine group had the highest concentration. This relation was found to be statistically significant. As for **Free testosterone** there is a statistically significant difference between studied groups as Nicotine group was the least finding then Nicotine with Vitamin C group followed by the Nicotine with Selenium group had the highest concentration **as shown in table (3) and figure (2).**

4.4 Haematological analysis

Regarding **red cell count (RBC'S)** there is no statistically significant difference between the Nicotine group which show the least concentrations then Nicotine with Selenium while Nicotine with Vitamin C group shows the best enhancement. As for **Hemoglobin** there is statistically significant difference between the Nicotine group which show the highest concentrations than Nicotine with Vitamin C group while decreased in Nicotine with Selenium group. **Total leukocyte count** shows statistically significant difference between the Nicotine group which shows the highest concentrations then Nicotine with Selenium group while Nicotine with Vitamin C group shows the best results **as shown in table (4) and figure (3).**

4.5 Oxidative parameters

There were no significant changes in the concentrations of (CAT, SOD and GSH) levels as well as MDA concentrations in tissue in the solvent group compared to their levels in the healthy control group. These results demonstrated that rat's antioxidant markers were unaffected by solvent injection. Moreover, there were no significant changes in the concentrations of (CAT, SOD and GSH) levels as well as MDA concentrations in tissue in the selenium group compared to their levels in the healthy control group, but there was a significant increase in SOD level in the serum in the selenium group compared to the healthy control group. Furthermore, no significant changes were observed in the MDA and GSH concentrations of the vitamin C group compared to their values in the healthy control group. However, there was significant difference in CAT, SOD level in the tissue and CAT level in the serum of the vitamin C groupcompared to their levels in the healthy control group **as shown in table (5) and figure (4) & (5).**

4.6 Histopathological analysis

The examination of the testes by H&E-stained sections examined under the power 100 and 400 of **control rats (positive and negative) in groups (1), (2)** showed normal histological architecture. The parenchyma of testis was formed of the well-arranged seminiferous tubules and interstitial tissue in between, seminiferous

tubules are normal with oval or rounded in outline. The germinal epithelium is mitotically active and showed different stages of maturation, arranged in many layers from basement membrane toward the lumen of the tubules, the three types of spermatogonia can be recognized, lumen is filled with spermatids and spermatozoa. Each tubule is surrounded by fibrous lamina called tunica. The limited interstitial spaces are filled with groups of well-arranged groups of pale stained interstitial Leydig cells normal interstitial tissue with no vacuolization, normal non congested blood vessels and no interstitial oedema ***(Figure 6).*** At the same time, the testis of rats treated with **Selenium, Vitamin c, (groups (4) & (5))** showed preserved histological structure, with no vacuolization, normal non congested blood vessels and no interstitial oedema. ***(Figures 7&8).***

Testicular tissue architecture, seminiferous tubules, and some spermatogonia not in order as in control rats were significantly lost in the testis of the rat group treated with **nicotine alone (group 3)**. They seem dispersed and hardly encircle the entire seminiferous tubule. Many seminiferous tubules demonstrated obvious hypocellularity at all stages, tubular degeneration, intercellular vacuolization, which indicates severe degeneration, and decreased numbers and occasionally absence of spermatids and mature sperm. Numerous different types of degenerative changes to the germ cells were seen, ranging from the loss of elongated spermatids to the disorganisation of germ cell layers, detachment and sloughing, and vacuolization of the seminiferous tubules, which eventually caused atrophy and the defoliation of numerous spermatocytes into the ST lumen ***(Figures 9 &10).***

While in rat group received **Vitamin C concomitant with Nicotine (group 7)** there was a slight restoration to the architecture of seminiferous tubules; germ cells lined the entire seminiferous tubule appearing mitotically active and showing different stages of maturation, arranged in many layers from the basement membrane towards the lumen of the tubules, few focal spermatogenic arrest noticed with much less atrophy and vacuolation, and there was a slight improvement in histopathological findings**.** ***(Figure 11).*** However, the improvement in histopathological findings was less in the rat group treated with nicotine and vitamin C than it was in the group receiving selenium concurrently (group 8), as most seminiferous tubules appear with very mild restore to architecture of seminiferous tubules with intercellular vacuoles, less numbers of spermatocytes and sperms filling lumen, and less atrophy ***(Figure 12).***

Table (1): Comparison between Positive control, negative control, Nicotine, Selenium and Vitamin C regarding body weight and testis weight.

