

Role of tocotrienol-rich palm vitamin E on pregnancy and preimplantation embryos in nicotine-treated rats

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Abstract

The present study observed the effects of palm vitamin E (PVE) on pregnancy and embryo development in the nicotine-treated rat model. Sprague Dawley rats weighing 160 - 240 g (aged 3 – 6 months) were divided into four groups. Group A (control group), Group B had nicotine (5 mg/kg in 0.2 ml corn oil) sc/day. Animals of Group C received nicotine concurrently with PVE at a dose of 60 mg/kg orally/day and Group D had PVE alone. To study the embryonic development, immature rats were superovulated following an identical treatment schedules as stated above.

Nicotine treatment during pregnancy (from day 1 pc until term) reduced the pregnancy outcome to 33.3% whereas oral supplementation with PVE in nicotine-treated rats increased the percentage of pregnancy outcome to 83.3%. It was moreover found that 25.68 % embryos developed into 2- and 4-cell stage in the nicotine plus PVE-treated animals. In conclusion, PVE, an antioxidant, is found to be beneficial in neutralizing the nicotine-related adverse impact on female reproduction.

Introduction

Female reproductive system is constantly exposed to multiple deleterious factors including nicotine exposure. Among the 3,500 chemical substances, nicotine being an addictive agent retards fetal development, causes low birth weight and delays parturition [1-3]. In addition, administration of nicotine for 5 consecutive days in pregnant rats could alter the rate of embryo proliferation, delays implantation and delivery process [4]. Nicotine when present in higher concentrations in oocytes cultured *in vitro*, causes perturbation in the first and second meiotic division [5].

The mechanism by which the nicotine could damage tissues is related to its effects on gamete cells' viability or change in the oviductal epithelial function [6]. Earlier reports showed that nicotine could act as free radicals. These free radicals could damage the polyunsaturated fatty acids that are present in the cell membrane or as side-chains in certain chemical species [7]. Furthermore, administration of nicotine in rats leads to higher lipid peroxidation with subsequent decrease in antioxidant enzymes [8]. Lipid peroxidation could lead to a variety of toxicological effects such as impaired mitochondrial func-

tions and inhibition of antioxidant enzymes. The end product of this process can be measured by using malondialdehyde (MDA) [9]. To counter this condition, the effect of antioxidant such as vitamin E has been highlighted. Vitamin E is known for its antioxidant properties since its discovery in 1922 [10]. Among all the components of vitamin E, α -tocopherol is widely reported to have the highest biological activities [11,12]. Recently, γ -tocotrienol has provoked many researchers to explore its beneficial effects in various human diseases [13].

A population study on the hypercholesterolemic patients treated with palmvitee (tocotrienol-rich fraction of palm oil capsule) demonstrates a significant reduction in total cholesterol (10%), low-density cholesterol (13%) and Apo B (7%) [14]. The significance of vitamin E in male reproduction has been emphasized when its deficiency is found to cause testicular degeneration in rats [15]. Evidences have therefore provided a new incentive to investigate the role of palm vitamin E in combating the radical-driven oxidative events.

Aim of the present study was to investigate the possible protective profile of tocotrienol-rich palm vitamin E

(PVE) on the embryo development and pregnancy outcome in nicotine-treated rats.

Materials and Methods

The experimental protocol was approved by the Universiti Kebangsaan Malaysia Animal Care and Use Committee (UKMACUC) and was conducted at the Animal Biotechnology Laboratory, Department of Physiology, Universiti Kebangsaan Malaysia (UKM).

Twenty-four fertile female Sprague Dawley rats weighing 160 – 240 g (aged 3 – 6 months) were obtained from UKM animal unit. Animals were kept in the animal house at 25 – 30°C and had free access to rat chow and drinking water. Animals were divided into four groups of 6 rats in each. Fertile male rats were caged together for the purpose of mating.

Palm vitamin E (PVE) was a complimentary gift from Mr. Gapor Mat Top, Malaysian Palm Oil Board. PVE per 100 g contained: α -tocotrienol (17.3%), γ -tocotrienol (21.6%), δ -tocotrienol (15.3%), α -tocopherol (18.9%) and palm olein (26.9%). PVE was diluted with tocopherol-stripped corn oil (ICN USA) to obtain the desired concentration of 500 mg/kg rat weight. The dispensing volume was 0.2 ml.

