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# ACUTE NICOTINE & PYRIDINE-3-CARBOXYLIC ACID SYNERGY PROTECTS NICOTINE INDUCED TESTICULAR DEGENERATION AND MODERATES MALONDIALDEHYDE LEVELS

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***Abstract:*** **Nicotine is a naturally produced alkaloid in the Solanaceae family of plants, widely used as a recreational substance or an anxiolytic and acts as a receptor agonist at most nicotinic acetylcholine receptors (nAChRs). Pyridine 3-carboxylic acid is a monocarboxylic derivative of pyridine. Also known as niacin, it is a water-soluble vitamin that is indispensable in the diet of humans and animals and has a variety of important applications, such as in matrices for matrix-assisted laser desorption ionization (MALDI) mass spectrometry analyses of large polypeptides. With few reported cases of negative effects of nicotine on reproductive health, this study examined the comparative micro-therapeutic effects of nicotine and nicotine - pyridine-3-carboxylic acid synergy on the testis microstructure and subsequent alterations in serum malondialdehyde levels using adult wistar rats.**

**Twenty five (25) adult male wistar rats were used for this study and they were divided into five groups, with five (5) animals in each. Group 1 is the normal control group which was given food and water only, group 2 was given 0.06mg of Nicotine only for 17 days, group 3 was given 0.06mg of Nicotine and 0.15mg of pyridine-3-carboxylic acid for 17 days, group 4 was given 0.06mg of Nicotine and 0.25mg of pyridine-3-carboxylic acid for 17 days, while group 5 was given 0.25mg of pyridine-3-carboxylic acid only for 17 days. The administration of Nicotine and pyridine-3-carboxylic acid was done orally. Results showed that nicotine & pyridine-3-carboxylic acid synergy had curative effects on the testis microstructure (severe interstitial tissue necrosis and germ cell degeneration) caused by nicotine, helped reduce oxidative stress levels in nicotine-induced oxidation.**

***Keywords:*** **Nicotine, pyridine-3-carboxylic acid, oxidative stress, malondialdehyde, wistar rats, testis.**

## 1. INTRODUCTION

Nicotine is a naturally produced alkaloid in the Solanaceae family of plants**,** widely used as a recreational substance or an anxiolytic and acts as a receptor agonist at most nicotinic acetylcholine receptors (nAChRs) . It is an amine composed of pyridine and pyrrolidine rings and can cross the biological membranes including the blood brain barrier to influence various functions in the central nervous system. Once absorbed, nicotine can widely be metabolized by the liver to a number of major and minor metabolites . The actions of nicotine have been extensively investigated in human, in animal, and in a variety of cell systems. The predominant effects of nicotine in the whole intact animal or human consist of an increase in pulse rate, blood pressure, and an increase in plasma free fatty acids, a mobilization of blood sugar, and an increase in the level of catecholamines in the blood . Nicotine concentrations of between 70 and 300 µg/L (0.43–1.85 µM) have been found in the seminal fluids of daily smokers  an indication of high transfer from the blood stream, and therefore, testicular cells

and spermatogenesis may be vulnerable to its effects. Also, daily i.p. injection of nicotine at 0.6 mg/kg body weight in rats for six weeks led to seminiferous tubule and spermatogenic derangement in the testes as well as reduced overall testicular weight also observed in orally administered rats . Current safety databases state that the lethal dose of ingested nicotine in adults is 60 mg for humans which is equivalent to the amount found in approximately five cigarettes . Despite the low lethal dose and high availability of nicotine in various forms, there are relatively few cases of fatal overdose reported. It is agreeable that nicotine is not a major contributing factor to the diseases associated with tobacco smoking as many research are still being carried out on this field to reposition facts, nicotine research continues to be an active field of study. This study gives preliminary insight into the roles of nicotine in the development of acute toxicity as it may adversely affect the testis microstructure.