Group	Negative control		Positive control		Selenium		Vitamin C		Nicotine		P value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Body weight	233.33	25.2	232.5	13.59	211.67	17.15	262.67	20.88	126.33	14.19	<0.001*
Testis weight	0.42	0.13	0.47	0.1	0.42	0.06	0.48	0.07	0.35	0.7	0.1

Table (2): Comparison between Negative control, Nicotine, Nicotine with Selenium, Nicotine with vitamin C regarding Reproductive abnormality.

Group / Semen analysis	Negative control		Nicotine		Nicotine with Selenium		Nicotine with vitamin C		P value
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
Motility (%)	81.67	5.16	10.67 [a]	9.83	60.83 [a, b]	4.92	73.33 [b, c]	9.31	<0.001*
Sperm count (million)	40	7.75	8.83 [a]	7.98	14.08 [a]	9.28	31.17 [b, c]	18.65	<0.001*
Livability (%)	93.37	3.01	28.42 [a]	7.38	32.78 [a]	2.54	82.01 [a, b, c]	8.16	<0.001*
Normal cells (%)	81.83	7.38	28.73 [a]	17.03	55.78 [a]	6.6	67.9 [b, c]	19.33	<0.001*
Head abnormality (%)	1.76	0.36	42.16 [a]	15.34	9.97 [a]	14.4	9 [b, c]	12.97	<0.001*
Tail abnormality (%)	14.67	5.14	31.2 [a]	11.93	20.17 [a]	19.34	19.11 [c]	8.35	0.03*

** Statistically significant (S).*
[a] significant from Negative control, [b] significant from Nicotine, [c] significant from Nicotine with Selenium.

Table (3): Comparison between Negative control, Nicotine, Nicotine with Selenium, Nicotine with vitamin C regarding Hormonal assay.

Group / Hormone	Negative control		Nicotine		Nicotine with Selenium		Nicotine with vitamin C		P value
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
Follicular Stimulating Hormone (FSH) (U/ml)	0.13	0.01	1.31	2.85	0.12	0.04	0.16	0.06	0.4
Luteinizing Hormone (LH) (U/ml)	0.6	0.1	7.37 [a]	6.92	0.66 [b]	0.38	0.57 [b]	0.29	0.005*
Testosterone (Free) (pg/ml)	57.08	11.16	29.15 [a]	11.09	73.17 [b]	8.61	68.67 [b]	28.54	<0.001*

** Statistically significant (S). [a] significant from Negative control, [b] significant from Nicotine, [c] significant from Nicotine with Selenium.*

Table (4): Comparison between Negative control, Nicotine, Nicotine with Selenium and Nicotine with vitamin C regarding Hematological profile.

Group / CBC	Negative control		Nicotine		Nicotine with Selenium		Nicotine with vitamin C		P value
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
Red cell count ($x10^{12}$ /L)	8.61	0.46	7.42 [a]	0.88	8.42 [b]	0.83	8.39 [b]	0.59	0.04*
Hemoglobin (g/dl)	18.18	0.72	14.02	1.09	18.9 [b]	1.55	16.72 [c]	1.68	0.001*
Total leukocyte count ($x10^9$/L)	12.47	1.25	22.4 [a]	2.43	17.98 [a, b]	2.12	15.63 [a, b]	1.99	<0.001*

** Statistically significant (S). [a] significant from Negative control, [b] significant from Nicotine, [c] significant from Nicotine with Selenium.*

Table (5): Comparison between Negative control, Nicotine, Vitamin C and Nicotine with Vitamin C. regarding Oxidative parameters.

