Maternal exposure to nicotine during pregnancy induces oxidative stress and growth retardation in F1 generation female Wistar rats.

Aderemi, A.V.[1], Olowu, R.O.[2], Adeyemi, D.H.[2], Arokoyo, D.S.[3], Onuoha, C.F.[2], Ogunsesan,G.R.[2], Ladele M.O.[2] and Oyeyipo I.P.[2]

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Nigeria
[2]Department of Physiology, Faculty of Basic Medical Sciences, Osun State University, Osogbo Nigeria
[3]Department of Physiology, Federal University of Technology, Akure, Nigeria

Article Info

Article type:
Original Article

Article history:
Received: January 31, 2025
Accepted: February 18, 2025
Published: April 30, 2025

Keywords:
Antioxidant, Nicotine, Lipids, Offspring, Tissue, Rats.

Corresponding author:
Aderemi, A.V.
adewale.aderemi@uniosun.edu.ng; walerem@yahoo.com

This article can be accessed at:
www.rjhs.org

http://dx.doi.org/10.4314/rejhs.v13i2.12

Abstract

Aim: We investigated the effects of prenatal nicotine exposure on selected parameters in first filial (F1) generation female rats.
Methods: Twenty-five adult female rats were divided into five groups and mated as follows: Group I, II, and III respectively received 0.2 ml/kg of normal saline, 0.5 and 1.0 mg/kg BW of nicotine daily for 28 days before mating and throughout gestation. Group IV (low-dose recovery) and V (high-dose recovery) received 0.5 and 1.0 mg/kg BW of nicotine daily for 28 days before mating only. Five female F1 generation pups were randomly selected from the groups and allowed to grow naturally until puberty (12 weeks) before sacrifice, followed by measurement of organ-weights and biochemical parameters.
Results: Results showed a dose-dependent reduction in percent weight gain in the F1 generation rats of the treated and recovery groups. Activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) were significantly decreased ($P<0.05$) while serum Malondialdehyde (MDA) concentration was significantly increased ($P<0.05$) in the female offspring of the 0.5 and 1.0 mg/kg nicotine-treated groups.
Conclusions: Prenatal nicotine administration is associated with impaired growth, reduced tissue and serum antioxidants activities in F1 generation female rats.

L'exposition maternelle à la nicotine pendant la grossesse induit un stress oxydatif et un retard de croissance chez les rats femelles Wistar de la génération F1

Résumé
Objectif de l'étude : Nous avons étudié les effets de l'exposition prénatale à la nicotine sur des paramètres sélectionnés chez des rats femelles de première génération filiale (F1).
Méthode de l'étude : Vingt-cinq (25) rats d'adultes ont été divisées en cinq groupes et accouplées comme suit : les groupes I, II et III ont reçu respectivement 0,2 ml/kg de solution saline normale, 0,5 et 1,0 mg/kg de poids corporel de nicotine par jour pendant 28 jours avant l'accouplement et tout au long de la gestation. Les groupes IV (récupération à faible dose) et V (récupération à forte dose) ont reçu 0,5 et 1,0 mg/kg de poids corporel de nicotine par jour pendant 28 jours avant l'accouplement uniquement. Cinq petits femelles de la génération F1 ont été sélectionnés au hasard dans les groupes et ont été laissés grandir naturellement jusqu'à la puberté (12 semaines) avant le sacrifice, suivi d'une mesure du poids des organes et des paramètres biochimiques.
Résultat de l'étude : Les résultats ont montré une réduction dose-dépendante du pourcentage de gain de poids chez les rats de la génération F1 des groupes traités et de récupération. Les activités de la glutathion peroxydase (GPx), de la superoxyde dismutase (SOD) et de la catalase (CAT) ont été significativement diminuées ($P < 0,05$) tandis que la concentration sérique de malondialdéhyde (MDA) a été significativement augmentée ($P < 0,05$) chez la progéniture femelle des groupes traités à 0,5 et 1,0 mg/kg de nicotine.
Conclusions : L'administration prénatale de nicotine est associée à une croissance altérée, à une réduction des activités antioxydantes tissulaires et sériques chez les rats femelles de la génération F1.

Mots-clés : Antioxydant, nicotine, lipides, progéniture, tissu, rats

INTRODUCTION

Tobacco smoking constitutes a major public health menace and remains a leading cause of avoidable death globally (1). The World Health Organization (WHO) has estimated that well over 8 million tobacco-related mortalities are recorded annually (2), more concerning is its increasing prevalence among the adolescents (3). Despite reduction in its prevalence in some parts of the world (2), the burden of cigarette smoking remains a cause for worry. In recognition of this potential threat to lives, the WHO 2023 report has shown that 74 countries are currently implementing smoke-free policies, up from just 10 nations that subscribed to these measures in 2007 (2). A few other countries are also taking measures such as introduction of tax increments on tobacco-related products, all with a view to significantly reducing by the year 2030 the prevalence of this global epidemic especially among individuals who are 15 years and above (2).