## 2. MATERIALS AND METHOD

Purchase of Nicotine and pyridine-3-carboxylic acid

100mls (containing 1.01g) of 95% Nicotine stock solution was purchased from sigma Aldrich with Batch No, 1922540, product No, 25140 and delivered to the Department of Anatomy, Enugu state university of Science and Technology, Enugu. It was kept in a shelf at room temperature. Pure nicotine is a clear liquid with a characteristic odour whereas it turns brown on exposure to air and can mix with an equal amount of water . Pyridine-3-carboxylic acid was purchased from Open haven Pharmacy, GRA, Enugu. Each tablet of pyridine-3-carboxylic acid is 50mg.

**Preparation of Nicotine/Toxicity Test**

3mls containing 30mg of 95% Nicotine was dissolved in 150mls of distilled water and this was the working solution after carrying out routine toxicity test using Karber's method . From the result of the toxicology test carried out, 40mg/kg was the LD50 of Nicotine for rats weighing 150g. Current safety databases state that the lethal dose of ingested nicotine in adults is 60 mg which is equivalent to the amount found in approximately five cigarettes .

**Preparation of pyridine-3-carboxylic acid**

Each tablet of pyridine-3-carboxylic acid purchased contained 50mg. To prepare the working solution, two tablets (100mg) of Niacin was dissolved in 200mls of water.

**Animal management**

Wistar rats used for this study were purchased from the animal house of the Faculty of Basic Medical Sciences, ESUCOM, Enugu State. There were kept under standard conditions with good ventilation and a clean environment in standard cages. There were fed with animal growers feed and water throughout and were allowed to acclimatize for 14 days prior to commencement of administration of Nicotine and pyridine-3-carboxylic acid.

**Research design**

Twenty five (25) adult male wistar rats were used for this study and they were divided into five groups, with five (5) animals in each group. Group 1 is the normal control group which was given food and water only, group 2 which was the negative control group was given 0.06mg of Nicotine only for 17 days, group 3 was given 0.06mg of Nicotine and 0.15mg of pyridine-3-carboxylic acid for 17 days, group 4 was given 0.06mg of Nicotine and 0.25mg of pyridine-3-carboxylic acid for 17 days, while group 5 was given 0.25mg of pyridine-3-carboxylic acid only for 17 days. The administration of Nicotine and pyridine-3-carboxylic acid was done orally.

**Table i: showing the summary of research design**

| GROUPS | NO OF RATS | DURATION FOR NICOTINE | DOSAGE FOR NICOTINE (mg/kg) | DURATION OF PYRIDINE -3- ACID | PYRIDINE -3- ACID DOSAGE (mg/kg/bwt) |
|---|---|---|---|---|---|
| **1 (Normal control)** | 5 | Feed + water | Feed + water | NIL | Feed + water |
| **2 (Nicotine only)** | 5 | 17 days | 0.06 | 17 days | NIL |
| **3 (Nicotine + Niacin)** | 5 | 17 days | 0.06 | 17 days | 0.15 |
| **4 (Nicotine + Niacin)** | 5 | 17 days | 0.06 | 17 days | 0.25 |
| **5 (Niacin)** | 5 | 17 days | NIL | 17 days | 0.25 |

After the 17 days administration period, animals were euthanized using 0.5mls of ketamine injection as anesthesia and then blood was collected via orbital method for oxidative stress test using Superoxide dismutase (SOD) and Malondialdehyde (MDA) biomarkers. Oxidative stress levels were carried out in the blood to access for the presence of free radicals across all the groups and the potential of Niacin to inhibit oxidative stress levels. Cigarette smoke which contains nicotine has been said to be one of the major causes of oxidative stress .