Group / Oxidative parameters		Negative control		Nicotine		Nicotine with Selenium		Nicotine with vitamin C		P value
		Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
Tissue	MDA (nmol/gm)	53.73	8.06	361.31 [a]	11.93	158.36 [a, b]	46.75	247.66 [a, b, c]	77.18	<0.001*
	CAT (U/gm)	532.2	51.35	392.12 [a]	42.48	737.16 [a, b]	170.45	640.22 [b]	75.73	<0.001*
	GSH (mg/gm)	10.54	0.92	4.97 [a]	0.66	7.25 [a, b]	0.64	5.91 [a, c]	1.12	0.001*
	SOD (U/gm)	427	23.29	350.6	80.82	387.98	102.39	350.16 [a]	18.49	0.049*

** Statistically significant (S).* [a] *significant from Negative control,* [b] *significant from Nicotine,* [c] *significant from Nicotine with Selenium.*

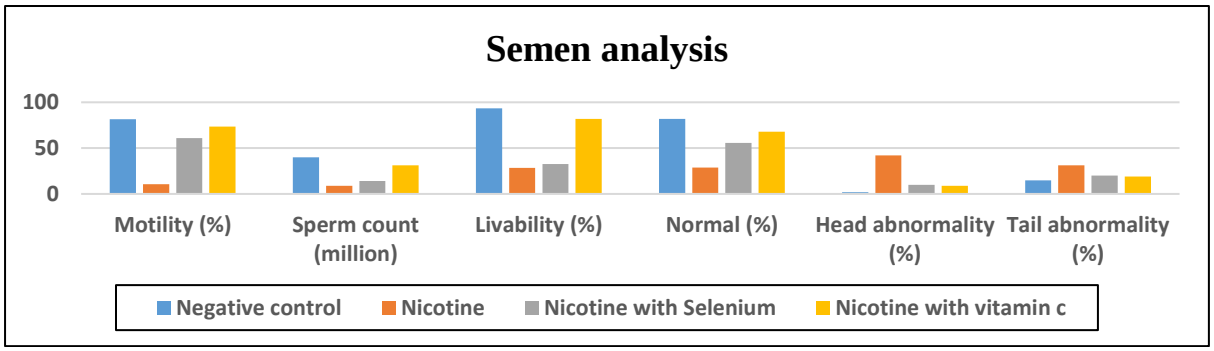

Figure (1): Bar chart shows comparison between Negative control, Nicotine, Nicotine with Selenium and Nicotine with Vitamin C regarding semen analysis.

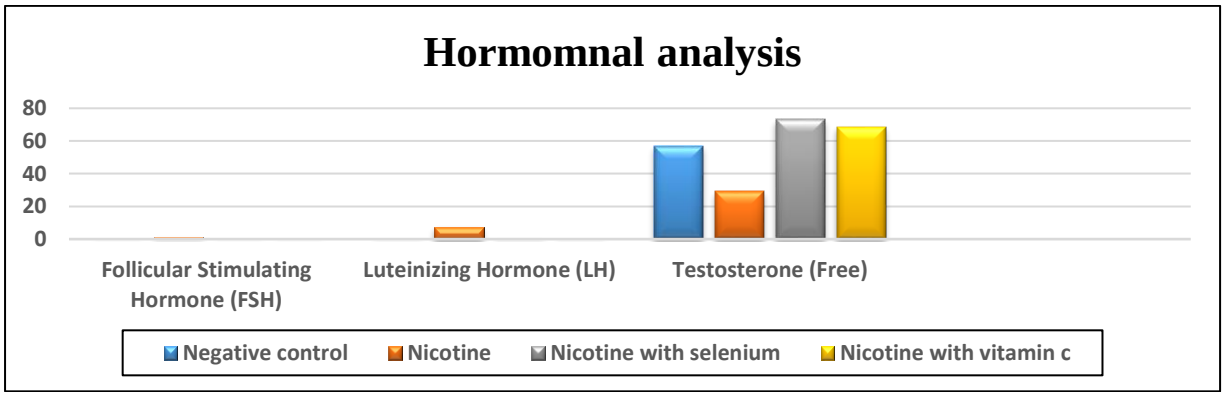

Figure (2): Clustered column chart show the comparison between Negative control, Nicotine, Nicotine with Selenium, Nicotine with vitamin C regarding Hormonal assay.

