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Vitamin E improves growth rate and reproductive functions in female rats exposed to nicotine

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ABSTRACT

This study investigates the effect of vitamin E on the deleterious effects of nicotine on female reproductive functions in rats. 56 albino wistar rats (42 female, 14 male) were used for the study. The female rats with synchronized ovulation were randomly selected into 7 groups. Group 1 received 0.2ml normal saline (control), groups 2 and 3 received 0.5mg/kg and 1.0mg/kg of nicotine respectively, group 4 received 0.5mg/kg of nicotine with 10mg/kg of vitamin E, group 5 received 1.0mg/kg nicotine and 10mg/kg Vitamin E, groups 6 and 7 were recovery for groups 2 and 3 respectively. All administrations were done orally. The weights of the female animals were monitored weekly throughout the experiment. At the 31st day, male rats were introduced to female in ratio 1:3 and treatments were discontinued in groups 6 and 7. The presence of sperm plug in vagina served as indicator for positive copulation and the day was taken as the first day of pregnancy. On the 13th day of pregnancy, animals in all groups were sacrificed and blood was collected for determination of plasma progesterone and estrogen levels. The brain, heart, kidney, liver, lungs, ovaries, uterine tube and vagina were harvested and weighed. Uterus was dissected to count the number of implantation sites. There was a significant reduction in body growth rate in the high dose nicotine treated group. Heart, lungs and vagina of all nicotine only treated groups were significantly decreased in weight when compared with the control. Also implantation sites were significantly decreased in both the nicotine treated rats and their recovery groups. The ratios of plasma estrogen to progesterone were significantly increased in nicotine groups, their recovery groups and high dose of nicotine with vitamin E when compared with control. In conclusion, vitamin E was able to minimize the deleterious effects of nicotine on body weight, reproductive organs and implantation in female.

1. Introduction

Nicotine is the principal alkaloid contained in tobacco. It is widely consumed through cigarette smoking and tobacco chewing in 30-40% of the world's population [1]. Within the body, nicotine is oxidized to its metabolite cotinine, which has a long half-life, and both the nicotine and cotinine adversely affected spermatogenesis, epididymal sperm count, motility, and the fertilizing potential of sperms [2].

Epidemiological studies have indicated that cigarette smoking has deleterious effects on visceral tissues lowers fertility in women [3, 4]. Along with its constituents particularly nicotine, cigarettes are toxicants affecting all stages of reproductive functions [5]. It has been associated with premature births, low birth weight in babies carried to full term, preterm-related deaths, deaths caused by sudden infant death syndrome [6, 7], decreased normal body weight gains with significant increases upon cessation in female rats [8] and effect on some visceral organs [9].

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The deleterious effects of nicotine are, at least in part, due to the increased production of reactive oxygen species (ROS) [10, 11]. Physiologic relevance of ROS is implicated in oocyte maturation, ovarian steroidogenesis, ovulation, implantation, formation of blastocyst, luteolysis and luteal maintenance in pregnancy [12, 13, 14]. An imbalance in the pro-oxidants (ROS) and the body's scavenging activity results in oxidative stress which exerts its pathological effects by various mechanisms including lipid damage, inhibition of protein synthesis, and depletion of ATP [15].

Vitamin E is a potent antioxidant that is useful in the body to maintain redox homeostasis and has been reported to have protective effect against endogenous oxidative DNA damage and membrane damage [16]. It is thus assumed that the application of this antioxidant as probable preventive agent could be targeted in therapeutic amelioration of nicotine-induced abnormalities.

Therefore, this study is aimed at investigating the effect of vitamin E on body weight, visceral and reproductive organs weights, implantation sites, plasma estrogen and progesterone level in nicotine treated female rats.

2. Materials and Methods

2.1. Nicotine Preparation

Nicotine hydrogen tartrate with product number 26140 (95% nicotine) was obtained from BDH chemical Ltd Poole England. Nicotine stock solution was prepared at concentration of 1mg/ml and it was stored in foil-wrapped glass bottle 40C for no longer than ten days.

2.2.Animals

Wistar strain albino rats (150-220g) obtained from the Central Animal House, College of Medicine, University of Nigeria, Enugu campus were used for this study. They were housed in wire mesh cage with free access to feed and water ad libitum. Animals were also maintained in a well ventilated room with a 12:12-hour light-dark at room temperature. The experiment was conducted in accordance with the Guidelines of the U.S National Institute of Health (NIH) on the care and use of laboratory animals. The experimental procedure started after acclimatizing the rats for one week in the Animal House, Faculty of Basic Medical Sciences, Madonna University, Elele.

