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PROTECTIVE EFFECT OF *MELIA AZEDARACH* ON NICOTINE INDUCED CHANGES IN REPRODUCTIVE TISSUE OF FEMALE ALBINO RATS

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ABSTRACT

The beneficial effects of ***Melia azedarach*** extract as an antioxidant has been assessed in nicotine administered rats to examine the effects of nicotine on the antioxidant defense systems in reproductive tissue of female albino rat. The rats were divided into 4 groups. Group I: control animals received normal saline, Group II: injected with nicotine 4mg/kg, Group III: injected with nicotine4mg/kg and *Melia azedarach* 100mg given orally. Group IV: given *Melia azedarach* 100 mg/kg orally. The experimental group were injected subcutaneously 4 mg/kg of nicotine tartrate daily for 30 days. The animals were sacrificed after 24 hours after the last treatment by cervical dislocation and isolated the ovaries and uterus tissue washed with ice-cold saline and stored 4^0c at for enzymatic assays. In the present study Glutathione (GSH) vitamin E, vitamin C and vitamin A nicotine treated rats in the reproductive tissue and enhancement was observed in the combination treatment (Nicotine+ *Melia azedarach*).The data obtained from this study speculated that 100 mg of *Melia azedarach* has the capacity to scavenge free radical and can protect against oxidative stress induced by Nicotine intoxication. Supplementation of *Melia azedarach* could be useful in alleviating antioxidant enzymes in nicotine induced reproductive tissue injury in female rats.

KEYWORDS: Nicotine, *Melia azedarach*, Glutathione (GSH) ,vitamin E, vitamin C and vitamin A

INTRODUCTION

The female reproductive system includes organs and organ systems that serve a number of functions related to the creation and maturation of a new organism. Each of the components

of the system may be subject to the toxic effects by xenobiotic agents. There is growing concern about the increasing prevalence of various abnormalities of the reproductive system in humans. Since the reproductive process is critical for perpetuation of any organism, factors or agents that alter or disrupt this process can have devastating consequences.

Nicotine, one of the most potent alkaloids found in significant amounts in tobacco leaves, is also present in lower levels in potatoes, tomatoes and eggplants. It is a naturally occurring alkaloid found in the nightshade family (*Solanaceae*) of plants, predominantly in tobacco plant (*Nicotiana tabaccum*).[1] Nicotine is the established endocrine disruptor reported to disturb the reproductive process in both males and females. The exposure of nicotine causes impaired fertility and ovarian dysfunction. Nicotine has been documented to alter the oxidant and antioxidant balance alters lipid peroxidation and antioxidant enzyme in plasma and ovaries of female rats.[2]

Meliaazedarach. L. belongs to the family Meliaceae is from west Asia. It has been used for various medicinal purposes.[3] The leaf juice is used as an anthelmintic. It is also to cure strangury, amenorrhoea, bronchitis, leprosy, eczema, asthma and as an antipyretic.[4] The purpose of this study was to investigate the toxic effects of nicotine in two major female reproductive organs, viz., ovary and uterus and to examine the possible beneficial role of ***Melia azedarach*** in preventing nicotine-induced female reproductive toxicity.

MATERIALS AND METHODS

Chemicals

Nicotine hydrogen tartrate were purchased from Sigma Chemicals, St. Louis, USA. All other required chemicals were purchased from Genie. Bangalore, India. All the chemicals and reagents used were analytical grade.

Experimental protocol

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 a balanced diet as prescribed by Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd.,Bangalore, India and water ad libitum at room temperature of 28 ± 2°C. Female rats were divided four groups. Group I control, group II nicotine induced, group III nicotine induced and *Melia azedarach* treated and group IV only drug *Melia azedarach* treated. Group II received a subcutaneous

injection of nicotine tartrate (4mg/kg bw per day for 30 days). Along with nicotine, *Melia azedarach* was given at the dosage of 100mg per kg body weight for Group III rats.

Sample collection

All the experimental rats were sacrificed by decapitation on the 31st day, 24 hours after the final dose. The body weight was recorded. Ovary and uterus was dissected out, freed from adherent tissue and weighed on an electronic balance. Glutathione (GSH).[5] vitamin E (Rosenberg,1992).[6] vitamin C (Roe and Keuther, 1943).[7] and vitamin A (Bayfield and Cole, 1980).[8] content of ovaries and uterus were determined in rats of all the four groups.

RESULTS

In nicotine treated rats the level of GSH was decreased in the ovary and uterus. On the other hand treatment with *Melia azedarach* with nicotine increased the activity of GSH gradually in group III rats (Table 1). The GSH level was normal in Group IV rats as that of group I. The value of vitamin E, C and A of ovary and uterus were given in the Table2,3 and 4. The level of vitamins was significantly reduced in nicotine exposed rats when compared to the normal group. The crude powder of *Melia azedarach* given along with the nicotine gradually increased the levels of vitamins. In group IV rats no changes in the levels was observed.