Nicotine, the primary active ingredient in cigarettes and with great potential for addiction (4), is consumed in different forms but more often as smoked tobacco. It is an alkaloid with potent stimulant and para-sympathomimetic effects (4). Out of the nearly 8-20 mg of nicotine present in one cigarette, just about one-tenth (accounting for roughly 1-2 mg) of this is said to find its way successfully into the human body where it mediates its harmful effects (5). As a result of the addictive properties of nicotine, its intake through cigarette smoking has become a very common occurrence and a serious health and economic issue in most societies. In animal models, nicotine has been reported to induce lipid peroxidation and increase nitric oxide levels in the blood of male Albino rats exposed to nicotine, in a dose-dependent manner (6). Nicotine's adverse effects, via oxidative stress induction on sperm quality and functions, have been well documented, but in females, the impact of oxidative stress on oocytes and reproductive functions remains unclear (7).

Oxidative stress is a state characterized by an alteration in the balance between pro-oxidant molecules (including reactive oxygen, ROS, and nitrogen species), and antioxidant defenses,(8) such as catalase, superoxide dismutase, glutathione peroxidase, etc., and may induce apoptosis in the affected cells leading to various disease conditions (7,9). This imbalance tends to favour overproduction of ROS or radicals such as hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-),and hydroxyl radical (OH) that have been linked to a number of gynecological conditions that predispose both genders to an increased risk of infertility (7). ROS has also been reported to have a direct damaging effect on lipids, such as cholesterol, glycolipids and phospholipids, with the hydroxyl (HO·) and hydroperoxyl (HOO·) radicals being the major culprits (8). Additionally, intrauterine exposure may lead to retardation in the growth of the embryo through perturbation of important cell components needed for cell division (9). However, it is debatable to say that, nicotine, being the main active ingredient in tobacco, is responsible for all the harmful effects traceable to free radical accumulation in the tissues. The reason is because some research has reported that nicotine might possess not just a pro-oxidant effect but also an antioxidant property (10).

In utero exposure to substances that are capable of altering the environment in which a growing fetus develops has been shown to cause structural, physiological and metabolic changes that can predispose the individual to cardiovascular and other disorders in adult life (11). The present study was designed to investigate the impacts of nicotine administration on growth, serum and tissue antioxidant activities, as well as lipid profile of the first filial (F1) generation of the female offspring of Wistar rats. The aim is to gain insights into the toxicological and potential generational programming effects of prenatal exposure to nicotine in female Wistar rats.

MATERIALS AND METHODS

Animals and treatment

Twenty-five (25) mature nulliparous female rats (12 weeks old) with body weights ranging between 150-180g were included in the study. Thirteen (13) fertile male animals of the same age and weight were cohabited for mating. The animals were procured and kept in the vivarium of the College of Health Sciences at Osun State University, Nigeria. The animals were housed individually in cages, fed with standard pellet diet, and offered water *ad libitum*. Throughout the experiment, the animals were maintained on a 12-hour light/12-hour dark cycle under constant room temperature. Pairing for mating was 1:2 for males/females. Mating was confirmed by the presence of a sperm-positive vaginal smear or a copulation plug in the females. The day after which either was found was considered as day 1 of gestation. The pregnant rats were randomly assigned to five groups, of 5 rats per group as shown in **Table 1.** After delivery, their offspring were allowed to grow naturally till puberty (12 weeks). Thereafter, the following parameters were measured using only the female offspring while their male counterparts were

excluded: whole body, visceral and reproductive organ weights, biochemical assays including antioxidant and lipid profiles in randomly selected five F1 generation female Wistar rats. By obtaining institutional ethical approval, utilizing minimal number of animals, and handling the animals in a very humane manner, the study was carried out in compliance with the National Institute of Health guidelines for the Care and Use of Laboratory Animals (12).

Animal sacrifice and sample collection

First filial generation female animals were sacrificed at the expiration of the 12 weeks post-delivery, using chloroform inhalation. This method was chosen over cervical dislocation or diethyl ether inhalation in that chloroform has milder effect on the rat, much easier to administer and gives the highest amount of blood especially for studies involving use of blood samples (13). The animals were dissected and blood samples were collected from the apex of the heart into plain sample bottles. The blood in the sample bottles were spun in a centrifuge at the rate of 3000 revolutions for 15 minutes. The uterus and ovaries were collected, cleared of adherent tissues, weighed and recorded.

