Implications of Chronic Nicotine Exposure on Pattern of Advanced Oxidative Protein Products and Pain Hypersensitivity in Albino Wister Rats

ABSTRACT

The effect of chronic administration of nicotine on pattern of advanced oxidative protein products and pain hypersensitivity in albino wistar rat was investigated in this research. Nicotine, a major alkaloid, is present in tobacco products as well as in smaller amounts in potatoes, tomatoes, and eggplants. The effect of chronic administration of nicotine on pattern of advanced oxidative protein products and pain hypersensitivity was studied using 25 adult albino rats which were divided into 5 different groups which includes; Group 1(control), Group 2(low dose nicotine treated), Group 3(medium dose nicotine treated), Group 4(high dose nicotine treated) and Group 5(epinephrine treated). The groups were kept in different compartments of the cage. Group 2 animals received 50µg/kg nicotine through inhalation, rats in Group 3 received 75µg/kg nicotine through inhalation and Group 4 received 100µg/kg nicotine through inhalation daily. Group 5 rats were administered 0.1ml/100g of epinephrine drug intravenously daily and this affected emotional behavior (aggressiveness). Experimental procedures were carried out using the electroconvulsive therapy for shock, passive avoidance test to access learning and memory, the analgesylmeter test to access pain hypersensitivity and the animals were observed for neurobehavioral performance. Lab analysis was carried out by homogenizing the brain matter to determine the effect of nicotine on oxidative stress, using the parameters, malondialdehyde, catalase, superoxide dismutase, glutathione reductase and advanced oxidation protein products (AOPP). The data obtained were presented as a mean value standard error of means (±sem) and statistical analysis was done using the Statistical Package for Social Sciences (SPSS) and excel and the results were significant at $P<0.05$. From the results of this study, it was demonstrated that, chronic administration of nicotine was found to significantly reduce memory capacity, caused pain hypersensitivity and also induced oxidative stress by protein oxidation (AOPP as a biomarker) or can also be a mild antioxidant by inhibiting lipid peroxidation (MDA as a biomarker). While administration of nicotine in low dose on the other hand enhanced memory, caused hyposensitivity to a mild hypersensitivity and is beneficial in eliminating neurotoxicity by reducing oxidative stress.

Keywords: Nicotine, Advanced oxidative Protein Products, pain hypersensitivity, epinephrine

Introduction

Nicotine has been found to constitute approximately 0.6–3.0% of the dry weight of tobacco. It is widely used recreationally as a stimulant and anxiolytic and as a pharmaceutical drug, it

is used for smoking cessation to relieve withdrawal symptoms [1]. Nicotine acts as a receptor agonist at most nicotinic acetylcholine receptors (nAChRs) [2]. Except at two nicotinic receptor subunits (nAChRα9 and nAChRα10) where it acts as a receptor antagonist. According to the World Health Organization, more than 1 billion people smoke and over 400 million people of these people are obese (BMI > 30km-2). Eating can be compulsive, and the neurobiological processes relating to over indulgence in food overlap with those involved in substance abuse and addition [3]. Smoking and obesity are major risk factors for many serious diseases. Eating and smoking are behavioral traits that are at least in part controlled by the same reward mechanism, [4] [5]. Recent studies suggest that nicotine may adversely affect cognitive development in adolescence, but the relevance of these findings to human brain development is disputed. At low amounts, it has a mild analgesic effect [6].

Nicotine in quantities similar to those in cigarette smoke can induce oxidative stress, as shown in vitro and in vivo [7]. Increased lipid peroxidation occurs in Chinese hamster ovary cells [8]. And pancreatic tissue of rats [9] that are incubated with nicotine. After intraperitoneal nicotine, the liver, lung and heart tissues of rats also show lipid peroxidation [10]. Furthermore, the plasma of smokers' shows increased products of lipid peroxidation [11]. The significant cognitive, intellectual and behavioral impairments in the offspring of mothers who smoke during pregnancy may be related to oxidative stress and oxidative cellular injury [12]. However, nicotine may also have antioxidant potential and neuroprotective effects. Research studies about the effects of chronic nicotine exposure have revealed great risk to the oxidative state, indicating increase in the oxidative stress, neurotoxicity, lipid peroxidation and DNA injury [8]. Drug abuse has been a major problem amongst adolescents, adults and even minors in this present day and age. In recent times, most smokers have believed that smoking cigarettes helps them to feel on top of the world thereby making them less sensitive to pain. Since nicotine is the major constituent in cigarette and there are fewer studies about the antioxidant power of nicotine as regards oxidative stress and pain hypersensitivity, it is better to understand the effects of nicotine on neurodevelopment, its neuroprotective properties, as well as its influence on learning and memory and pain hypersensitivity.

Materials and Methods

COLLECTION OF EXPERIMENTAL ANIMALS

25 adult albino rats weighing between 80-180g were purchased from the animal house of the Department of Pharmacology, Faculty of Basic Medical Science, University of Port Harcourt, where they were also housed. The house was maintained at 23ºC and 50% relative humidity, with a 12h/12h light/dark cycle. Based on the treatments, the rats of matching weights were divided into 5 groups of 5 rats each, which comprised of Control Group (Group 1), Low Dose Nicotine Group (Group 2), Medium dose Nicotine group (Group 3), High Dose Nicotine Group (Group 4) and the Epinephrine treated group (Group 5). These groups of rats housed in plastic cages and allowed to feed on rodent chow ad libitum with Top feed Finisher mash and clean water. The experiment was conducted in accordance with the National Institute of Health Guild for the care and use of laboratory animals. These guides were approved by the institutional ethics committee for animal experiment.