## 3. RESULTS

**Result of Histological findings**

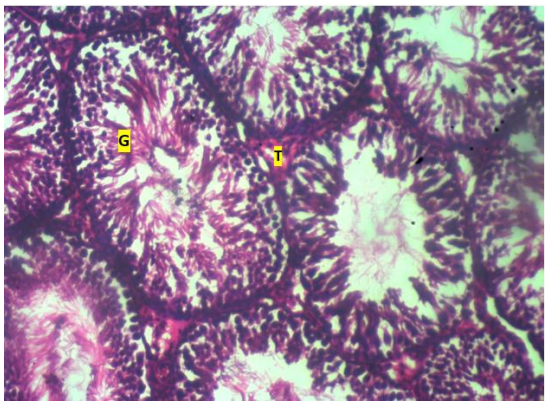


**Fig 1. (GROUP 1): PHOTOMICROGRAPH OF THE TESTIS SHOWING INTERSTITIAL TISSUE (T) AND SEMINIFEROUS TUBULES FILLED WITH GERM CELLS (G). TISSUE APPEARS NORMAL. H & E. X300**

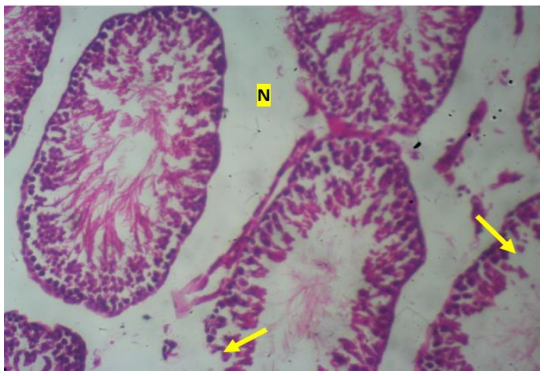


**Fig 2. (GROUP 2): PHOTOMICROGRAPH OF THE TESTIS SHOWING SEVERE INTERSTITIAL TISSUE NECROSIS (N) AND GERM CELL DEGENERATION (ARROW). H & E. X300**

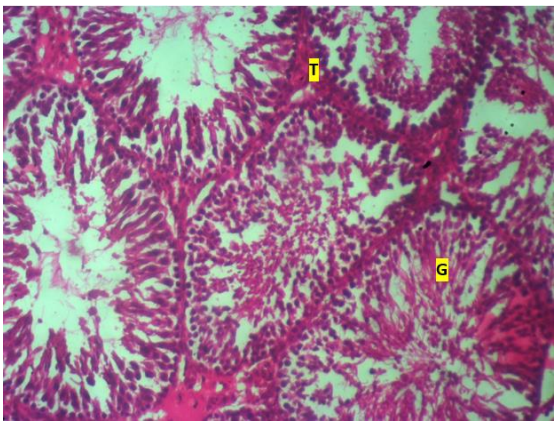


**Fig 3. (GROUP 3): PHOTOMICROGRAPH OF THE TESTIS SHOWING INTERSTITIAL TISSUE (T) AND SEMINIFEROUS TUBULES FILLED WITH GERM CELLS (G). TISSUE APPEARS NORMAL. H & E. X300**

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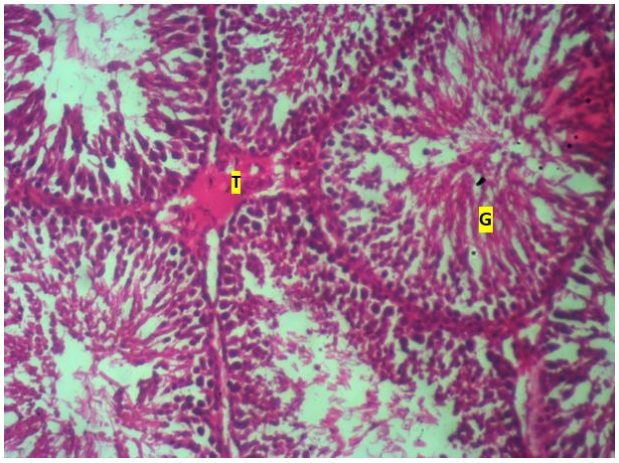