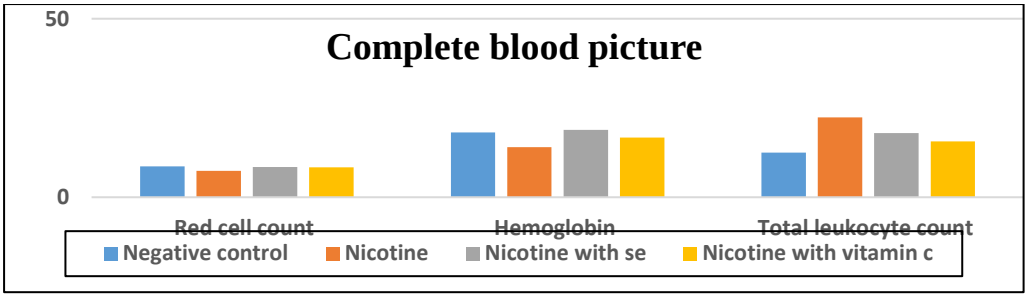

Figure (3): Clustered column chart shows the comparison between Negative control, Nicotine, Nicotine with Selenium, Nicotine with vitamin C regarding Hematological profile.

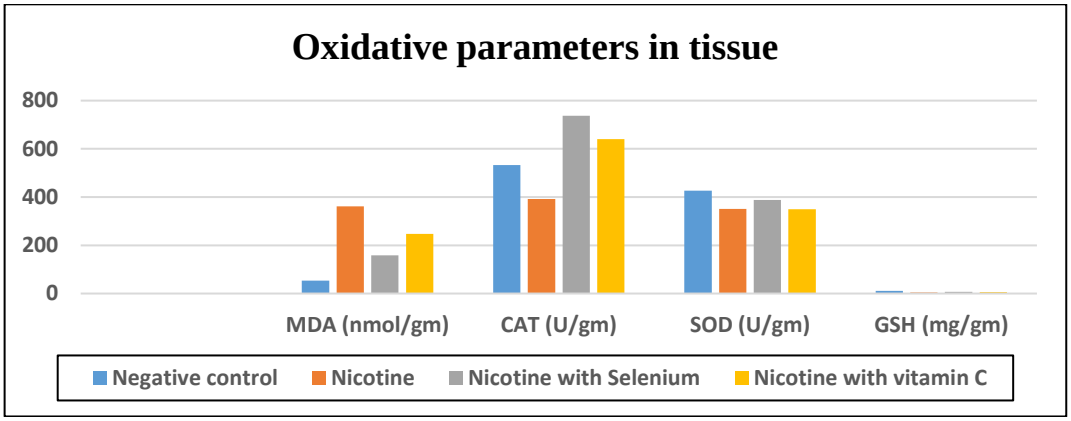

Figure (4): Clustered column chart shows the comparison between Negative control, Nicotine, selenium and Nicotine with Vitamin C regarding Oxidative parameters in tissue.

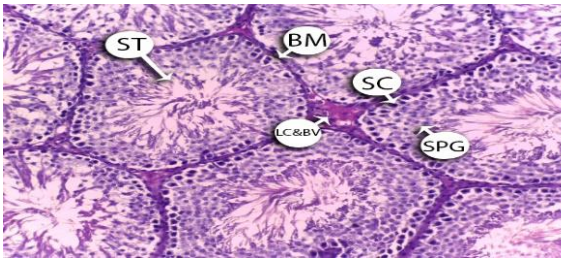

Figure (5): A photomicrograph of a section from rat's testis of a control group showing normal architecture with well-arranged closely matted seminiferous tubule (S) and normal interstitial tissues which contain Leydig cell and Blood vessels (LC&BV) & normal spermatogonia (SPG) & Sertoli cells (SC) with normal Basement membrane (BM) (Hx & E x 200)

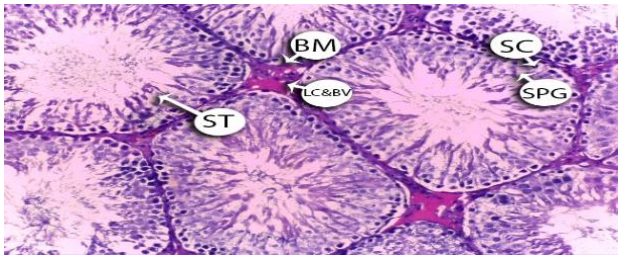

Figure (6): A photomicrograph of a section from rat's testis of a Selenium treated group showing normal architecture with well-arranged closely matted seminiferous tubule (ST) and normal interstitial tissues which contain Leydig cell and Blood vessels (LC&BV) & normal spermatogonia (SPG) & Sertoli cells (SC) with normal Basement membrane (BM) (Hx & E x 200).