ACCLIMATIZATION OF ANIMALS

The rats were acclimatized for a period of one week at the animal house and pre-exposed to the laboratory environment. This was done to enable the rats adapt to the environment and to also observe for overt signs of ill health before the commencement of the experiment under standard laboratory condition.

PREPARATION OF TREATMENTS

- A solution containing 0.5% (0.5ml/100ml) of raw liquid nicotine was prepared from Nicotine ([-]-1-Methyl-2-[3-Pyridyl] pyrrolidine), EC No. 200-195. Produced by SIGMA-ALDRICH CO. After preparation, it was kept at a temperature of 2-8ºC during usage.
- 0.1ml of Epinephrine drug was diluted into 100ml of distilled water.

EXPERIMENTAL DESIGN

The experimental rats were divided into five (5) groups, with 5 rats per group and treated for a period of twenty-one (21) days.

Group 1: Control group

Group 2: Low dose nicotine treated

Group 3: Medium dose nicotine treated

Group 4: High dose nicotine treated

Group 5: Epinephrine treated

Nicotine was administered to the Wister rats through sniffs. Each rat was exposed to nicotine by inhalation. According to Grant and Makintosh (1963), each sniff contains 25µg/kg of Nicotine.

- 50µg/kg (2 sniffs) of nicotine was administered through inhalation to group 2 daily
- 75µg/kg (3 sniffs) of nicotine was administered through inhalation to group 3 daily
- 100µg/kg (4 sniffs) of nicotine was administered through inhalation to group 4 daily
- 0.1ml 0f epinephrine drug was administered intravenously to group 5 daily

ELECTROCONVULSIVE THERAPY (ECT) TEST

The electroconvulsive therapy test was used to administer shock in the rats, intentionally triggering a brief seizure after small electric current was passed. Each wistar rat received ECT once daily for the period of the experiment (0.5mA, for 2s with pulse width 0.2ms) via placement of the electrode earclips on each ear of the wistar rat and electric current switched on. The rats were monitored after treatment to ensure that it had brief seizure indicating that it received the shock. The testing was performed in three trials per session which lasted for a period of three weeks.

PASSIVE AVOIDANCE TEST

The passive avoidance test was used to evaluate learning and memory in the rats. The passive avoidance box consists of two compartments (25x15x15cm high), one illuminated and one dark and both equipped with a grid floor. The two compartments were separated by a sliding door. In the acquisition trials, each rat was placed in the illuminated (light) compartment and was voluntarily allowed into the dark compartment using the door. When the rat entered the dark compartment, a foot shock was applied through the grid floor and the rat reacted by either escaping back into the light compartment or by freezing itself in the dark compartment. When the rat moves back to the light compartment, a timer is used to record the retention latency until

300 seconds had elapsed. The retention latency is the time it takes for the rat to move back from the light compartment into the dark compartment after it had received a foot shock. The testing was performed in three trials per session which lasted for a period of three weeks.

ANALGESY-METER TEST

The analgesy-meter test was used to evaluate the pain hypersensitivity in the wistar rats. After each rat completed the passive avoidance test, they were individually subjected to pain using the analgesy-meter apparatus by placing the sharp and pointed part of the analgesy-meter on the finger of the rat. The pain threshold of each rat was recorded when the rat removed its hand from the grip. The testing was performed in three trials per session which lasted for a period of three weeks.

SAMPLE COLLECTION

At the end of the experiment after chronic nicotine exposure (3 weeks), 2 rats from each group were randomly selected and deeply anesthetized using chloroform by inhalation. A cardiac puncture was carried out immediately and the brain tissue was carefully removed and emptied into a plain bottle. The brain tissue was stored with ice.

Homogenization of brain tissue and collection of supernatant

0.5g of the brain tissue was homogenized under a pH of 7.4 using a buffer. The homogenate was centrifuged using a cold centrifuge at 4ºC at 5000Rpm for 10 minutes to get the supernatant. The supernatant was stored in a refrigerator until biochemical analysis.

SAMPLE ANALYSIS

Determination of Malondialdehyde (MDA)

Malondialdehyde, which is the final product of lipid peroxidation was determined using the method Ohkawa et.al., (1979).

Preparation of Reagent:

A mixture of 7.5g of Trichloride acetic acid (TCA) (15%), 0.3g of thiobabituric acid (TBA) (0.65%) and 1.015g of Hydrochloric acid (HCl) (0.25%) was used to make a whole solution of 50ml. That is, distilled water was added to the mixture to bring the total volume to 50ml. The mixture was subjected to heat to dissolve the solution.

Procedure:

0.3ml of sample was emptied into a test tube. 3ml of the reagent (MDA) was added into the test tube and was boiled for 15 minutes. The absorbance was measured at 532nm using a spectrophotometer.

Determination of Catalase

Materials:

0.05M of sodium potassium phosphate buffer with pH of 7.2 and 0.036M of hydrogen peroxide (H_2O_2).

Preparation of buffer:

0.8g of disodium hydrogen phosphate was added to 50ml of distilled water to make solution A. 0.7g of potassium dihydrogen phosphate (K_2HPO_4) was added into 50ml of distilled water to make solution B. Solution B was emptied into the beaker containing solution A and measured to a pH of 7.3.

Preparation of hydrogen peroxide:

0.3g of 6% H_2O_2 was emptied into a beaker and distilled water was added to make a whole solution of 50ml in volume.

Procedure*:*

A mixture of 100µl of sample and 4ml of buffer was created. The mixture was read at 480nm at every 30seconds and 3 minutes using a spectrophotometer.

Determination of Superoxide dismutase (SOD)

Materials:

0.05M Carbonate buffer with pH, 10.2.

Preparation of Carbonate buffer:

For a 50ml carbonate buffer, distilled water was added to a mixture of 0.05g of sodium bicarbonate ($Na_2\ HCO_3$) and 0.45g of sodium carbonate monohydrate ($Na_2\ CO_3$) to sum it up to a volume of 50ml.