2.3.Experimental procedures and Methods

42 female and 14 male rats were used for the study. The female rats of synchronized ovulation were randomly and equally divided into 7 groups. Animals in group 1 served as control and were given 0.2ml of normal saline. Group 2 and 3 were treated with 0.5mg/kg (low) and 1.0mg/kg of nicotine (high) per body weight respectively. Group 4 was treated with 0.5mg/kg of nicotine with 10mg/kg of vitamin E. Group 5 received 1.0mg/kg of nicotine with 10mg/kg of vitamin E. While group 6 and 7 (recovery groups) received treatment in similar way to group 2 and 3 respectively, but the treatment was withdrawn on day thirty. The vehicle and drugs were administrated daily via oral route for forty-three days in groups 1 to 5, and thirty days in groups 6 and 7.

After 30 days of treatment, male rats were introduced to the female in ratio 1:3, (i.e. 1 male to 3 female). The vaginal smears were examined daily in the morning between 8-10a.m for the presence of sperm plug, which indicates positive copulation. The animals which showed thick clumps of spermatozoa in the vaginal smear were separated and that day was designated as Day 1 of pregnancy. Body weight of the animals was monitored weekly throughout the period of the study and growth rate per week was calculated according to Iranloye and Bolarinwa [9] as;

$$\frac{\text{Weight by week 5} - \text{Weight by week 1}}{5}$$

On the 13th day of pregnancy, the animals were injected 0.3ml of 0.5% Evans blue dye through the tail vein, stabilized for 15 minutes post injection and;

1. Blood samples were collected through cardiac puncture into heparinized tubes at 4000rmfor 15 minutes. Plasma was collected into plain tubes for estrogen and progesterone assay using Enzyme Linked Immunosorbent Assay (ELISA) method.
2. The animals were dissected and uteri were opened to ascertain implantation sites as described by Iranloye and Owokunle [17]. The dye sites were counted and recorded as implantation sites per rat.
3. The brain, heart, kidneys, liver, lungs, uterine tubes, ovaries and vagina were harvested and weighed.

2.4.Statistical Analysis

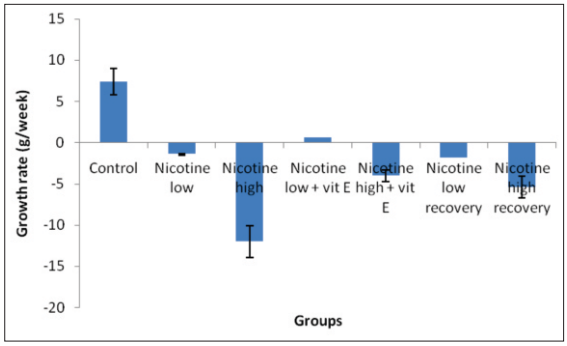
All statistical analysis was performed using one way analysis of variance (ANOVA) followed by the LSD post hoc tests for pair-wise comparisons were performed using SPSS 17.0 version. All data were expressed as Mean ± Standard Error of mean (SEM) and $p<0.05$ was considered significant.

3. Results

3.1.Effect of nicotine and vitamin E on growth rate

The growth rate of the animals was presented in figure 1. The growth rate of the animals in groups 2 to 7 was significantly reduced when compared with control ($p<0.05$). The growth rate of the animals treated with nicotine and vitamin E was improved when compared with nicotine treated groups (figure 1).

Figure 1: Effect of nicotine and vitamin E on growth rate



Values are expressed as Mean ± SEM of 6 rats per group. Bars with super script (*) are considered Significant at $p<0.05$.

3.2.Effect of nicotine and vitamin E on visceral organs weight

Table 1 revealed that there was no significant difference in the brain, kidneys, and liver weight of rats in all the groups (p>0.05), while heart and lungs weight showed significant reduction in group 2 and 4 when compared with control (p<0.05).

Table 1: Effect of nicotine and vitamin E on visceral organs weight

Brain (g)	Heart (g)	Kidneys (g)	Liver (g)	Lungs (g)
1.20±0.01	0.70±0.01	1.11±0.01	5.20±0.08	1.30±0.06
1.31±0.05	0.59±0.03*	1.15±0.05	5.36±0.22	0.94±0.04*
1.30±0.09	0.55±0.02*	1.15±0.02	4.54±0.28	0.99±0.12*
1.41±0.08	0.70±0.04	0.99±0.33	5.70±0.41	1.30±0.14
1.36±0.06	0.66±0.04	1.17±0.03	5.20±0.25	1.20±0.10
1.30±0.09	0.67±0.05	1.14±0.05	5.29±0.40	1.21±0.07
1.34±0.08	0.64±0.02	1.04±0.03	4.75±0.09	1.22±0.05

Values are expressed as Mean ± SEM of 6 rats per group. Values with super script (*) are considered Significant at p<0.05. Group 1given normal saline (control), Group 2 was treated with 0.5mg/kg of nicotine (low), Group 3 was treated with 1.0mg/kg of nicotine (high), Group 4 rats treated with 0.5mg/kg of nicotine with 10mg/kg of vitamin E, Group 5 rats were given 1.0mg/kg of nicotine with 10mg/kg of vitamin E, Group 6 was recovery for 0.5mg/kg of nicotine, Group 7 was recovery for 1.0mg/kg of nicotine.