Nicotine N-3876 (SIGMA chemical company, USA) was prepared weekly at a concentration of 20 mg/ml saline and stored away from light.

Effects of PVE on pregnancy outcome in rats treated with nicotine

Pregnant females were treated daily from day 1 pc until term. Animals of Group A (control) received 0.5 ml 0.9% sodium chloride (sc) and 0.2 ml tocopherol-stripped corn oil orally. Animals of Group B received nicotine [5 mg/kg body weight (sc)] concurrently with 0.2 ml tocopherol-stripped corn oil orally. Group C had nicotine [5 mg/kg body weight (sc)] and PVE at 60 mg/kg body weight orally. Group D received 0.5 ml 0.9% sodium chloride (sc) and PVE at a dose of 60 mg/kg body weight orally. Vaginal smears were taken daily and subjected for Schorr's staining. Sperm-positive vaginal smear was cou-

nted as day 0 of pregnancy. Upon delivery, anthropometric measurements were performed on each pup.

Effects of PVE on embryo development in rats treated with nicotine

The distribution of animals groups was the same as described in the previous experiment. Immature female rats were 9-12 weeks old and weighing between 120-140g. Treatments were administered for 30 consecutive days. Animals were superovulated by an intraperitoneal injection of 150 IU/kg body weight of PMSG and 48h later by another injection (sc) of hCG (75 IU/kg body weight). Animals were then mated with fertile males and sacrificed at 48 h post mating. The embryos were flushed from the fallopian tubes and examined under a dissecting microscope.

Venous blood samples were taken from the orbital sinus after the pregnant rats had delivered. Plasma levels of malondialdehyde (MDA) were measured as an indicator of lipid peroxidation [16]. In addition, vitamin E in plasma was also measured [17]. Cotinine level in urine was determined using a spectrophotometer according to the published protocol [18].

Values are given as median and the comparisons were analyzed by non-parametric Kruskal-Wallis analysis of variance on rank. Probability levels of less than 0.05 were taken as statistical significance.

Results

Nicotine treatment until term reduced the rate of pregnancy outcome to 33.3% (Table 1). The pregnant (2/6) had longer duration of gestation (median 23.5 days) compared to controls (median 21.7 days). The harmful effect of nicotine as viewed by the lowest number of embryos survived in Group B which was 26 out of 183 (14.21%) [Table 3]. The extended length of gestation in the nicotine-primed rats could possibly be due to delayed embryo cleavage in which 61.54% of retrieved embryos remained uncleaved. The excretion of cotinine as the metabolite of nicotine in the urine in Group B animals was found to be significantly higher compared to the controls ($p < 0.05$) [Table 2]. The MDA levels were similarly recorded to be higher in Group B animals ($P < 0.05$) [Table 2].

Table 1. The percentage of pregnancy, gestation period and postnatal development of pups following treatment throughout the pregnancy. $n = 6$ in each groups.

Groups	Percentage of pregnancy (%)	Gestation period (median in days)	Total number of pups
Control (group A)	100	21	9.2 ± 0.4
Nicotine only (group B)	33.3	23.5	5.5 ± 3.5
Nicotine + PVE (group C)	83.3	22	7.8 ± 1.36
PVE only (group D)	100	22	6.7 ± 0.8

Supplementation of PVE in Group C rats improved the rate of pregnancy to 83.3% with the gestation length of median 22 days (Table 1). The number of retrieved embryos accounted for 47 out of 183 (25.68%). However, 76.56% of them were found to be in 2-cell and 4-cell stage (Table 3). The benefit of PVE supplementation was

more obvious in Group D animals, 100% of them became pregnant with the highest number of retrieved embryos (57 out of 183). Furthermore, all the embryos obtained in this group were fertilized and cleaved. Ninety percent of them was found to be in 2-cell and 4-cell embryos and 7% had reached 8-cell and 16-cell stage.