Procedure:

100µl of sample was mixed with 4ml of buffer and incubated at 37ºC for 5 minutes. The reaction started by adding 100µl of adrenaline and immediately read at 480nm at every 60 seconds interval. That is, the reaction was read at 30 seconds and 1:30 seconds by the use of a spectrophotometer.

Determination of Glutathione reductase

750µl of tissue supernatant was mixed with 750µl 4% sulfosalicylic acid. The mixture was incubated in the refrigerator for 20 minutes and centrifuged at 5000rpm for 10 minutes at 4ºC. The supernatant was transferred into a test tube. 500µl of 0.1M phosphate buffer (pH of 7.4) and 500µl of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) was added to the supernatant. The resultant mixture was read at 412nm including set of standards and control.

Determination of Advanced oxidative protein products (AOPP)

400µl of sample was diluted with 1600µl of phosphate buffer saline (BPS). 200µl of 1.16M potassium iodide (KI) was added to start the reaction. The reaction was stopped after 5 minutes with 100µl of acetic acid. Standards were prepared with Chloroamine-T. The samples and standards were read at 340nm using a spectrophotometer.

STATISTICAL ANALYSIS

Data obtained from the study were expressed as mean ± SEM. The differences between each group were analyzed using ANOVA (Analysis of Variance). Values of $P < 0.05$ were considered significant with a confidence level of 95. The analysis was done using Statistical Packages for Social Sciences (SPSS version 23).

Results

Table 1 Pattern of memory test analysis of the test groups as compared to the control group during the three weeks of Passive Avoidance test

Groups	Treatment	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
1	Control	300.00 ±00	130.00 ±69.57	135.00 ±67.75	180.00 ±73.49	179.40 ±69.88	180.00 ±73.49	180.00 ±73.49	180.00 ±73.49	240.00 ±60.00
2	50µg/kg nicotine	240.18 ±59.82	240.00 ±60.00	240.00 ±60.00	284.40 ±15.60	299.40 ±0.60	300.00 ±00	195.04 ±65.35	228.40 ±45.81	240.00 ±60.00
3	75µg/kg nicotine	240.00 ±60.00	240.00 ±60.00	219.00 ±58.40	240.00 ±60.00	240.00 ±60.00	240.00 ±60.00	240.00 ±60.0	120.09 ±73.45	231.00 ±58.40
4	100µg/kg nicotine	120.00 ±73.49*	180.00 ±73.49	165.60 ±68.87	180.00 ±73.49	120.00 ±73.49	120.00 ±73.49	180.00 ±73.49	180.00 ±73.49	180.00 ±73.49
5	Epinephrine	180.00 ±73.49	180.00 ±73.49	180.00 ±73.49	180.00 ±73.49	180.00 ±73.49	180.00 ±73.49	180.00 ±73.49	180.00 ±73.49	180.00 ±73.49

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All values are presented as mean ± sem. N=5. * means values are statistically significant when compared to the control values.

Table 2 Pattern of pain hypersensitivity analysis of the test groups as compared to the control group during the three weeks of Analgesy-meter test.

Analgesy-meter Test (g ± sem)

		Week 1			Week 2			Week 3		
Groups	**Treatment**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**
1	**Control**	18.04 ±4.37	21.36 ±3.64	25.00 ±0.00	22.62 ±2.28	18.30 ±4.55	13.64 ±3.01	21.40 ±3.60	25.00 ±0.00	23.70 ±1.30
2	**50µg/kg nicotine**	18.00 ±3.01	15.54 ±4.64	25.00 ±0.00	19.94 ±3.12	23.20 ±1.10	24.74 ±0.26*	21.40 ±3.60	25.00 ±0.00	23.70 ±1.30
3	**75µg/kg nicotine**	14.56 ±4.33	15.10 ±4.27	11.62 ±4.91*	18.00 ±3.16	19.80 ±2.93	19.72 ±2.86	22.80 ±1.65	23.62 ±1.38	25.00 ±0.00
4	**100µg/kg nicotine**	19.90 ±4.98	5.42 ±1.45*	15.00 ±4.85*	17.24 ±5.07	19.90 ±4.98	20.00 ±5.00	16.24 ±4.59	18.86 ±4.82	18.30 ±4.85
5	**Epinephrine**	25.00 ±0.00	25.00 ±0.00	25.00 ±0.00	25.00 ±0.00	25.00 ±0.00	25.00 ±0.00*	25.00 ±0.00	25.00 ±0.00	25.00 ±0.00

All values are presented as mean ± sem. N=5. * means values are statistically significant when compared to the control values.