Determination of MDA concentration

The concentration of malondialdehyde (MDA), which is a measure of oxidative stress from lipid peroxidation, was measured as previously documented (14,15) with modifications.

Assessment of serum and tissue antioxidant enzymes activities

The activities of the enzymatic antioxidants, namely glutathione peroxidase (GPx) (14), superoxide dismutase (SOD) (14,16) and catalase (CAT) (17,18) activities, were estimated from the serum and tissue samples in accordance with established protocols for the determination of antioxidant and lipid peroxidation.

Determination of lipid profile

Triglycerides (TG), Total Cholesterol (TC) and high density lipoprotein cholesterol (HDL-cholesterol) concentration were evaluated according to the instruction of manufacturer of the assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA). According to Friedewald formula (19). Very low density lipoprotein cholesterol (VLDL-cholesterol) and low density lipoprotein cholesterol (LDL-cholesterol) were calculated as:

VLDL cholesterol = TG/5 and

LDL cholesterol = TC – (VLDL+HDL cholesterol).

Statistical Analysis

Data obtained were expressed as Mean ± S.E.M and analyzed using One-way Analysis of Variance (ANOVA) in the Statistical Package for the Social Sciences (SPSS) version 20. Values with $P<0.05$ were considered statistically significant for all experiments.

RESULTS

Effect of nicotine on the overall body weight

The result showed an increase in weight from birth to the time of sacrifice in the 0.5 mg/kg and 1.0 mg/kg treatment groups with an overall gain in body weight of 80 % and 19 % respectively compared to the control with a gain of 36 % (**Table 2**). However, in the recovery groups, a 6.3 % reduction in weight was observed in the 0.5 mg/kg group, while a paltry 1.7 % gain in weight was recorded for the 1.0 mg/kg group.

Effect of nicotine on reproductive organs weight of F1 generation female Wistar rats.

Two reproductive organs (ovary and uterus) from both the treated and the recovery groups were examined. Result showed no significant changes in the weight of the ovaries of rats in the treated as well as the recovery groups when compared with the control group. However, a slight but statistically insignificant increase in weight was observed in the uterus category in the 0.5 mg/kg and 1.0 mg/kg treated and recovery groups compared to the control, after the experimental period. (**Figure 1**).

Effect of nicotine on visceral organ weight

Table 3 gives a summary of the effects of nicotine on selected visceral organs of the FI generation female Wistar rats. Except for the spleen and the liver in the 0.5 mg/kg treated groups, where a slight reduction and increase respectively were observed, no significant changes in weight of the visceral organs were noted when compared with the control. In the 0.5 mg/kg and 1.0 mg/kg recovery groups, however, an overall but statistically insignificant decrease ($p>0.05$) in organ weights are seen especially with the liver and the heart when compared with the control.

Effects of nicotine on serum GPx, SOD, CAT activities, and MDA concentration

The serum antioxidant parameters (GPx, SOD, CAT, and MDA) were tested as described in the methodology. The result showed a dose-dependent decrease in the mean GPx serum activities in female offspring of rats in the treated and the recovery groups (**Figure 2 A)**. However, the observable effect is only statistically significant in the 1.0 mg/kg treated group when compared to the control group. For the serum SOD assay (**Figure 2 B**), the result showed a dose-dependent decrease in the mean serum SOD activities in the treated (0.5 mg/kg and 1.0 mg/kg) groups, while a slightly higher and a little lower values were observed in the 0.5 mg/kg and 1.0 mg/kg recovery groups respectively when compared to the control. In both the treated and recovery groups, however, the observed differences were not statistically significant ($p>0.05$). A comparison of the mean serum CAT activities in the treated and the recovery groups to the control (**Figure 2 C**) showed a significant decrease ($p<0.05$) in the 0.5 mg/kg and 1.0 mg/kg treated and 1.0 mg/kg recovery groups. No significant change in value was observed in the 0.5 mg/kg recovery group. **Figure 2 D** shows the result of the effect of nicotine on the serum MDA levels. A significant, dose-dependent increase ($p<0.05$) in the mean serum MDA level was noted in the treated groups (0.5 mg/kg and 1.0 mg/kg) compared to the control. In the recovery groups, the mean serum MDA value of the 0.5 mg/kg group is essentially the same as the control, while a statistically significant ($p<0.05$) increase was noted for the 1.0 mg/kg group.

