

RESEARCH ARTICLE

POTENTIAL AMELIORATION OF *MELIA AZEDARACHL* AGAINST NICOTINE-INDUCED OXIDATIVE STRESS IN THE FEMALE RATS

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Abstract:

The protective effect in female rat by oral administration of *Melia azedarach* (100 mg/kg bw /day) against ovarian and uterine oxidative stress induced by subcutaneous injection of nicotine (4mg/kg bw/day), was studied. After 30 days, nicotine treatment increased production of malondialdehyde in the nicotine-treated group which was accompanied by marked alterations in the levels of activities of superoxide dismutase, catalase, GSH, vitamin C and E. Compared to nicotine alone, the combined treatment of nicotine with *Melia azedarach* significantly lowered the level of lipid peroxides and enhanced the antioxidant status. The group of rats given *Melia azedarach* without exposure to nicotine exhibited no significant changes in the above indices. Thus the results from this study showed that *Melia azedarach* exerted a protective action against nicotine-induced oxidative stress and disturbance of ovarian functions in the rat.

Keywords: Nicotine, ovary toxicity; *Melia azedarach* , ovarian oxidative stress.

Introduction

Cigarette smoke contains a mixture of 4000 toxic chemicals, including nicotine, addictive components, carbon monoxide, and several recognized carcinogens and mutagens (1). Smoking has deleterious effects on cardiovascular, pulmonary physiology and reproductive system. In women, smoking is associated with infertility, spontaneous abortion, menstrual abnormalities, ectopic pregnancies and early onset of menopause (2).

Nicotine is a highly toxic substance and it is quickly absorbed through the respiratory tract, mouth mucosa and skin. Nicotine is extensively metabolized to a number of metabolites by the liver. Quantitatively, the most important metabolite of nicotine in mammalian species and humans is the lactam derivative cotinine. In humans, about 70 to 80% of nicotine is converted to cotinine (3).

In adult humans, cotinine has been detected in the follicular fluid of women who smoke, demonstrating that nicotine reaches the ovary and developing follicles. Cotinine, has also been detected in granulosa lutein cells. Treatment of rats with nicotine is associated with a decrease in estrogen dependent parameters, including uterine weight, myometrial and endometrial diameter and thickness.

There are different forms of nicotine administration for evaluating its absorption pharmacokinetics such as smoking, nasal spray, gum, inhaler, sublingual tablets, tooth patch, transdermal patch, intravenous and subcutaneous injections, oral capsule, oral solution and enema. In animal models, nicotine is usually administered subcutaneously, intraperitoneally or orally. Intraperitoneal and subcutaneous administrations of nicotine are more effective than the oral route.

This may be due to the fact that intraperitoneal or subcutaneous routes facilitate the rapid absorption of the substance. All of this evidence indicates that nicotine can affect gamete cell function. (4).

Nicotine disrupts antioxidant mechanism (5) by enhancing Reactive Oxygen Species (ROS) production and thereby decreases the antioxidant level causing peroxidative tissue damages (6). Oxidative stress (OS) affects multiple physiological processes, from oocyte maturation to fertilization, embryo development and pregnancy. It has been suggested that the age-related decline in fertility is modulated by the OS. OS plays a role during pregnancy and normal parturition and in the initiation of pre-term labor. The OS can affect sperm and oocyte quality, the fertilization process, and the embryo. This article highlights the adverse effects of free radicals and how they can affect female fertility. It also addresses how the OS can adversely affect assisted reproduction, a technique which is utilized to help infertile couples conceive their own biological child. Antioxidants act as scavengers to neutralize free radicals, and have generated considerable interest in overcoming the adverse and pathological results of the OS. The OS can cause direct damage to oocytes in developing follicles, or the embryo in the fallopian tube, (7) or through an imbalance in redox potential leading to luteal regression that result in lack of luteal support to pregnancy. Overcoming the pathological effects of the OS may be achieved by reducing the generation of ROS or increasing the amounts of antioxidants available. There are literature reports on the utilization of nutritional supplements and antioxidants such as vitamin E supplementation in patients with infertility. However, there is lack of consensus on the type and dosage of antioxidants to be used. Clinical evidence on the benefits of antioxidant supplementation is equivocal.