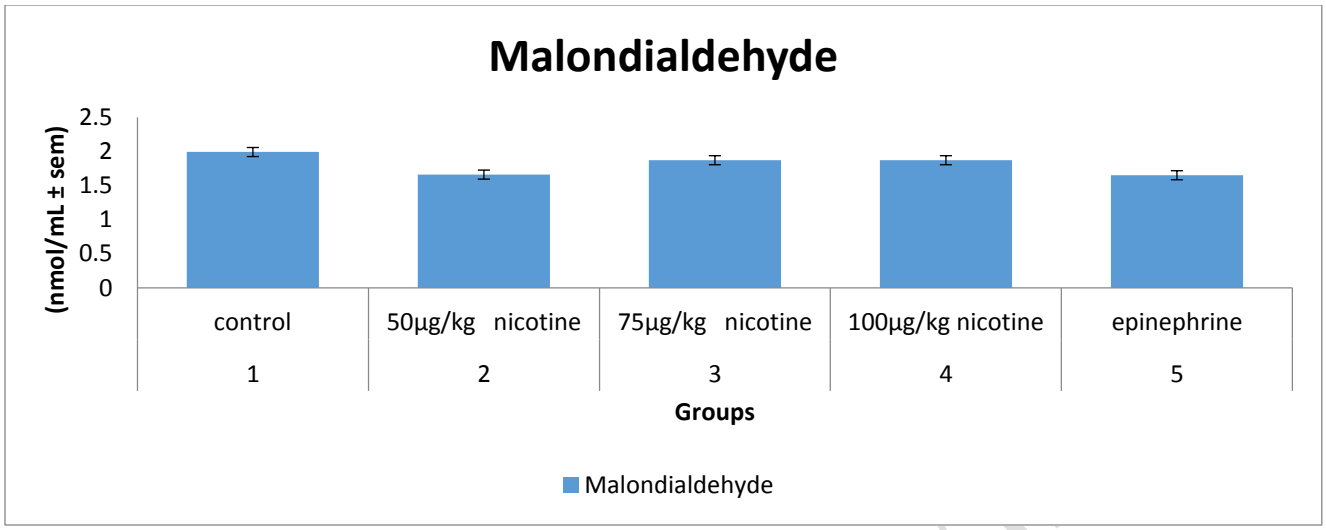

Figure 1. Analysis of oxidative stress of the test groups during the Malondialdehyde test, caused by repeated nicotine treatment and epinephrine drug

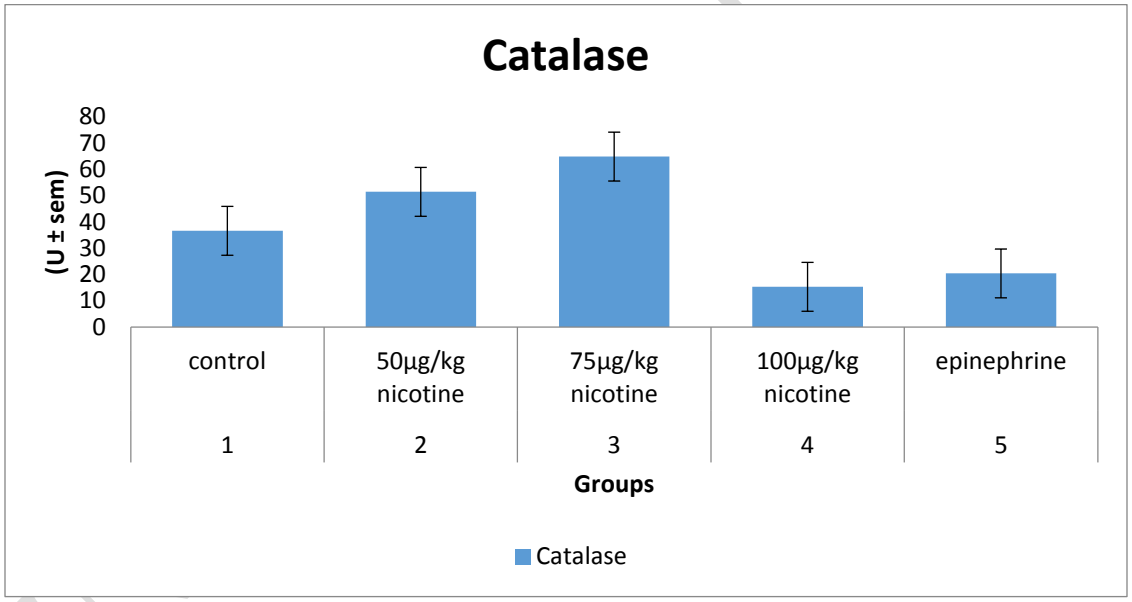

Figure 2 Analysis of oxidative stress of the test groups during the Catalase test, caused by repeated nicotine treatment and epinephrine drug.

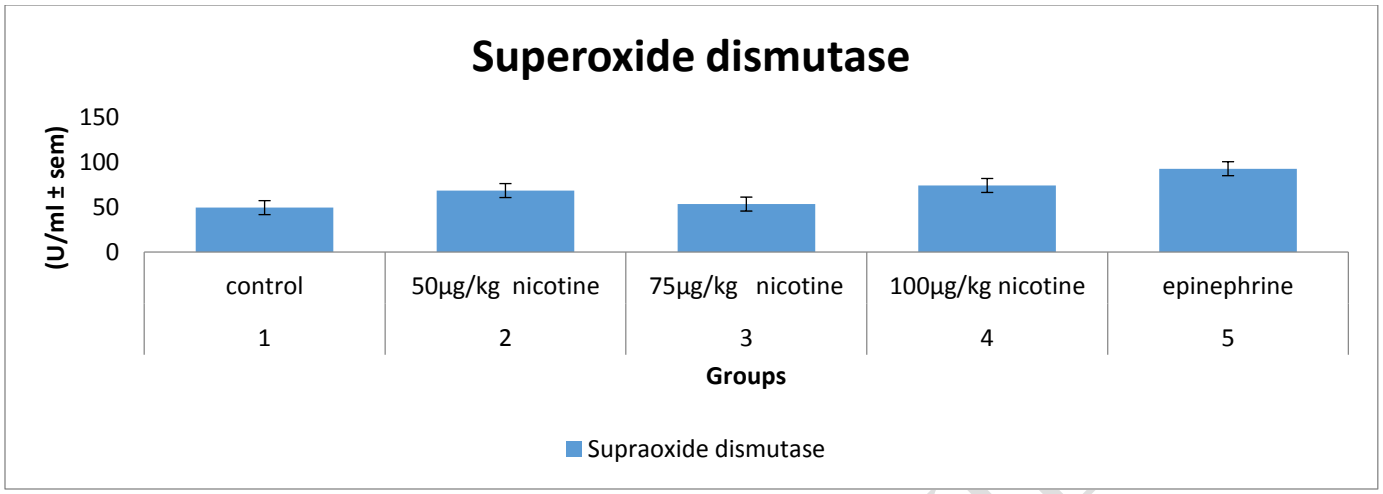

Figure 3 Analysis of oxidative stress of the test groups during the Superoxide dismutase test, caused by repeated nicotine treatment and epinephrine drug.