Effects of nicotine on the tissue GPx, SOD, CAT activities and MDA level

The results of the effects of nicotine on the tissue activities of GPx, SOD, CAT and MDA are reported. The results showed a statistically insignificant dose-dependent increase ($p>0.05$) in the mean tissue GPx activities in both 0.5 mg/kg and 1.0 mg/kg treated groups, and to a much lower extent in the recovery groups, when compared to control group (**Figure 3 A**). For the SOD activities (**Figure 3 B**), a statistically insignificant dose-dependent decrease was observed in the treated (0.5 mg/kg and 1.0 mg/kg) groups when compared to control group. Conversely, a near total recovery to the value in the control group was noticeable in the recovery groups, with the effect being more pronounced in the 1.0 mg/kg group. **Figure 3 C** summarizes the effect of nicotine on the mean tissue activities of CAT. Here, the result showed a statistically significant increase ($p<0.05$) only in the 0.5 mg/kg group. A slightly lower mean CAT value was recorded for the 1.0 mg/kg treatment group, and the two recovery groups but the observed differences compared to the control, are not statistically significant. The result for the tissue MDA level (**Figure 3 D**) showed an insignificant increase ($p>0.05$) and decrease in the mean values of 0.5 mg/kg (treated) and 0.5 mg/kg and 1.0 mg/kg (recovery groups) respectively. However, a lower, but statistically insignificant, value was noted for the 1.0 mg/kg treated group, compared to the control.

Effect of nicotine on the serum lipid profiles

The serum lipid profiles of the F1 generation of the female Wistar rats, including the total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) were tested in both the treated as well as the recovery groups. Slightly higher but statistically insignificant ($p>0.05$) total cholesterol values were detected in the 0.5 mg/kg treated and 0.5 mg/kg recovery groups, compared to the control group. On the other hand, no noticeable difference was observed in the 1.0 mg/kg treated as well as the 1.0 mg/kg recovery groups compared to the control (**Figure 4 A**). The result showed no significant change in the level of triglycerides of 0.5 mg/kg and 1.0 mg/kg nicotine treated and recovery groups of F1 generation female Wistar rat compared to the control (**Figure 4 B**). Compared to the control group, somewhat higher but statistically insignificant ($p>0.05$) serum LDL values were noticeable in the 0.5 mg/kg treated and 0.5 mg/kg recovery groups while values obtained in the 1.0 mg/kg treated and the 1.0 mg/kg recovery groups were essentially the same as that recorded for the control group (**Figure 4 C**). When compared to the control, the HDL assay result showed a dose-dependent but statistically insignificant increase ($p>0.05$) in the serum levels of the lipoprotein both in the 0.5 mg/kg and 1.0

DISCUSSION

Cigarette or tobacco smoking constitutes a major health hazard especially among chronic users. It has been variously identified as the major culprit in the development of chronic obstructive pulmonary disease (20). lung cancers and other disease conditions affecting the heart and other organs in human body (21). For those who suffer from such cancers, cigarette smoking plays a major role in their survival, exacerbates inflammatory disease of the lung and impacts negatively on their ability to fight bacterial

infections (20,22). Tobacco smoking has also been linked to an increased risk of developing dyslipidemia, hypertension and coronary artery disease, thereby contributing considerably to cardiovascular disease morbidity and mortality in man (23). Nicotine is the major toxic constituent in tobacco and it is absorbable through the nose, mouth and skin (24). Nicotine has a very strong additive effect in those that are exposed to it (25), and it is this potential for addiction that makes it practically difficult for users to quit smoking.

In humans, studies have shown that children born to mothers that smoked during pregnancy are at higher risk of becoming hypertensive (26) and diabetic (27) later in life. The harmful effects of cigarette smoking on hematology, lipid profiles and hepatic enzymes have also been documented in animal models (24,28). For instance, Kolawole *et al* (24) have shown that nicotine exposure has a damaging effect on the reproductive organs in male Wistar rats. Exposure to nicotine in early neonatal life has also been linked to abnormal metabolic alterations such as reduced insulin level possibly secondary to its effects on pancreatic islet β cells (29).

In the present study, we examined the antioxidant activities and lipid profile in the F1 generation female rats of mothers prenatally exposed to nicotine as well as the effect of such exposure on their overall general body weight, visceral weight and reproductive organ weight, using Wistar rats as models. Gains in the general body weight from birth to the time when the rats were sacrificed were noticeably poorer in the high-nicotine treated and recovery groups compared to the low-dose nicotine treated and control groups. The reduction in percent weight gain in the former categories is indicative of a dose-dependent growth retardation. This finding is consistent with earlier study in which significant nicotine-induced weight reduction was noticed in mice that were fed with high fat diet, and the observed effect is believed to be secondary to a reduction in calorie intake by the exposed mice (30). We also recorded slight differences in the weight of some visceral organs of the F1 generation rats. In keeping with the poor percent general body weight in the recovery groups, a decrease in weight of the liver, the main organ for nicotine metabolism, and of the heart, was particularly noted. Our result does not agree with a previous study, in which an increase in the weight of liver, presumably due to a nicotine-induced fat accumulation in the hepatic parenchymal cells, was reported (31) We reason that the decrease in liver weight recorded in this study may be associated with a reduction in liver cell (hepatocytes) density and glycogen content, two important effects that have been documented for nicotine exposure in rats (32). The reduction in weight of the heart of the F1 generation of mothers prenatally exposed to nicotine was equally observed, which is in agreement with earlier studies (31).