**Fig 4. (GROUP 4): PHOTOMICROGRAPH OF THE TESTIS SHOWING INTERSTITIAL TISSUE (T) AND SEMINIFEROUS TUBULES FILLED WITH GERM CELLS (G). TISSUE APPEARS NORMAL. H & E. X300**

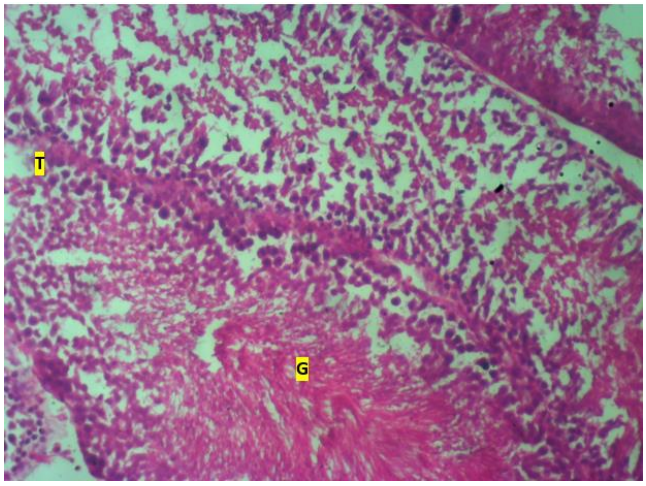


**Fig 5. (GROUP 5): PHOTOMICROGRAPH OF THE TESTIS SHOWING INTERSTITIAL TISSUE (T) AND SEMINIFEROUS TUBULES FILLED WITH GERM CELLS (G). TISSUE APPEARS NORMAL. H & E. X300**

**Table ii: Result of Oxidative Stress Test**

| BIOMARKER | GROUP | MEAN±SD | P VALUE | |
|---|---|---|---|---|
| SOD (u/mg protein) | 1 | 34.96±0.714 | 0.10* | Significant |
| | 2 | 32.95±0.318 | | |
| | 3 | 31.98±0.905 | | |
| | 4 | 31.07±1.937 | | |
| | 5 | 33.42±0.933 | | |
| | **TOTAL** | 32.87±1.613 | | |
| | | | | |
| MDA(mmo/mgprotein) | 1 | 5.74±0.205 | 0.16* | Significant |
| | 2 | 6.86±0.219 | | |
| | 3 | 6.59±0.636 | | |
| | 4 | 6.72±0.205 | | |
| | 5 | 6.61±0.460 | | |
| | **TOTAL** | 6.50±0.506 | | |

Table ii: Showing the descriptive statistics of the oxidative stress markers (SOD & MDA) across all the five groups. Group one express the highest mean concentration of SOD (u/mg protein) (34.96±0.714) closely followed by group 5 (33.42 ±0.933). However there is a fair distribution of the mean value concentration across all groups in MDA with group 2 expressing the highest value of 6.86±0.219 mmo/mgprotein. n=5

## 4. DISCUSSION

Findings from this study indicate that nicotine- pyridine-3-carboxylic acid combined interactions had cytoprotective effects on the testicular microstructure. Animals in group 2 which were administered with 0.06mg of Nicotine only for 17 days showed necrosis of the inter-tubular tissue and depletion of germ cells (Fig 2). A study showed that daily i.p. injection of nicotine at 0.6 mg/kg body weight in rats for six weeks led to seminiferous tubule and spermatogenic derangement in the testes as well as reduced overall testicular weight with contributing factors including DNA damage and there was a reduction in the number of germ cells at several generations in the sperm cycle and abnormalities to sperm morphology were also significantly increased, particularly in the sperm head and formation of a “banana-like” shape . A Very similar findings have been shown in a recent study in adolescent rats, which were administered 0.6 mg/kg of nicotine via i.p. injection over 12 weeks . A higher concentration of subcutaneously injected nicotine in rats (2.5 mg/kg body weight) also resulted in smaller testes and smaller seminiferous tubule diameter, as well as reduced serum testosterone compared to untreated controls .