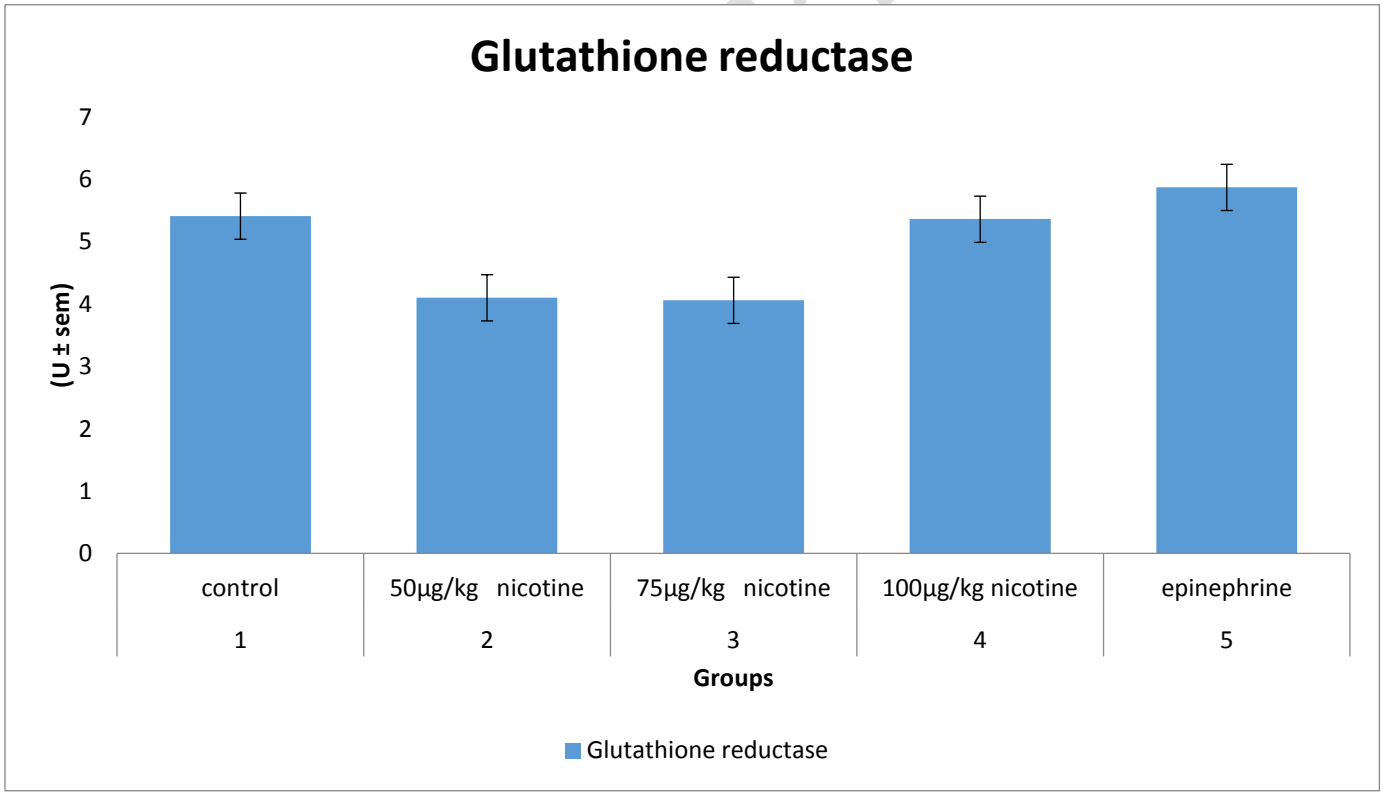

Figure 4 Analysis of oxidative stress of the test groups during the Glutathione reductase test, caused by repeated nicotine treatment and epinephrine drug.