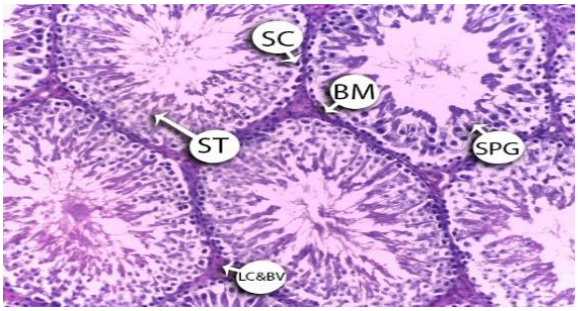

Figure (7): A photomicrograph of a section from rat's testis of Vitamin C treated group showing normal architecture with well-arranged closely matted seminiferous tubule (ST) and normal interstitial tissues which contain Leydig cell & Blood vessels (LC&BV) & normal spermatogonia (SPG) & Sertoli cells (SC) with normal Basement membrane (BM) (Hx & E x 200).

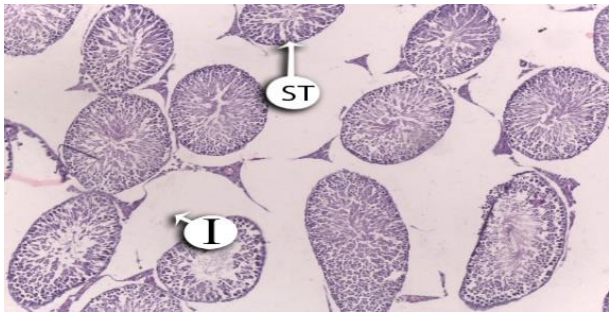

Figure (8): A photomicrograph of a section from rat's testis of Nicotine treated group showing wide interstitial space, disruption of ST, atrophy of cells, necrosis of spermatocytes, defoliation of many spermatocytes into lumen of the seminiferous tubules (ST) (Hx & E x 100).

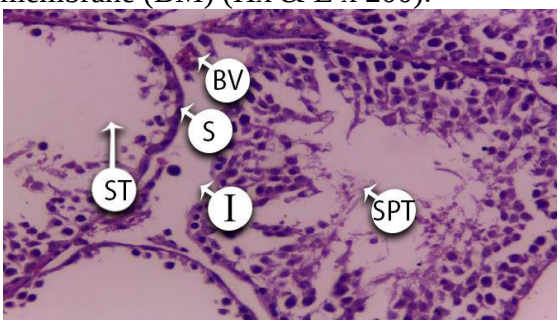

Figure (9): A photomicrograph of a section from rat's testis of Nicotine treated group showing interstitial edema (I) with congested blood vessel (BV), seminiferous tubules (ST) showed degeneration of germ cells with decrease in spermatid (SPT) count (H&E x 400).

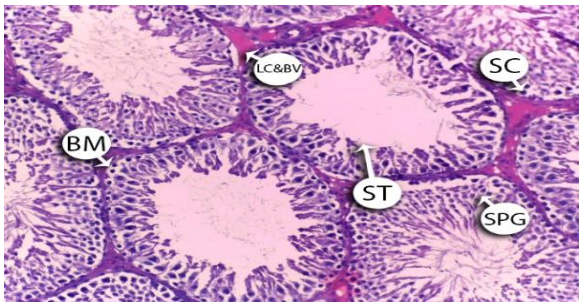

Figure (10): A photomicrograph of a section from rat's testis of Selenium and Nicotine group showing mild amelioration of normal architecture of seminiferous tubule (ST), decrease in spermatogonia (SPG) and thick basement membrane (BM) (Hx & E x 200).