Table 1: Effect of nicotine and *Melia azedarach*L. on GSH of female rats

GROUPS	GSH (µ moles /g protein)	
	OVARY	UTERUS
Group I	2.46±0.03	1.62±0.08
Group II	0.92±0.14**	0.85±0.08**
Group III	1.87±0.04**	1.23±0.04**
Group IV	2.5±0.14	1.87±0.04

Values are Mean ± S.E. ** P < 0.01, * P < 0.05

Table2: Effect of nicotine and *Melia azedarach* L. on Vitamin E of female rats

GROUPS	Vitamin E (mg /g)	
	OVARY	UTERUS
Group I	2.67±0.1	2.03±0.05
Group II	1.2±0.11**	0.73±0.12*
Group III	1.99±0.05**	1.41±0.06**
Group IV	2.69±0.09	2.57±0.13

Values are Mean ± S.E. ** P < 0.01, * P < 0.05

Table 3: Effect of nicotine and *Melia azedarach*L. On Vitamin C of female rats

GROUPS	Vitamin C(mg /g)	
	OVARY	UTERUS
Group I	2.47±0.2	1.7±0.11
Group II	1.07±0.13**	0.9±0.04**
Group III	2.0±0.18*	1.32±0.07*
Group IV	2.56±0.17	1.82±0.09

Values are Mean ± S.E. ** P < 0.01, * P < 0.05

Table 4: Effect of nicotine and *Melia azedarach* L. On Vitamin A of female rats

GROUPS	Vitamin A (µg /g)	
	OVARY	UTERUS
Group I	16.63±0.04	11.4±0.5
Group II	11.37±0.20**	6.9±0.5**
Group III	14.14±0.27**	8.4±0.06**
Group IV	16.27±0.13	10.5±0.16

Values are Mean ± S.E. ** P < 0.01, * P < 0.05

DISCUSSION

GSH is synthesized during oocyte maturation which has been reported in mouse by.[9] Yoshida *et al* .,1993 ROS produced in the follicle causes apoptosis whereas GSH and follicle stimulating hormone counterbalance this action in the growing follicle. Depletion in GSH caused increase in H2O2 concentration and DNA lesions.[10] Hydrogen peroxide formed during the depletion of GSH lowered both cAMP dependent and non-cAMP dependent steroidogenesis.In the present study the level of GSH was reduced in the ovary and uterus of the rats exposed to nicotine. Nicotine toxicity was counterbalanced by *Melia azedarach* which has vitamin E.

Vitamin E (α-tocopherol) is a lipid soluble vitamin with antioxidant activity. The table.2 depicts low vitamin E level in group II when compared to treated samples (group III).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. It is thus assumed that the application of this antioxidant as probable preventive agent could be targeted in therapeutic amelioration of nicotine-induced abnormalities.

Ascorbic acid (Vitamin C) and other anti-oxidative substances help to prevent oxidative stress and the production of free radicals that interfere with progesterone production. Vitamin C possesses some functions such as collagen synthesis increment, impact on sexual hormones

production, and protection of sexual cells against free radicals associated with infertility.[11] Collagen synthesis is required for follicle growth, for repair of the ovulated follicle, and for corpus luteum development.[12] Ascorbate will also be needed for secretion of collagen and proteoglycans into follicular fluid .Ovaries have cycles of tissue changes and steroid and peptide secretion which are correlated to vitamin C. Ovaries are the main sites of vitamin C reserve with the highest concentrations in theca interna cells, granulosa, and luteal part. Studies show that in women who are receiving steroid contraceptive drugs, plasma and leukocytes ascorbic acid levels are reduced. Furthermore, low plasma levels of ascorbic acid (i.e., Vitamin C) was observed in women who habitually miscarry. Ascorbic acid deficiency produces ovarian atrophy and extensive follicular atresia. Similar lower levels of vitamin C is seen in group II when compared to treated samples (group III). At the outset markable changes in the level of the enzymes were observed in the results. The improvision of antioxidant property after treatment was also observed. This clearly indicates that the leaf extract of *Melia azedarach* has an immense antioxidant property.

Vitamin A is a fat soluble vitamin, which is essential for growth, maintenance of visual function, reproduction and differentiation of epithelial tissue. Its metabolites affect ovarian follicular growth, uterine environment and oocyte maturation. Vitamin A and its metabolites play a crucial role in regulating the differentiation and proliferation of epithelial cells. There is clear *in vivo* evidence that vitamin A is required for the normal onset of meiotic prophase in ovarian germ cells.[13] When severe vitamin A deficiency is imposed prior to mating, cornified cells are continuously present in vaginal smears.[14] and reproduction fails prior to implantation .VAD female rats continue to ovulate and form corpora lutea irregularly or at normal intervals, however, degenerated eggs are found in the last portion of the tube, and there is no evidence that blastogenesis has occurred. Rats reared on a vitamin A-deficient diet have a reduced ability to secrete progesterone and 20cr-hydroxypregn-4-en-3-onineto the ovarian venous blood on day**9** and **15** of pregnancy.[15] In the group III rats the levels of the nonznymatic antioxidant were reversed by the treatment with *Melia azedarach.*It is concluded the *Melia azedarach* is an effective antioxidant in protecting the reproductive tissues.

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