3.3.Effect of nicotine and vitamin E on some female reproductive organs weight

Table 2 showed that nicotine and vitamin E have no significant effect on ovaries and uterine tube weight (p>0.05), while the vagina weight was significantly reduced in rats treated with high dose of nicotine and it recovery group when compared with control (p<0.05).

Table 2: Effect of nicotine and vitamin E on female reproductive organs weight

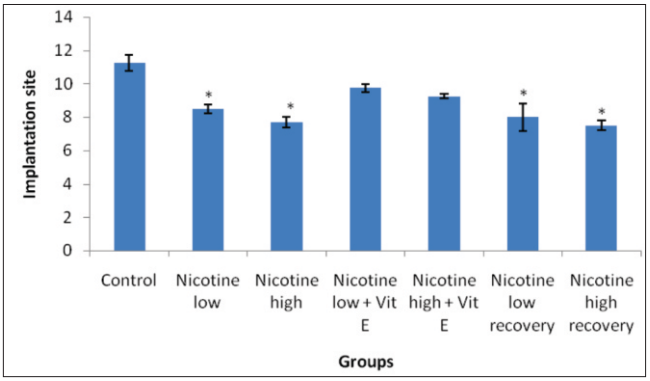
Groups	Ovaries (g)	Uterine tube (g)	Vagina (g)
1	0.10±0.01	0.34±0.01	0.08±0.00
2	0.14±0.02	0.33±0.08	0.07±0.01
3	0.13±0.01	0.32±0.03	0.06±0.03*
4	0.10±0.01	0.40±0.12	0.09±0.02
5	0.10±0.02	0.39±0.06	0.08±0.01
6	0.14±0.02	0.35±0.03	0.07±0.01
7	0.13±0.04	0.40±0.05	0.06±0.01*

Values are expressed as Mean ± SEM of 6 rats per group. Values with super script (*) are considered Significant at p<0.05. Group 1given normal saline (control), Group 2 was treated with 0.5mg/kg of nicotine (low), Group 3 was treated with 1.0mg/kg of nicotine (high), Group 4 rats treated with 0.5mg/kg of nicotine with 10mg/kg of vitamin E, Group 5 rats were given 1.0mg/kg of nicotine with 10mg/kg of vitamin E, Group 6 was recovery for 0.5mg/kg of nicotine, Group 7 was recovery for 1.0mg/kg of nicotine.

3.4.Effect of nicotine and vitamin E on Implantation Sites

Figure 2 revealed that there was a significant decrease in implantation site of pregnancy rats that were treated with low and high dose of nicotine when compared with the control (p<0.05). Similarly, the implantation site in recovery groups was significantly decreased when compared with the control (p<0.05) (figure 2).

Figure 2: Effect of nicotine and vitamin E on implantation sites



Each bar is expressed as Mean ± SEM of 6 rats per group. Bars with super script (*) are considered Significant at p<0.05.

3.5.Effect of nicotine and vitamin E on estrogen and progesterone

As shown in Table 3 there was significant increase in plasma estrogen level in group 2, 3, 5 and 5 when compared with the control (p<0.05). Also, the plasma progesterone was significantly increased in group 3, 5 and 7 relative to the control (p<0.05). The ratio of estrogen to progesterone was significantly increased (p<0.05) in group 2, 3, 5 and 7 when compared with the control.

Table 3: Effect of nicotine and vitamin E on estrogen and progesterone level in pregnant rats

Estrogen (ng/ml)	Progesterone (ng/ml)	Ratio of estrogen to progesterone
0.42 ± 0.023	7.3 ± 0.16	0.058 ± 0.0018
0.69±0.025*	7.8±0.07	0.089±0.0028*
0.82±0.017*	6.5±0.13*	0.126±0.0025*
0.45 ± 0.007	6.9 ± 0.09	0.065 ± 0.0011
0.67 ± 0.016*	8.5 ± 0.20*	0.080 ± 0.0033*
0.66 ± 0.009*	9.0 ± 0.11*	0.073 ± 0.0031*
0.80 ± 0.011*	9.5 ± 0.09*	0.085 ± 0.0017*

Values are expressed as Mean ± SEM of 6 rats per group. Values with super script (*) are considered Significant at p<0.05. Group 1given normal saline (control), Group 2 was treated with 0.5mg/kg of nicotine (low), Group 3 was treated with 1.0mg/kg of nicotine (high), Group 4 rats treated with 0.5mg/kg of nicotine with 10mg/kg of vitamin E, Group 5 rats were given 1.0mg/kg of nicotine with 10mg/kg of vitamin E, Group 6 was recovery for 0.5mg/kg of nicotine, Group 7 was recovery for 1.0mg/kg of nicotine.