Table 2. Biochemical analysis of plasma MDA and vitamin E with urinary cotinine. All values are expressed in median.

Groups	Plasma MDA (nmol/g protein)	Plasma vitamin E (mg/ml)	Urinary cotinine (µg/ml)
Control (group A)	2.63 ^a	11.74	2.16 ^a
Nicotine only (group B)	5.37 ^b	11.71	21.75 ^b
Nicotine + PVE (group C)	2.63 ^a	12.20	32.02 ^b
PVE (group D)	1.50 ^a	11.89	1.28 ^a

Statistically significant ($P < 0.05$) for the values labelled with different superscript within the same column.

Table 3: Number and percentage of oocytes and embryo derived from all the groups .

Cell stages (Groups)	Unfertilised single cell	Fertilised ovum	2-cell	4-cell	8-cell	16-cell	Total
Control (group A)	1 (1.89%)	4 (7.55%)	7 (13.21%)	38 (71.7%)	3 (5.66%)	-	53 (28.96%)
Nicotine only (group B)	1 (3.85%)	15 (57.69%)	9 (34.62%)	1 (3.85%)	-	-	26 (14.21%)
Nicotine + PVE (group C)	7 (14.89%)	4 (8.51%)	19 (40.42%)	17 (36.17%)	-	-	47 (25.68%)
PVE (group D)	-	2 (3.51%)	10 (17.54%)	41 (71.93%)	3 (5.26%)	1 (1.75%)	57 (31.15%)

Discussion

Present study particularly concentrated on the factors that could affect female reproduction. The idea was to investigate the effects of nicotine, a component of cigarette smoke, on pregnancy outcome. In addition, the effect of nicotine on pre-implantation embryo development had been programmed in order to observe whether the nicotine could perturb the process of embryogenesis by free radical-mediated oxidation [7]. Many epidemiological studies have documented the negative outcomes between maternal smoking and pregnancy and the long-term effects of nicotine dependence to the child's behavior [2]. Despite all these known risks of smoking, there are still high percentage of women smoke during pregnancy [3]. Vitamin E acts as an antioxidant thereby protects the cell membranes by inactivating free radicals [12]. A study on thirteen healthy smokers who received α -tocopherol supplementation for two weeks, showed a significantly reduction of lipid peroxidation [19].

It is evident from our preliminary study that nicotine treatment on first 7 days of pregnancy does not exert any

remarkable impact on pregnancy in rats. However, nicotine treatment from day 1 of pregnancy till term caused significant pregnancy wastage, only 33.3% of pregnancy continued until term. These findings correlate with the lesser number of embryos as retrieved (14.21%) from the superovulated rats that had nicotine treatment for 30 consecutive days. It has moreover been documented that the nicotine exposure (5 mg/kg body weight) for 4 consecutive days produced less number of blastocyst as compared to control [20]. Subsequently, in another study the number of collected oocytes for cytogenetic analysis is found to be lower among smokers [21]. Mice exposed to the cigarette smoke showed low fertilization and embryo development rate compared to the non-exposed animals ($p < 0.05$) [12]. This effect is most likely to be nicotine related because nicotine could be detected in the uterine fluid and also around the blastocyst [22].

Another obvious parameter affected by nicotine administration is the gestation length. Rats treated with nicotine throughout the duration of pregnancy showed a slightly longer gestation period (median 23.5 days) compared to controls (median 21.7 days). This extended length of ges-

tation could possibly be the consequence of delayed embryogenesis, because forty-eight hours following superovulation, the embryos are supposed to attain a 4-cell stage.

In control animals (Group A) the number of retrieved embryos at 4-cell stage was 71.7%. However, nicotine treatment (Group B) remarkably attenuated the rate of embryo cleavage, 61.54% retrieved embryos remained as a single cell (not cleaved). A previous study also documented that nicotine injection for 5 consecutive days resulted in delayed blastocyst hatching and loss of zona pellucida with delayed implantation [23]. Results of our study therefore conclude that PVE with a higher content of tocotrienol [24] is able to reverse the nicotine-induced retarded embryogenesis and consequently pregnancy loss in rats.

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