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PROTECTION OF *Curcuma aromatica* ON ANTIOXIDANT ENZYMES IN REPRODUCTIVE TISSUES OF FEMALE RATS TREATED WITH NICOTINE

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ABSTRACT

Nicotine is known to induce oxidative stress in rat reproductive tissues and the antioxidant properties of *Curcuma aromatica* have been reported. Four groups of four rats each were divided into: Group I: (control) received 0.2ml of 0.9% normal saline, group II (received nicotine 0.4mg/kg b.w subcutaneously), group III (received nicotine 0.4 mg/kg b.w + *Curcuma aromatica* 100mg/kg b.w orally), and group IV (received *Curcuma aromatica* 100mg/kg b.w orally). All animals were treated for 30 days. The ovary and uterus of the animals were harvested, weighed and homogenized. Non enzymatic antioxidant such as reduced glutathione (GSH),Vitamin C,E and A were then measured. The activities of GSH Vitamin C, and A significantly decreased in group II (nicotine only) rat tissues, while it was significantly increased in group III and IV rat tissues. The study showed that *Curcuma aromatica* extract (100mg/kg b.w) administration prevented oxidative damage in rat tissues treated with nicotine.

Keywords: *Nicotine, Vitamin E, Curcuma aromatica, antioxidant*

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INTRODUCTION

Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen derived oxidants commonly known as reactive oxygen species. Normal cellular aerobic metabolism resulted in the generation of ROS. Minimal levels of ROS act through signaling pathways, which is necessary for normal physiological functions in the female reproductive tract (1). Physiologically, ROS are increased in ovary after the preovulatory gonadotrophin surge and also in corpus luteum (CL) during steroidogenesis which involves the cytP450 system (2). Physiologic relevance of ROS is implicated in oocyte maturation, ovarian steroidogenesis, ovulation, implantation, formation of blastocyst, luteolysis and luteal maintenance in pregnancy .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 .There should be delicate balance between ROS and antioxidant enzymes in the ovarian and uterine tissues.

Nicotine disrupts antioxidant mechanism (3) by enhancing Reactive Oxygen Species (ROS) production and thereby decreases the antioxidant level causing peroxidative tissue damages.(4) Oxidative stress (OS) affects multiple physiological processes, from oocyte maturation to fertilization, embryo development and pregnancy. Antioxidants act as scavengers to neutralize free radicals and have generated considerable interest in overcoming the adverse and pathological results of the OS. 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, (5) 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.

Curcuma aromatica is distributed throughout India and is widely used as a flavouring agent, condiment and a source of yellow dye. Medicinally, it possesses strong antimicrobial effect. It is a well listed drug in Ayurveda and other indigenous systems of medicine. The rhizomes of *C. aromatica* possess a reputed property to promote health conditions by arresting ageing and have immunomodulatory effects. From ancient times, it is being used as an antibiotic against various microbial infections. (6) Historically, rhizomes are used as tonic, carminative, and externally in combinations with astringents, bitters and aromatics to brusises, in sprains and in snake-bite. They are also used for skin eruptions and infections. (7) Present study was undertaken to investigate the fertility activity of *Curcuma aromatica* 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 ± 2°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.

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 *Curcuma aromatica*. All the experimental rats were sacrificed by decapitation on 31st day, 24 hours after the final dose.

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 Reduced Glutathione (Moron *et al*., 1979) (8) vitamin E (9), vitamin C (10) and vitamin A (11).

RESULTS AND DISCUSSION

The levels of non enzymatic antioxidants like GSH, Vitamin E, C and A in ovary and uterus of control and experimental rat 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 *Curcuma aromatica* 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.

Nicotine is a highly addictive alkaloid induced oxidative stress both *in vivo* and in *vitro* (12). In the present study, the effects of nicotine in the rat ovary were detected by antioxidant measurement. It was shown that *Curcuma aromatica* reversed the adverse effects of nicotine in rat ovaries ant uterus. The maintenance of high redox potential is a prerequisite for assuring the reproductive system functions in a healthy organism (13). Physiologically, ROS are increased in ovary after the preovulatory gonadotrophin surge and also in corpus luteum (CL) during steroidogenesis which involves the cyt P450 system (14).However, the detoxification of ROS would particularly be important for the oocyte maturation and embryo development.

Glutathione being an important cellular reductant involves in protection against free radical**s,** peroxides and toxic compounds. Therefore depletion of GSH not only impairs cell defense against toxic compounds but also results in enhanced oxidative stress and tissue damage (15). 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 (Table 1).

The table1depicts 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 (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.

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 *Curcuma aromatica* has an immense antioxidant property. In the group III rats the levels of the nonznymatic antioxidant were reversed by the treatment with *Curcuma aromatica*. In conclusion, the antioxidant effect of garlic on the tissues reported in this study suggests that its consumption may reduce the oxidative stress induced by nicotine.

Table1 Effect of *Curcuma aromatica* on ovary in nicotine induced experimental rats

Parameters	Group I	Group II	Group III	Group IV
GSH	2.3±0.02	0.9±0.008**	1.6±0.16**	2.4±0.02
Vitamin E	2.67±0.1	1.21±0.11**	1.98±0.05**	2.6±0.08
Vitamin C	2.42±0.2	1.44±0.21**	2.16±0.08*	2.5±0.1
Vitamin A	16.63±0.04	11.375±0.20**	14.14±0.27**	16.27±0.13

GSH µ moles/g wet tissue; Vitamin E&C mg/g; Vitamin A µg /g
Values are Mean ± S.E. ** = P < 0.01,* = P < 0.05

Table 2 Effect of *Curcuma aromatica* on Uterus in nicotine induced experimental rats

Parameters	Group I	Group II	Group III	Group IV
GSH	1.4±0.05	0.83±0.04*	1.27±0.03*	1.5±0.1
Vitamin E	2.03±0.05	0.72±0.2*	1.4±0.06*	2.5±0.3
Vitamin C	1.5±0.11	0.69±0.19*	1.35±0.04*	1.72±0.11
Vitamin A	11.4±0.5	6.9±0.5**	8.4±0.06**	10.5±0.16

GSH µ moles/g wet tissue; Vitamin E&C mg/g; Vitamin A µg /g
Values are Mean ± S.E. ** = P < 0.01,* = P < 0.05

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