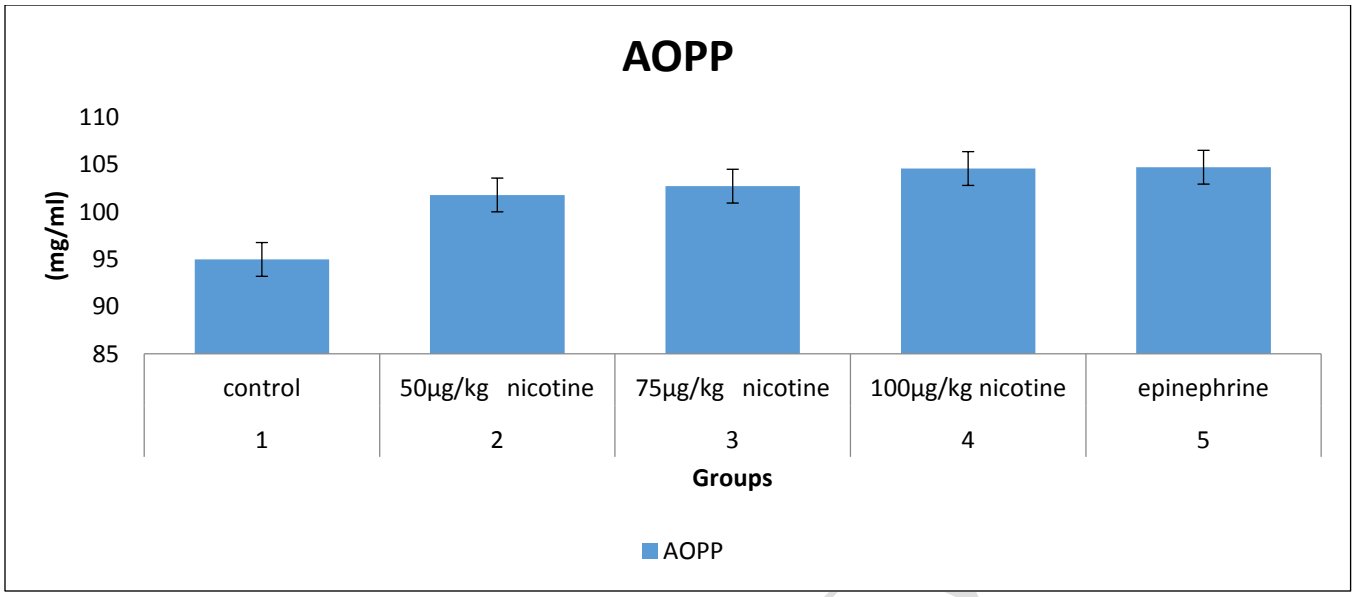

Figure 5 Bar chart showing analysis of protein oxidation of the test groups during the AOPP test, caused by repeated nicotine treatment and epinephrine drug.

Discussion

The present study evaluated the chronic effects of nicotine on pattern of advanced oxidative protein products and pain hypersensitivity in wistar rats. During the course of the research, behavioral alterations indicating the effects of nicotine on learning and memory were observed in the passive avoidance test. The ability of the wistar rats to withstand pain after treated with varying degrees of nicotine drug was observed in the analgesy-meter test. Also, the effects of chronic administration of nicotine on oxidative stress and advanced oxidation protein products were determined at the end of the study. The passive avoidance test is a fear-aggravated test used to evaluate learning and memory in rodent models. The test was conducted in a two-compartment box: one light (safe) compartment and one dark (unsafe) compartment, connected by a sliding door. During training, the rat was placed in the light compartment and after a defined time interval, gained access to the dark compartment (training latency), where it was subjected to a brief aversive stimulus (unconditioned stimulus, US; foot shock) [13]. A retention test was conducted later, where the rat was returned to the light compartment and the latency to enter the shock compartment, was measured. This measure (retention latency) was used to infer the animal's memory for the fearful experience. The longer the retention latency, the better the

memory. Multiple studies have shown that nicotine has a positive effect on memory and learning in humans and non-human animals, and one of the areas of the brain most affected by nicotine administration is the hippocampus. The hippocampus plays a key role in learning and memory, particularly learning and memory involving spatial information [14]. In the first week of the passive avoidance test, the results showed high retention latencies in Group 2 and Group 3 as compared to other test groups with the exception of the first trial of the control group (300.00±00) (Table 1), although there was no significant change. This result shows that the administration of nicotine in low and medium doses improved the memory and is in line with [32], who proposed that experiments performed with rats in a passive avoidance test demonstrated that both chronic and acute nicotine administration had cognitive-enhancing effects as a result of 7-nAChR stimulation in the hippocampus, thus, improving memory. Release of glutamate by nicotine helps to improve learning and memory. Also, there was a significant decrease in the retention latency of rats in Group 4 (Trial 1) as compared to the control group. This is in line with the report that high doses of nicotine do not demonstrate nicotine's positive effects due to Acetylcholine receptor desensitization. Receptor desensitization occurs when a neurotransmitter binds to a receptor but the ion channel, through which positively charged ions would normally travel to lead to an excitatory postsynaptic potential (EPSP), remains closed. Thus, though the neurotransmitter is bound to the receptor, an action potential does not occur and the neuron does not fire. Because of this desensitization (which occurs when rats are administered high levels of nicotine 100µg/kg/day for this particular study), important receptors involved with the release of both glutamate and dopamine will be affected and the effect of the nicotine on memory and learning will be inhibitory [15]. For this reason, it makes sense that large amounts of nicotine may actually decrease neurogenesis in the brain. Lower doses of nicotine, for example, 50µg/kg/day, did not show this desensitization, and thus still shows cognitive enhancement in spatial learning studies [16].

Correspondingly, from Table 1, the result suggests that administration of epinephrine to the rats in week one had an enhancing effect on memory consolidation of the epinephrine group. This is because although epinephrine does not readily cross the blood–brain barrier, its effects on memory consolidation appear to be initiated by activation of b-adrenoceptors located on vagal afferents in the periphery that project to noradrenergic cell groups within the nucleus of the solitary tract (NTS) in the brainstem [17][18]. These noradrenergic cells directly innervate brain

regions involved in memory consolidation, including the amygdala. The NTS may also influence noradrenergic activation via its projection to the locus coeruleus (LC), which has noradrenergic cells with more widespread projections to forebrain regions, including the hippocampus, cortex, and amygdala. Also, epinephrine which is a stress hormone can modulate the memory strength for one-trial Passive Avoidance training when injected after training at the time when they are normally released by the adrenal glands following an emotional experience.

From the analysis carried out in week two, it showed that there was a contrast in the memory capacities of the test groups as compared to the control group. Group 2 (284.40 ±15.60, 299.40 ±0.60, 300.00 ±00, for Trials 1, 2, and 3 respectively) showed an insignificant increase in the retention latencies of the test group. Group 3 (75µg/kg nicotine) maintained almost the same result from week one. That is, there was a slight improvement on memory of the rats. Rats treated with epinephrine (Group 5) showed no significant difference when compared to the control group. The high dose nicotine group (Table 2) showed an insignificant decrease in the memory capacity of the rats as compared to the control group. This alteration was seen particularly in Trial 2 (120.00 ±73.49) and Trial 3 (120.00 ±73.49) of the high dose nicotine group.