Melia azedarach (meliaceae; Neem) is an indigenous plant possessing several medicinal properties. *Melia azedarachinn* (synonym: media dubia Cav, Indian lilac, Persian lilac) belongs to the family meliaceae and is a tree found in India. It is popular as Indian lilac. Different phyto chemicals have been isolated from fruit include melianoninol (I), melianol (II), melianone (III), meliandiol (IV), vanillin (V) and vanillic acid (VI)(8).The plant is traditionally used for the treatment of leprosy, inflammations, and cardiac disorders. Its fruits extracts possess ovidical (9) and larvicidal activity (10).The leaf extracts also possess antiviral (11). As the role of free radicals has been documented in many of these conditions, the present study was directed to investigate the antioxidant activity of *Melia azedarach* leaf extract in rats. Present study was undertaken to investigate the fertility activity of *Melia azedarach*. in nicotine induced reproductive toxicity by oxidative stress.

Experimental Design

Normal cycling, healthy albino female rats of 80 days were used for the experiment. The animals were maintained in the standard laboratory conditions and fed with balanced diet as prescribed by Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd., Bangalore, India and water ad libitum at room temperature of $28 \pm 2^\circ\text{C}$. The animals were divided into four groups, each consisting of 4 animals. Based on the earlier studies in our laboratory the effective dose 0.4 mg/100g body weight was selected for nicotine. The treatment was started from estrous phase of the cycle only as the ovarian and uterine activities change markedly from one phase to another phase.

Female rats were divided four groups. Group I control, group II nicotine induced ,group III nicotine induced and drug treated and group IV only drug treated .Group II received subcutaneous injection of nicotine tartarate (4mg/kg bw per day for 30 days). Along with nicotine herbal drug was given at the dosage of 100mg per kg body weight for Group III rats. The group IV rats received only 100 mg of *Melia azedarach*. All the experimental rats were sacrificed by decapitation on 31st day, 24 hours after the final dose.

The body weight was recorded. Ovary and uterus were dissected out, freed from adherent tissue and weighed on Anamed electronic balance. The ovaries and uterus were homogenized in chilled 0.1M Tris-Hcl buffer in a Potter-Elvehjem Teflon Homogenizer. The homogenate was used for the assay of the activities of LPO (12)superoxide dismutase (Misra and Fridovich,1972) (13), catalase (Takaharaet al.,1960) (14), Reduced Glutathione(Moron et al.,1979) (15) vitamin C and E (16)

Results and discussion

The levels of enzymatic antioxidants like superoxide dismutase, catalase, in ovary and uterus of control and experimental mice were showed in Table 1 and 2. The levels of these enzymes in ovary and uterus are significantly depleted ($P<0.01$) in the nicotine induced group compared to normal control group. Treatment with herbal drug *Melia azedarach* showed significant increase of those enzymes in group 3 treated animals when compared to group 2. No alterations in the levels of these enzymes in group 4 when compared to group 1.Levels of non enzymatic antioxidants ovary and uterus of control and experimental animals were showed in Table1 and 2. The levels of these enzymes ovary and uterus are significant decreased ($P<0.01$) in the nicotine induced group compared to normal control Group. But the levels of LPO showed increased in nicotine induced Group. Treatment with herbal showed significant increase in group 3 animals when compared to group2. No variation in the levels of these enzymes in group 4 when compared to group 1.

Nicotine is a highly addictive alkaloid induced oxidative stress both *in vivo* and *in vitro* (17). In the present study, the effects of nicotine in the rat ovary were detected by antioxidant measurement. It was shown that *Melia azedarach* reversed the adverse effects of nicotine in rat ovaries and uterus. The maintenance of high redox potential is a prerequisite for assuring the reproductive system functions in a healthy organism (18). Physiologically, ROS are increased in ovary after the preovulatory gonadotrophin surge and also in corpus luteum (CL) during steroidogenesis which involves the cytochrome P450 system (19). However, the detoxification of ROS would particularly be important for the oocyte maturation and embryo development. If free radicals are not neutralized by endogenous or exogenous antioxidant molecules such as SOD, then lipid peroxidation would occur at the cell membranes. In these cells, unsaturated lipids converted to peroxides would produce degradation products with toxic aldehyde moieties such as MDA. These subsequently interfere with the ovarian reproductive functions.