Our study also showed that nicotine administration produced marked oxidative impact on the F1 generation as shown by the decrease in the activities of serum antioxidants GPx, SOD, CAT and a raised level of the serum MDA. The lower activities of the antioxidant enzymes observed in the current study may be due to nicotine-induced generation of free radicals, which in turn may have overwhelmed the antioxidant defense mechanism leading to a reduction in the activities of the enzymes. This observed perturbation is in keeping with the previously documented evidence of inhibitory effects of nicotine in male albino (6). GPx has been reported to have a broad protective spectrum, with the suppression of its activity often associated with downregulation in its serum level in rats treated with nicotine (6). The suppression in the activity of serum GPx was also recorded in this study in the female offspring of rats treated with nicotine. The study also shows that the activity of SOD, an enzyme that catalyzes the dismutation of superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2) (15,16), was down-regulated in the serum of female offspring (F1 generation) of rats of both the low- and high-nicotine treated groups, implying a dose-dependent effect. This observed reduction in the serum SOD activity may be a consequence of decreased *de novo* synthesis of the enzyme or its oxidative inactivation (6). A decrease in CAT activity in the serum was also recorded, and we theorize that this decrease may be connected with an inefficient elimination of toxic hydrogen peroxide (H_2O_2) by the GPx in the tissues. Female offspring of nicotine treated rats showed an elevation in MDA level when compared with the control group. This rise in the serum MDA concentration may be suggestive of its reduced production in the tissues of the nicotine-treated rats, a development that has been shown to shift the equilibrium in favour of reactive oxygen species (ROS) generation (6). It is noteworthy here that the values of the antioxidant parameters recorded for the recovery groups were in most instances comparable to those recorded for the control, indicating that the effect of nicotine on antioxidant enzyme could be ameliorated by nicotine cessation in the offspring of the nicotine treated rats.

The effects of the nicotine administration

on the tissue antioxidant activities of the enzymes were also assessed in this study. Our result showed that whereas the mean GPx activity in the serum was significantly reduced, an elevation in its tissue activity of the rats was noted both in the treatment and recovery groups, compared to the control. The activity of SOD was also down-regulated both in the low- and high-dose nicotine treated groups in the tissues of the F1 generation rats, and the effects were also dose-dependent as recorded for serum SOD. An elevation in the CAT activity in tissue was recorded in female offspring of rats of the low-dose nicotine treated group and a decrease in its activity for the offspring of the high-dose treated group when compared with control. This suggests that reduced CAT activity is only noticeable at high nicotine administration. For the tissue MDA concentration, an increase in level was recorded in the F1 generation rats in the low-dose nicotine treated group and a decrease in its activity for the offspring of the high-nicotine treated group when compared with control. The values in the recovery groups are essentially the same as for the control group. While this may suggest a restorative effect of cessation, it is hard to say with certainty that this is the case as the observed effects in the treatment group were not statistically significant when compared to the control.

To determine the effects of maternal exposure to nicotine during pregnancy on the lipid profile of their female F1 generations, we assayed the serum levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol cholesterol (LDL-cholesterol) and high density lipoprotein cholesterol (HDL-cholesterol). Our results indicate that although some changes in the lipid profiles of the treated groups are noticeable, for example an increase in the TG level of the 0.5 mg/kg treated group compared to the control, the impact of maternal nicotine exposure on the lipid profile of the female F1 generation rats was insignificant. This is somewhat at variance to an earlier study involving human subjects in which significantly higher TG and TC but lower HDL-cholesterol concentrations were found in smokers compared to their non-smoker cohort (32). We postulate that the observed differences in the human and animal models may be due to genetic make-up, duration of exposure (33,34) or simply a reflection of other confounding factors that are differentially present in the two study populations – rats and human. For example, in two different studies, an increase in the HDL-cholesterol was observed in one group of smokers (35), while a decrease in value was observed in another (32) when compared to their non-smoker cohorts.