In the third week of the passive avoidance test, the result showed no significant difference in the test groups when compared to the control group although there were contrasting retention latencies. Group 4 and Group 5 treated with high dose nicotine and epinephrine respectively, showed little or no changes. In addition to desensitization of nicotinic receptors, chronic administration of nicotine caused seizures, involuntary muscle twitching, as well as abnormal heart rhythms, a slow heart rate and fluctuating blood pressure.

The analgesy-meter test was used to access the pain hypersensitivity in wistar rats. In the first week of the analgesy-meter test (Figure 2), the result showed that Group 2 had no significant change when compared to the control group. Group 3 showed a slight decrease in the mechanical sensory threshold in the 3rd trial (11.62 ±4.91) compared to the control group. This indicates that acute administration of nicotine caused a very mild sensitivity to pain. Also, Group 4, more evident in Trial 2 (5.42 ±1.45) showed a significant decrease in the mechanical sensory threshold. This result shows that the administration of nicotine in chronic states caused a more intense pain hypersensitivity of the wistar rats.

As the week progressed, that is, in the second week of nicotine administration, the result showed that Group 2 had the highest mechanical sensory threshold when compared to the control group. This indicates that rats in group 2 were hyposensitive to pain. Also, administration of epinephrine in week 2 (Trial 3) showed a significant increase in the mechanical sensory threshold indicating pain hyposensitivity.

From the result of the analgesy-meter test, it can be deduced that short-term or acute nicotine exposure causes an increase in mechanical sensory threshold which is also termed hyposensitivity. This is due to the fact that acute nicotine exposure produces analgesic effect by increasing ɣ-aminobutyric acid (GABA), a major inhibitory neurotransmitter which exerts an inhibitory influence on pain pathways and by affecting the expression of glutamate decarboxylase 67 (GAD67), (GAD65), endorphins and μ-opioid receptors (MOR). GAD67 and GAD65 are enzymes that synthesize glutamic acid to GABA. Endorphins are natural pain killers in the body which inhibit the transmission of pain signals by binding to μ-receptors, thus blocking the release of the neurotransmitter, substance P. Also, chronic nicotine exposure causes increased pain hypersensitivity (hyperalgelsia) leading to chronic pain by decreasing the expression of GAD67, GAD65, MOR and GABA. This is in line with [19].

Nicotine induced oxidative stress in the brain is determined by an increase in malondialdehyde (MDA) level. This is because MDA is an indication of lipid peroxidation. It is a key index of membrane pathology, because it is formed from the decomposition of primary and secondary lipid peroxidation. That is, it is the end product of lipid peroxidation. From the result gotten in Figure 1, it shows a slight reduction in the malondialdehyde level in Group 2 (1.66 ±0.03), Group 3 (1.87 ±0.02) and Group 4 (1.87 ±0.06) when compared to the control group (1.99 ±0.16), with the most significant being group 2. This explains that nicotine exposure in both acute and chronic doses did not induce oxidative stress and thus, indicating that nicotine reduces lipid peroxidation. This supports the neuroprotective effect of nicotine [20]. This MDA decrease was also evidently seen in the epinephrine group (1.65 ±0.00) indicating the absence of oxidative stress.

On the other hand, Figure 4 showed that administration of nicotine in low and medium doses increased the catalase activity as compared to the control group. There were significant increase in groups 2 and 3 and a significant decrease in the group that had a high dose of nicotine, Group

4 (15.29 ±2.27) when compared to the control group (36.60 ±7.38). This shows that chronic nicotine administration induces oxidative stress because the role of catalase is to scavenge the hydrogen peroxide (H_2O_2) that was generated by free radicals.

Figure 3 showed an increase in the level of Superoxide dismutase (SOD) in low, medium and high doses of nicotine, with the most significant in Group2 (68.33 ±2.04) and Group 4 (high dose nicotine group) (74.00 ±4.49) as compared to the control group (49.33 ±4.49). Due to increased SOD levels in all groups administered with nicotine, it can be deduced that nicotine did not cause oxidative stress. This is because SODs are proteins that constitute a very important antioxidant defense against oxidative stress in the body [38]. They help the body fight against reactive oxygen species (ROS)-mediated injury by catalyzing the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen and hydrogen peroxide (H_2O_2) thereby decreasing O_2^- level which damages the cells at excessive concentration. Contrastingly, Figure 4.10 showed a significant decrease in the glutathione reductase levels in groups 2 and 3 and an increase in group 5 (epinephrine group) when compared to the control group. A decreased glutathione level thus, indicates the presence of free radicals in the brain tissue, which leads to oxidative stress. This is due to the fact that glutathione reductase catalyzes the reduction of glutathione disulfide (GSSG) to glutathione (GSH), which plays a role in maintaining the reducing environment of the cell by resisting oxidative stress.

Also, from the result obtained in Figure 4.11, it shows a progressive increase in the AOPP level from Group 2 (101.79±0.65), Group 3 (102.72 ±0.00) and Group 4 (104.58 ±3.76) in comparison to the control group (94.98 ±6.70). This suggests that the AOPP increased as the nicotine dose increased. This significant increase of the AOPP levels is a clear indication that a chronic low dose of nicotine induces oxidative stress through protein oxidation in the brain tissue of rat. In comparison with the epinephrine group, there was also an increase in the AOPP level, hence, oxidative stress occurred.