Results of oxidative stress tests done with Superoxide dismutase (SOD) and Malondialdehyde (MDA) biomarkers showed that there was a significant difference in the levels of oxidation amongst all groups (P=0.10 and 0.16 respectively) (Table ii). A related study showed that daily i.p. injection of nicotine at 0.6 mg/kg body weight in rats for six weeks led to an increase in reactive oxygen species . In nicotine administered rats, the concentrations of free fatty acids and the level of malondialdehyde, and hydroperoxides increased. It has also been shown that nicotine administration results in a decrease in the activities of free radical scavenging enzymes superoxide dismutase, catalase, and glutathione reductase . Result of the malondialdehyde test carried out in this study showed a significant increase in the mean oxidation levels of animals in group 2 which were administered with 0.06mg/kg of nicotine (6.86±0.219 mmo/mgprotein) compared to that of the control (5.74±0.205 mmo/mgprotein) (Table ii). Animals in groups 3 and 4 which were given different doses of combined nicotine and pyridine-3-carboxylic acid showed a significant decrease in mean malondialdehyde levels (6.59±0.636 and 6.72±0.205 mmo/mgprotein respectively) while animals in group 5 which were given 0.25mg/kg of pyridine-3-carboxylic acid only had a lower malondialdehyde level (6.61±0.460 mmo/mgprotein) than those in group 2 (6.86±0.219 mmo/mgprotein). This, thus is an indication that pyridine-3-carboxylic acid helped reduce oxidative stress levels in nicotine-induced oxidation. Generally, the mean malondialdehyde levels in animals in the control group was significantly lower than all the other four experimental groups (Table ii).

The mean Superoxide dismutase (SOD) levels was highest in the animals in the control group (34.96±0.714 u/mg protein) and lowest for animals in groups 3 and 4 which was given different doses of combined nicotine and pyridine-3-carboxylic acid only (31.53±1.421 u/mg protein)(table ii). Proteins represent a wide target for Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) generated under normal or oxidative stress conditions and can be considered as general scavengers of these species. Several amino acidic residues can undergo oxidative modifications including oxidation of sulphur-containing residues, hydroxylation of aromatic and aliphatic groups, nitration of tyrosine residues, nitrosylation and glutathionylation of cysteine residues, chlorination of aromatic groups and primary amino groups, and conversion of some amino acid residues to carbonyl derivatives . If the oxidative modifications of protein residues are not properly repaired or removed, they could affect the three-dimensional structure and physicochemical properties of the protein that may also become toxic.

## 5. CONCLUSION

Results of the oxidative stress tests showed that Malondialdehyde (MDA) test showed a reduction in oxidation especially in the groups given combined nicotine and pyridine-3-carboxylic acid compared to the group given nicotine only. Superoxide dismutase (SOD) levels rather reduced in all the groups given nicotine only and nicotine- pyridine-3-carboxylic acid compared to the control group. This study is an acute study on the cytoprotective effects of nicotine - pyridine-3-carboxylic acid synergy following nicotine – induced testicular degeneration in adult wistar rats. It proffered a preliminary approach and suggestion to synergistic treatment in the management of cases of testicular malfunctions that are linked to microstructural degenerations as well as in management of age-related testicular degeneration. Nicotine- pyridine-3-

carboxylic acid synergy in this study has laid a rudimentary report on the management of testicular degeneration cases as it affects the germ cells in the seminiferous tubules. It may be suggested that the pyridine-3-carboxylic acid may cushion any antagonistic physiological and histopathological effects nicotine may cause and resultantly utilize the physiological synergy in bringing the protective effects. It would be in proper place to, in further research in this topic to also incorporate hormonal assays to also support and possibly verify the observations seen in this research.

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