CONCLUSIONS

Overall, the results in this study suggest that nicotine exposure during pregnancy retards growth, induces significant oxidative stress and may be associated with gonadotoxicity in the F1 female offspring Wistar rats. Furthermore, whereas a decrease in the body weight of the treated rats as well as in some of their visceral organs is noticeable, this was not associated with significant perturbations in lipid profiles. The data also indicate that nicotine withdrawal for a particular period of time could ameliorate the observed effects, however, further investigations are needed to validate this.

Ethics statement: The institutional ethical approval for this study was obtained from Osun State University Health Research Ethics Committee with approval number UNIOSUNHREC 2024/008B

Conflicts of interest: Authors declare no competing interest that may have influenced the conduct, result or publication of this study.

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Table 1. Grouping and Design of the Experimental Animals

Group	Designation	Treatment
I	Control	Administered 0.2 ml/kg body weight of normal saline daily throughout the gestational period
II	Low-dose treatment	Administered 0.5 mg/kg body weight of nicotine daily for 28 days before mating and throughout the gestation period;
III	High-dose treatment	Administered 1.0 mg/kg body weight of nicotine daily for 28 days before mating and throughout the gestation period
IV	Low-dose recovery	Administered 0.5 mg/kg body weight of nicotine 28 days before mating only
V	High-dose recovery	Administered 1.0 mg/kg body weight of nicotine 28 days before mating only

Table 2: Effects of nicotine on the overall body weight in F1 generation female Wistar rats.

Parameters	Control	Group			
		0.5 mg/kg (Treated)	1.0 mg/kg (Treated)	0.5 mg/kg (Recovery)	1.0 mg/kg (Recovery)
Initial weight (g)	97.0 ± 12	80.0 ± 8.4	121 ± 13	128 ± 9.7	117 ± 11
Final weight (g)	132 ± 6.3	144 ± 5.8	144 ± 9.9	120 ± 7.1	119 ± 1.1
Weight change (g)	35.0 ± 5.5	64.0 ± 2.6	23.0 ± 2.9	8.00 ± 2.6	2.00 ± 0.3
% Weight increase	36.0 ± 4.2	80.0 ± 4.9	19.0 ± 6.5	6.30 ± 3.4	1.70 ± 6.0

Table 3: Effect of nicotine on visceral organ weight in first filial (F1) generation female Wistar rats.

Groups	Weight of the Organs (g)				
	Spleen	Heart	Lungs	Liver	Kidney
Control	0.71 ± 0.09	0.63 ± 0.05	1.21 ± 0.1	5.51 ± 0.71	1.03 ± 0.05
0.5 mg/kg (treated)	0.59 ± 0.04	0.63 ± 0.03	1.34 ± 0.08	6.10 ± 0.51	0.99 ± 0.07
1.0 mg/kg (treated)	0.71 ± 0.07	0.62 ± 0.06	1.28 ± 0.11	5.42 ± 0.43	0.92 ± 0.09
0.5 mg/kg (recovery)	0.67 ± 0.03	0.54 ± 0.03	1.23 ± 0.1	4.60 ± 0.31	0.86 ± 0.04
1.0 mg/kg (recovery)	0.68 ± 0.09	0.54 ± 0.04	1.12 ± 0.12	4.78 ± 0.43	1.01 ± 0.11

**Values that are statistically significantly different ($p<0.05$) from the control.*
Values are expressed as Mean ± S.E.M

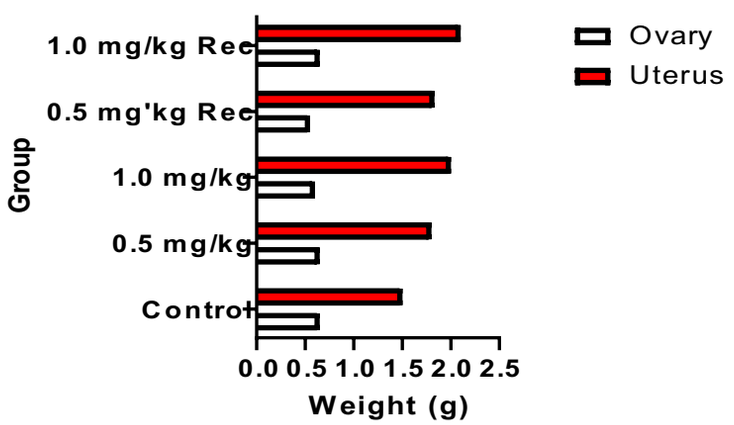

Figure 1. Weights of the reproductive organs of the F1 generation femal Wistar ratsResult shows no significant differences in weight between th control and the treatment/recovery groups for ovary, but a slightly higher va in the treatment and recovery groups compared to the control for the uterus.