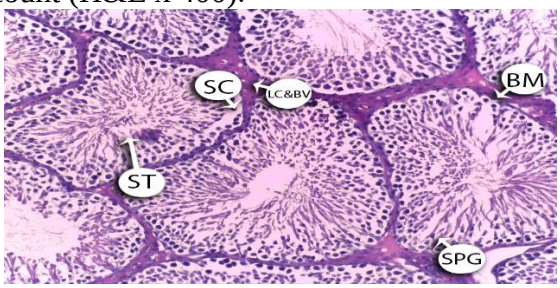

Figure (11): A photomicrograph of a section from rat's testis of Vitamin C and Nicotine group showing mild amelioration of normal architecture of seminiferous tubule (ST) and thick basement membrane (BM) (Hx & E x 200).

5.DISCUSSION

The **body weight** increased in all the studied groups at the end of the fourth week (final body weight), except for the nicotine group, which experienced a weight loss. These groups included the negative control group, the positive control group, the selenium group, the vitamin C group, the nicotine with selenium group, and the nicotine with vitamin C group. This was in line with ***Abdel-Hamid (2018)*** assertion that nicotine-treated rats' body weights were significantly lower than those of the control and nicotine-plus-vitamin-C groups. Also, ***Iranloye and Bolarinwa1 (2009)*** reported that after nicotine treatment, the body weight gradually decreased.

Our findings demonstrated that there was a statistically significant difference in the mean **testicular weight** between the tested groups at the end of the four-week period, with the Nano Selenium/Vitamin C group showing a small improvement over the Negative Control group. ***Oyeyipo et al., (2010)*** shown that the mean testicular weight significantly decreased after nicotine delivery. The testis weight of the rats dropped in the nicotine-administered group when compared to the control group, according to ***Seema, et al, (2007)*** The weight increased in contrast to the nicotine group after selenium supplementation and concurrent delivery of nicotine and selenium.

Regarding **reproductive abnormality** our results showed that considering **sperm count (S.C.C)** there was statistically significant difference as Nicotine with Vitamin C group showed the highest concentrations. For **motility** there was statistically significant difference as Nicotine with Vitamin C group had the highest concentrations. As for **Livability** there was statistically significant difference in which Nicotine with Vitamin C group which had the highest concentrations. **Normal cells** showed statistically significant difference as Nicotine with Vitamin C group which has the highest concentrations. **Head abnormality** showed statistically significant difference as Nicotine with Selenium group had the highest concentrations.

In accordance with our results ***Mosbah et al. (2015)*** found that nicotine had a significant impact on sperm quality, as shown by a significant decrease in the number of spermatids, sperm count, sperm motility, daily sperm production, and testosterone levels, while a significant increase in sperm abnormality. Similar to our study, ***Ezzatabadipour et al. (2012)*** discovered that sperm concentration was lower in all treatment groups, particularly the nicotine group, when compared to either the intact or control groups. Sperm concentration was equivalent across the intact and control groups.

Tizabi et al., (2014) stated that there were increased abnormalities of sperm heads in nicotine-treated rats compared to control rats, demonstrating the detrimental effect of nicotine on spermatozoa. Our data confirmed that nicotine induction significantly reduced sperm count, sperm motility, and livability percentages with increased sperm abnormality.

Kaur and Bansal (2005) noted a decline in the capacity for reproduction in selenium-deficient mice. Selenium, particularly the selenoenzyme phospholipid glutathione peroxidase, is important for spermatogenesis (PHGPx, GPx-4)., ***Okon and utuk, (2016)*** came to the same conclusion about vitamin C's considerable influence on male reproductive morphology in accordance with the findings of this study.

Regarding **Hormonal analysis, Follicular stimulating hormone (FSH)** there was no statistically significant difference between the studied groups as the Nicotine group had the highest concentrations. Regarding **Luteinizing hormone (LH)** Nicotine group had the highest concentration. This relation was

found to be statistically significant. As for **Free testosterone** there is a statistically significant difference between studied groups as Nicotine group was the least finding and Nicotine with Selenium group had the highest concentration.