4. Discussion

The effect of nicotine and cigarette smoked on body weight lost is well documented [8, 18, 19]. Results of this study were similar to previous report on effect of nicotine on body weight. There was a significantly decreased in body weight of animals treated with high dose of nicotine, their recovery and high dose of nicotine with vitamin E ($p< 0.05$). The growth rate of animals treated with high dose of nicotine was very low in relative to others. The decreased in body weight observed in high dose nicotine treated rats showed that the effect of nicotine on body weight was dose dependent. There was an improvement in growth rate when nicotine and vitamin E was co-administered relative to nicotine treated groups and their recovery groups. Nicotine increase body metabolism by sympathoadrenal activation [20], increases energy expenditure, lost of appetite, release of body stored fat and cholesterol into the blood stream [8, 19], all these factors may be the mechanism through which nicotine decreases body weight.

The brain which has been reported to achieve high concentration of nicotine after it administration [1] showed no significant change in weight. The weight was slightly increased but insignificant in co-administer of nicotine and vitamin E. This result was contrary to Iranloye and Bolarinwa [9] who reported reduction in brain weight of rats treated with nicotine. Kidneys and liver are organs for metabolizes nicotine showed no significant change in weight. Heart weight was significantly reduced in rats treated with nicotine, this was in agreement with Iranloye and Bolarinwa [9] report. The reduction in heart weight has been associated to effect of nicotine in increase epinephrine release that may cause vasoconstriction and reduced blood supply to the heart which may result in necrosis of myocardium. In this study, Vitamin E was able to attenuate the toxic effect of nicotine on heart weight and also, the withdrawal of nicotine. The significant decrease in lungs weight observed in nicotine treated groups was reversed in vitamin E co administered with nicotine and recovery groups and this is in agreement with Proskocil, et al. [21] report which showed that antioxidant attenuate toxic effect of nicotine on lungs functions. The female reproductive organs (ovaries and uterine tubes) weight showed no significant change, while there was significant decrease in vagina weight in high dose nicotine treated and its recovery. Iranloye and Bolarinwa [9]; Patil, et al., [22] reported that nicotine decreased female reproductive organs weight of rats treated with nicotine. Audi et al., [18] associated the decreased in these female reproductive organs weight to inadequate availability of gonadotropin hormone in rats exposed to nicotine. No significant change in weight of these organs in this study may be due to antioxidant effect of vitamin E which may neutralize the toxin action nicotine. Also, estrogen which is necessary for maintaining the size and weight of reproductive organs during the reproductive stage of live was moderately increased in the rats treated with nicotine and vitamin E.

Yoshinaga, et al., [23] reported delayed in the time of implantation of the blastocyst in nicotine treated rats. The present study showed significantly decreased in implantation sites in pregnant rats treated with nicotine and the recovery groups. While, there was no significant difference in implantation site of rats treated with nicotine and vitamin E. One of the mechanism of action of nicotine in reducing implantation sites may be due to it effect on alter the motility of the female reproductive tract [24]. Also, Zavos, et al., [25] in their review, showed that cigarette smoking cause destruction of oocyte in dose dependent manner, this is in agreement with our finding.

During pregnancy, plasma level of estrogen and progesterone usually increased. Corpus luteum and placenta secrete more of these hormones for the sustenance of the pregnancy. The results of this study showed a significantly higher in plasma estrogen level in pregnant rats treated with nicotine, recovery groups for nicotine and high dose of nicotine with vitamin E. This is in support of Sheung, et al. [26], although before this time Petridou, et al. [27] reported a decreased in estrogen level in smoker. The plasma level of progesterone was also significantly increased in high dose nicotine with vitamin E, recovery groups for nicotine treated pregnant rats, while significant reduction was observed in high dose nicotine treated group. The estrogen and progesterone ratio is very important for the maintenance of pregnancy. In this study, there was a significant increase in ratio of estrogen to progesterone in the pregnant rats exposed to nicotine, their recovery animals and high nicotine with vitamin E. This may be one of the reasons for reduction in implantation sites observed in nicotine treated rats and their recovery. The increased in estrogen-progesterone ratio may alter the motility of reproductive tract in the pregnant rats and result in reduction of implantation site [26].The plasma level of progesterone which is well know to antagonist the effect of estrogen during pregnant may not be enough in rats treated with high dose of nicotine with vitamin E.

In conclusion, this study showed that vitamin E moderately attenuated the toxic effect of nicotine on growth rate, some visceral and reproductive organs weight, implantation site, plasma estrogen and progesterone level.

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