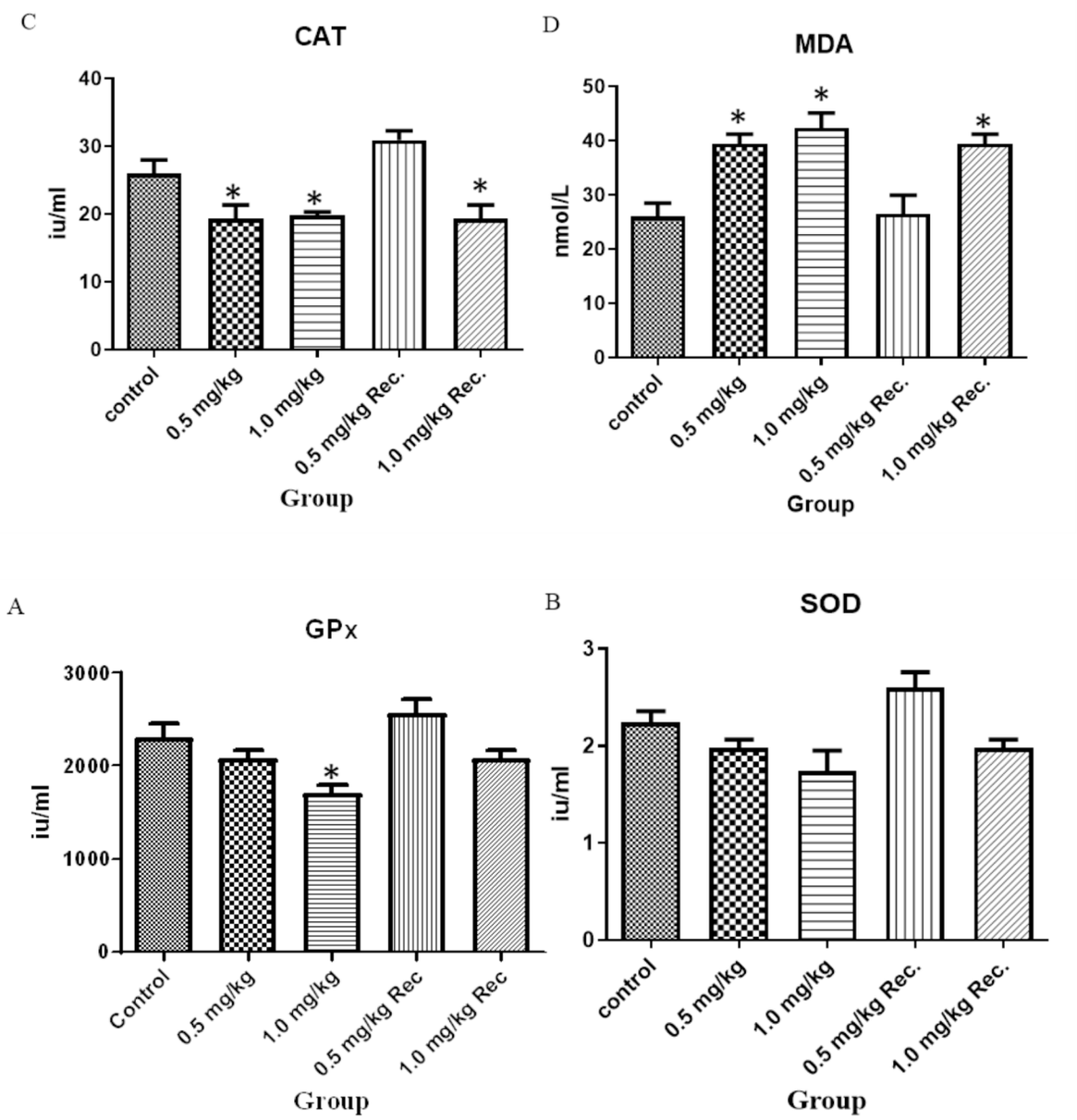

Figure 2. Serum antioxidant parameters of F1 generation rats of mothers exposed to Nicotine prenatally**. A**) Serum GPx activities, showing statistically significant ($p<0.05$) effect only in the 1.0 mg/kg nicotine treated group. B) Serum SOD activities, showing a dose-dependent, but statistically insignificant ($p>0.05$) decrease in the treated compared to the control group. C) Serum CAT activities, showing an almost equal statistically significant reduction in the 0.5 and 1.0 mg/kg treated, and 1.0 mg/kg recovery groups compared to the control. D) Serum MDA levels, a statistically significant dose-dependent increase in the treated groups, as well as the 1.0 mg/kg recovery group. Values are expressed as Mean ± S.E.M. *$p<0.05$ indicates significant differences when compared with control. Rec. stands for Recovery Group