Regarding the nicotine results on our side, ***Sydney and Theresa (2015)*** found that treated cigarette smoke, smokeless tobacco, and nicotine exposed mice had higher serum levels of LH and FSH. These results concur with those from ***Heidary et al., (2012)*** who found that rats who smoked cigarettes considerably increased their serum FSH levels compared to control animals while hookah smokers saw only a small rise. This finding was in accordance with ***Trummer, et al (2002)*** They demonstrated that, when compared to the control group, the mean serum testosterone levels of rats given either a low or high dose of nicotine over the course of four weeks were significantly lower. Our results were in line with those of ***Eissenberg and Shihadeh (2009)*** who discovered that testosterone levels in the serum were lower in hookah or cigarette smoking rats than in non-smoking rats.

Notifying to the selenium results ***Chattopadhyay et al. (2003)*** discovered that selenium supplementation in combination with nicotine treatment caused hormonal levels (FSH, LH, and Testosterone) to be close to basal levels, similar to the control group. This was explained by the hypothesis that nicotine-induced reproductive toxicity may be caused by the induction of oxidative stress or free radical generation, and since selenium is a crucial dietary antioxidant, it may prevent this toxicity.

Regarding Vitamin C results ***Azeez (2021)*** claimed that the level of FSH and LH was 29% lower in rats treated with nicotine alone. Also, he revealed that vitamin C protects the testes from the toxicity of nicotine and that it has antioxidant characteristics, since nicotine + vit C resulted in significant reductions in FSH, LH levels of 21% and 12%, respectively, as compared to the control and nicotine groups. Vitamin C significantly reduced mRNA damage and improved testicular antioxidant, endocrine, and sperm quality.

Considering **Haematological analysis** Regarding **red cell count (RBC'S)** there is no statistically significant difference as the Nicotine group showed the least concentrations. As for **Hemoglobin** there is statistically significant difference as the Nicotine group showed the highest concentrations.

Nicotine is known to cause the release of adrenaline and increases leukocytes in the peripheral blood, bone marrow and spleen ***(Chang et al., 2010).*** This association has been attributed to bronchopulmonary inflammation and/or infection. Increased WBC counts in the present study may suggest chronic inflammatory changes in various tissues, due to exposure to toxic oxidative substances in nicotine ***(Richter et al., 2008).***

Total leukocyte count showed statistically significant difference as the Nicotine group had the highest concentrations.

Also, ***Rajasekhar et al., (2007)*** reported that when compared to the controls, the experimental rats had lower RBC and higher WBC counts.

Also, in agreement to our study ***Abouelghar et al. (2020)*** who treated mice with selenium via oral administration for two weeks, found that it had positive effects by raising the levels of hematological parameters such as RBC and hemoglobin (HGB).

Our finding was in accordance with ***Mongi et al., (2011)*** who showed that the vitamin C supplementation reversed the abnormal hematological effects and attained the hematological parameter changes to normal levels. Vitamin C is a potent scavenger of free radicals which stimulate activation of NOS activity and increase NO synthesis in endothelial cells. These effects could contribute to improved

hematological indices, re-establish the disordered conditions and counteracting the toxicity induced alteration caused by nicotine exposure ***(Mongi et al., 2011).***

Regarding **Oxidative parameters** the **MDA concentrations in tissue** there is statistically significant difference as the Nicotine group showed the highest results. As for **CAT concentrations in tissue** there was statistically significant difference as the Nicotine group Vitamin C group and Nicotine with Vitamin C group showed the highest results. Considering **GSH concentration in tissue** showed statistically significant difference as the Vitamin C group showed the highest results. While **SOD concentrations in tissue** showed statistically significant difference as Vitamin C group showed the highest results. As for **CAT concentrations in serum** showed statistically significant difference as the Vitamin C group showed the highest results. Regarding **SOD concentrations in serum** showed statistically significant difference as the Nicotine with Vitamin C group showed the highest results.

On our side ***Jana et al., (2010)*** stated that the testicular content of MDA, the product of lipid peroxidation of the polyunsaturated fatty acid present in cell membrane, was significantly elevated concomitantly with significant increase in the generation of testicular hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH─) after chronic nicotine exposure with respect to the controls, indicating the testicular ROS generations and induction of oxidative stress and also it has been shown that the amount of the GSH significantly decreased with nicotine treatment in the testis with respect to the control reflecting a state of apparent oxidative stress.