It has been reported that the nicotine disrupts the mitochondrial respiratory chain leading to an increase in generations of superoxide ions and hydrogen peroxide (20). Superoxide anion and hydrogen peroxide are the main sources of the nicotine induced free radical generation and depletion of the cellular antioxidant (21). Glutathione being an important cellular reductant involved in protection against free radicals, peroxides and toxic compounds (22). Therefore depletion of GSH not only impairs cell defense against toxic compounds but also results in enhanced oxidative stress and tissue damage (23). Our observation shows that nicotine treatment more significantly ($p < 0.01$) depletes GSH level of ovary of nicotine treated groups indicating higher level of tissue damage (24) (Table 1). Decreased SOD levels, which means that the consumption of this antioxidant enzyme is increased due to nicotine or its metabolites. In other words, oxidation means the overproduction of the free radicals; they covalently bind to DNA and increase the proapoptotic signals (25). These death signals allow cytochrome c to leak out of mitochondria into the cytosol and then cause the caspase 9 to activate the caspase (cysteine-aspartic acid protease) cascade which then leads to cell death (26). Caspase 3 is an "effector" enzyme that functions in this cascade to promote the cell death. It is located in the cytoplasm of luteal and theca cells in CL and healthy follicles and also in the granulosa cells of follicles undergoing apoptosis (27). As a result, nicotine causes all these reactions which deteriorate the ovarian and uterine antioxidant status, induce lipid peroxidation, and promote the apoptotic cell death (28). A lack of vitamin E can result in shrinkage of reproductive organs including the uterus and ovary.

In conclusion, present results demonstrate that the herbal drug *Melia azedarach* has antioxidant activities due to the presence of phenolic compounds. The ameliorative effect of *Melia azedarach* is due to its scavenging or neutralizing free radical activities that inhibits peroxidation of membrane lipids and maintains cell membrane integrity and functions (Ritukumari *et al.*, 2002).

Conclusion

The present study reported the beneficial effects of *Melia azedarach* on ovarian toxicity induced by nicotine induction.

Table 1 Effect of nicotine and *Melia azedarach* L. on antioxidant enzymes in ovary

Parameters	Control	Nicotine	Nicotine & Melia Azedarach .	Melia Azedarach
SOD	5.87 ±0.28	3.53 ±0.04**	4.93 ±0.10**	5.8 ±0.27*
CATALASE	6.35±0.08	3.54±0.21**	4.26±0.10*	6.65±0.09
GSH	2.46±0.03	0.92±0.14**	1.87±0.04**	2.5±0.14
VITAMIN C	2.47±0.2	1.07±0.13**	2.0±0.18*	2.56±0.17
VITAMIN E	2.67±0.1	1.2±0.11**	1.99±0.05**	2.69±0.09
LPO	1.6±0.08	3.4±0.15**	2.23±0.05	1.8±0.08

SOD-units/mg protein
 Catalase μ moles of H₂O₂ decomposed/min/mg protein
 GSH μ moles/g wet tissue
 VITAMIN C&E mg/g
 LPO n moles of MDA/mg protein
 Values are Mean ± S.E. ** = P < 0.01, * = P < 0.05

Table 2 – Effect of nicotine and *Melia azedarach* L. on antioxidant enzymes in uterus

Parameters	Control	Nicotine	Nicotine & <i>Melia</i>	<i>Melia</i> Azedarach L.
SOD	3.24±0.14	1.67±0.10**	2.50±0.03**	3.5±0.09
CATALASE	1.6±0.079	0.8±0.08**	1.3±0.04*	1.50±0.11
GSH	1.62±0.08	0.85±0.08**	1.23±0.04**	1.87±0.04
VITAMIN C	1.7±0.11	0.9±0.04**	1.32±0.07*	1.82±0.09
VITAMIN E	2.03±0.05	0.73±0.12**	1.41±0.06	2.57±0.13
LPO	0.63 ±0.08	2.9±.3**	1.73±0.23*	0.8±0.08

SOD-units/mg protein
 Catalase μ moles of H₂O₂ decomposed/min/mg protein
 GSH μ moles/g wet tissue
 VITAMIN C&E mg/g
 LPO n moles of MDA/mg protein
 Values are Mean ± S.E. ** = P < 0.01, * = P < 0.05

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