CONCLUSION

From the results of the study, it can be deduced that administration of epinephrine can significantly enhance the memory of the rats, decrease pain hypersensitivity, and can induce oxidative stress through protein oxidation, although it can be a potent antioxidant by reducing

lipid peroxidation. Also, chronic administration of nicotine can significantly reduce memory capacity, increase pain hypersensitivity, induce oxidative stress through protein oxidation (AOPP as a biomarker) and can also be a mild antioxidant, by reducing lipid peroxidation (MDA as a biomarker). While administration of nicotine in low dose on the other hand can significantly enhance memory, causes hyposensitivity to a mild hypersensitivity and is beneficial in eliminating neurotoxicity by reducing oxidative stress.

In conclusion, the daily administration of nicotine in high doses from these results points to a significant negative effect of nicotine on memory, pain hypersensitivity and to some extent, oxidative stress. Therefore, it is advisable for individuals to minimize their nicotine intake as chronic exposure poses great risk to human health.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCE

1. Sajja R.K., Rahman S., Cucullo L. "Drugs of abuse and blood-brain barrier endothelial dysfunction: A focus on the role of oxidative stress". *Journal of Cerebral Blood Flow and Metabolism*. 2016; 36 (3): 539–54.
2. Landoni J.H. "Nicotine (PIM)". INCHEM. *International Programme on Chemical Safety*. 2019; 7 (9): 811–9.

3. Volkow N.D., O'Brien C.P. Issues for DSM-V: should obesity be included as a brain disorder. *American Journal of Psychiatry*; 2007; 164:708–710.
4. Haslam D.W., James W.P. Obesity. *Lancet*; 2005; 366:1197–1209.
5. Volkow N.D., Wang G.J., Fowler J.S., Telang F. Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. *Philos Trans R Soc Lond B Biol Sci*; 2008; 363:3191–3200.
6. Mayer B. "How much nicotine kills a human? Tracing back the generally accepted lethal dose to dubious self-experiments in the nineteenth century". *Archives of Toxicology*. 2014; 88 (1): 5–7.
7. Caponnetto P., Campagna D., Papale G., Russo C., Polosa R. "The emerging phenomenon of electronic cigarettes". *Expert Review of Respiratory Medicine*. 2012; 6 (1): 63–74.
8. Smith, T.T., Rupprecht, L.E,. Cwalina, S.N., Onimus, M.J., Murphy, S.E., Donny, E.C., Sved, A.F. "Effects of Monoamine Oxidase Inhibition on the Reinforcing Properties of Low-Dose Nicotine". *Neuropsychopharmacology*. 2016; 41 (9): 2335–2343.
9. Jain R., Mukherjee K., Balhara Y.P. "The role of NMDA receptor antagonists in nicotine tolerance, sensitization, and physical dependence: a preclinical review". *Yonsei Medical Journal*. 2008; 49 (2): 175–88.
10. Miyasato K. "Psychiatric and psychological features of nicotine dependence". Nihon Rinsho. *Japanese Journal of Clinical Medicine*. 2013; 71 (3): 477–81.
11. Parrott A.C. "Nicotine psychobiology: how chronic-dose prospective studies can illuminate some of the theoretical issues from acute-dose research". *Psychopharmacology*. 2006; 184 (3–4): 567–76
12. Parrott A.C. "Cigarette-Derived Nicotine is not a Medicine". *The World Journal of Biological Psychiatry*. 2003; 4 (2): 49–55.
13. Schraufnagel D.E. "Electronic Cigarettes: Vulnerability of Youth". *Pediatric Allergy, Immunology, and Pulmonology*. 2015; 28 (1): 2–6.
14. Solak Z.A., Kabaroglu C., Cok G., Parildar Z., Bayindir U., Ozmen D. *et al*. Effect of different levels of cigarette smoking on lipid peroxidation, glutathione enzymes and paraoxonase 1 activity in healthy people. *Clin Exp Med.* 2005; 5: 99–105.

15. Yildiz D., Ercal N., Armstrong D.W. Nicotine enantiomers and oxidative stress. *Toxicology*. 1998; 130: 155–165.
16. Wetscher G.J., Bagchi M., Bagchi D., Perdikis G., Hinder P.R., Glaser K. *et al*. Free radical production in nicotine treated pancreatic tissue. *Free Radic Biol Med.* 1995; 18: 877–882
17. Helen A., Krishnakumar K., Vijayammal P.L., Augusti K.T. A comparative study of antioxidants S-allyl cysteine sulfoxide and vitamin E on the damages induced by nicotine in rats. *Pharmacology*. 2003; 67: 113–117.
18. Kharb S., Singh G.P. Effect of smoking on lipid profile, lipid peroxidation and antioxidant status in normal subjects and in patients during and after acute myocardial infarction. *Clin Chim Acta*. 2000; 302: 213–219.
19. Ernst M, Moolchan E.T., Robinson M.L. Behavioral and neural consequences of prenatal exposure to nicotine. *J Am Acad Child Adol Psychiat*. 2001; **6**: 630–641.
20. O¨ gren S.O. Evidence for a role of brain serotonergic neurotransmission in avoidance learning. *Acta Physiol Scand*., 1985; 125(544):1–71
21. Izquierdo, I. (1995). The role of the hippocampus, amygdala, and entorhinal cortex in memory storage and expression.
22. Uzum G., Diler A.S., Bahcekapili N., Tasyurekli M., Ziylan Y.Z. Nicotine improves learning and memory in rats: morphological evidence for acetylcholine involvement. *Int J Neurosci, 2004; 114*(9), 1163-1179.
23. Benwell M.E., Balfour D.J., Birrell C.E. Desensitization of the nicotine induced mesolimbic dopamine responses during constant infusion with nicotine. *Br J Pharmacol, 1995; 114*(2), 454-460.
24. Schreurs J., Seelig T., Schulman H.. Beta 2-adrenergic receptors on peripheral nerves. J. *Neurochem*. 1986; 46 (1), 294–296