Similarly, to our results ***Mendelson et al. (2003)*** In the nicotine group, the results revealed a significant decrease in GSH levels as well as CAT and SOD activities, whereas CAT and SOD activities were significantly higher in the antioxidant group. On the other hand, there was no statistically significant difference between the control group and the nicotine and antioxidant group for the mean values of the oxidative stress indicators.

Selenium (Se) supplementation may prevent the formation of free radicals and the process of lipid peroxidation ***(El-Demerdash and Nasr, 2014).*** Se has been found to operate as a substrate for several enzymes, including glutathione peroxidase, and is crucial for the metabolism of sulphur amino acids, which serve as antioxidants and protect the body from a number of ailments. The antioxidant activity of selenium and the stimulation of cellular antioxidant enzymes may both contribute to its protective impact against nicotine-induced tissue damage ***(Lamia et al., 2010).***

While ***Zhuo et al (2010)*** stated that they discovered that nicotine administration significantly decreased the catalase activity (CAT) compared to the control group. Vitamin C supplementation raised CAT activity in nicotine-treated groups to normal levels. Moreover, selenium therapy increased CAT activity, while it was still higher than in the control group.

Referring to the histopathology, the examination of the testes by H&E-stained sections examined under the power 100 and 400 of **control rats (positive and negative)** showed normal histological architecture. Testis of rat group treated with **Nicotine alone** showed marked loss of testicular tissue architecture seminiferous tubules with some spermatogonia not in order as in control rats. While in rat group received **Vitamin C concomitant with Nicotine** mild improvement in histopathological findings was detected as there was mild restore to architecture of seminiferous tubules. However, in rat group received **Selenium concomitant with Nicotine**, the improvement in histopathological findings was less than that was seen in Vitamin C treated group.

In the line of our findings ***Mosadegh et al., (2017)*** demonstrated that a microscopic inspection of the testicles of control rats revealed their normal

architecture. In contrast to testis from rats treated only with selenium, testis from rats treated alone with hazardous chemical displayed severe interstitial congestion.

In agreement to our results ***(Ahmed et al., 2014)*** stated that the spermatogenic cells and Sertoli cells in the seminiferous tubules of vitamin C treated group and control group rats were observed in normal structure as vitamin C improves antioxidant enzyme activity and can increase the level of plasma testosterone and the sperm concentration, motility, and morphological structure. In accordance to our result ***(Binsawad et al., 2011)*** observed that selenium administration as an antioxidant substance to cigarette smoking exposed animals, resulted in amelioration in the histological and histochemical changes caused by nicotine

6. CONCLUSION

Our study suggests that oral nicotine administration causes toxic effects, hormonal changes, and hematological damage in the testes. These negative effects may be related to the induction of oxidative stress because nicotine increases ROS by rupturing the mitochondrial respiratory chain and prevents testosterone biosynthesis in rat Leydig cells. By inhibiting the expression of the steroidogenic acute regulatory protein, nicotine's increased ROS generation reduces steroidogenesis at the initial stage of cholesterol transport to the mitochondria. These improvements in the delivery of selenium and vitamin C along with nicotine, which protect against the detrimental effects of nicotine, can be extremely useful instruments in the practise of toxicology.

6. RECOMMENDATIONS

Depending on the results of this study, the following guidelines are recommended:

- Implementing a nationwide policy that prohibits the use of nicotine in all theatres.
- Smoking-related health education initiatives should consider the empowerment of non-smokers and include culturally acceptable means for them to express their wish for a smoke-free atmosphere.
- Extensive public education on the negative consequences of smoking on one's health, with a focus on the toxin it has on the reproductive system.
- Creating a smoking cessation message based on research.
- Selenium functions as a powerful antioxidant that can reverse oxidative damage inside the tissue, making dietary intake of selenium supplements together with increased intake of foods and fruits rich in selenium extremely important.
- Daily vitamin C administration as part of treatment for male infertility helps to improve testicular integrity and hormonal function.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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