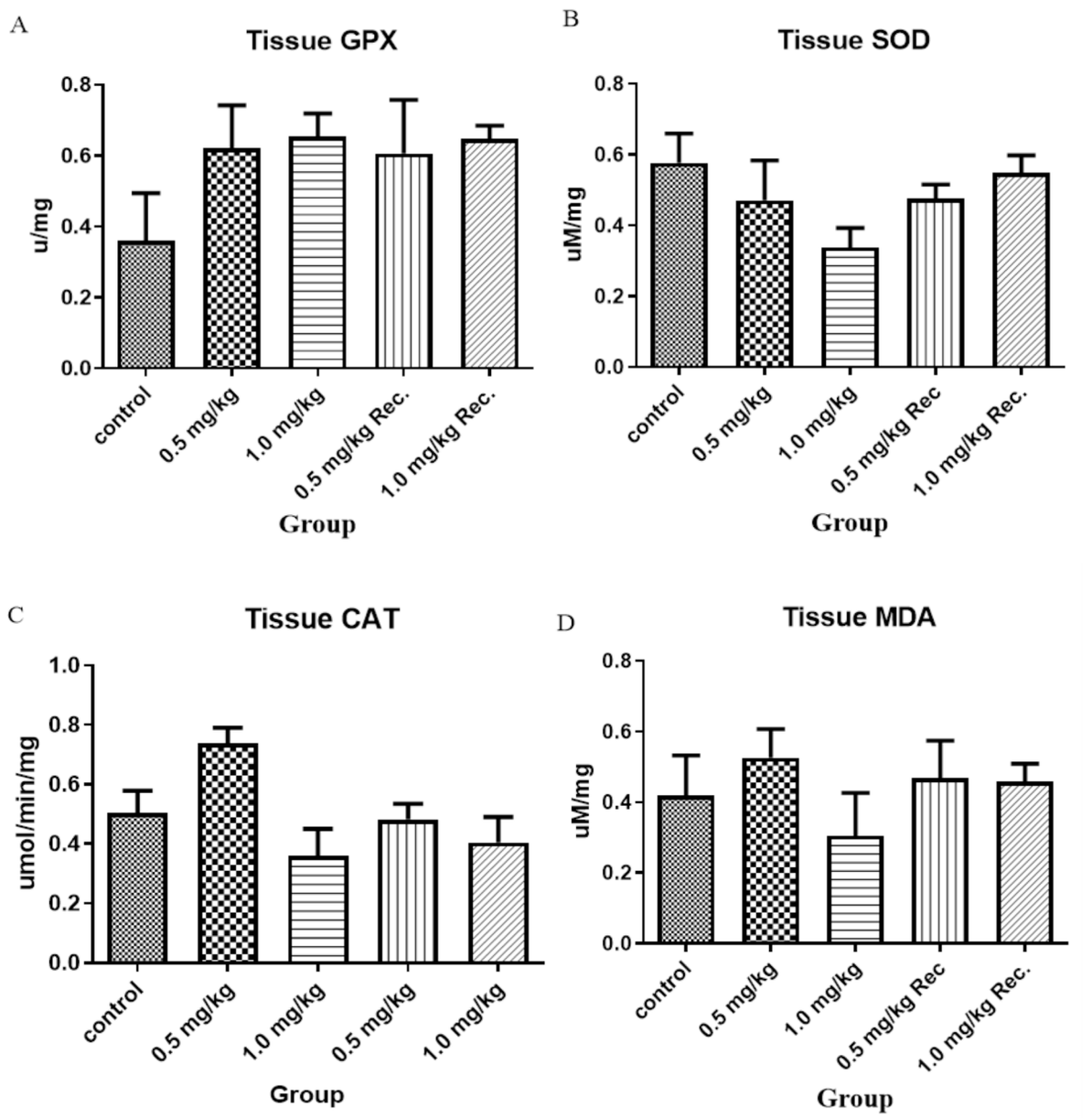

Figure 3. Tissue antioxidant parameters of F1 generations of female Wistar rats exposed to Nicotine during pregnancy. A) Effect on tissue GPx, showing a dose-dependent but statistically insignificant ($p>0.05$) increase in both the treated and the recovery groups. B) Tissue SOD activities of F1 generation rats exposed to nicotine, showing a dose-dependent decrease in the treated, but increase in the recovery groups. The observed effects in both cases are not statistically significant. C) Tissue CAT activities of F1 generation rats exposed to nicotine, showing no significant changes in both treated and the recovery groups. D) Tissue MDA levels of F1 generations rats exposed to nicotine. The mean MDA concentration is slightly raised in the 0.5 mg/kg treated group, but almost same as for the control group in the recovery groups**.** Values are expressed as Mean ± S.E.M. Rec. stands for Recovery Group