

STUDIES ON OVARIAN ACTIVITY IN PINUS LAMBERTIANA TREATED RAT

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INTRODUCTION

Pinus lambertiana (Pinus I.) is a homoeopathic medicine, prepared from the sugar pine. Recently Paul, Deb and Banik¹ reported that, treatment with 0.1 ml mother tincture of Pinus I. resulted in drastic alteration of estrous cycle and ultimately leading to its arrest in diestrous phase. Physiological effects and mechanism of action of this drug is not yet clear. In the present paper the effect of Pinus I. on the ovarian activities of the albino rats (whister strain) have been studied.

Thirty-six closed colony bred adult albino female rats of 140 to 150 g bodyweight used. The animals were kept in the laboratory with standard laboratory diet and sufficient water for one week. Before starting the experiment three consecutive estrous cycle (4-day cycle) were studied by vaginal smear technique. The animals were divided into three groups, each comprising 12 rats. 0.1 ml mother tincture of Pinus I. were fed to one group of animals daily for 10 days. Among the other two groups, one group treated with 96% alcohol and the other one with only distilled water in the same amount and in same manner. In all the animals treatment started at the diestrous phase. 24 hours after the application of last dose, all the animals were killed and the ovaries and uterus were dissected out and their weights were recorded. Ovarian $\Delta^3\beta$ hydroxysteroid dehydrogenase ($\Delta^3\beta$ HSD) was demonstrated histochemically, using dehydroepiandrosterone as substrate². Parallel sections were incubated in corresponding substrate-free media and served as controls. Histological studies of ovary and uterus were performed after staining it with haematoxylin and eosin. The ascorbic acid content of ovaries were estimated by following the method of Roe and Kuether³ and the cholesterol according to Sperry and Webb⁴. The right and left ovaries of 6 animals were taken for estimation of cholesterol and ascorbic acid, respectively.

RESULTS

After treatment with Pinus I. the weights of ovaries and uterus were reduced significantly (Table 1). On histological examination it was observed that graafian follicles were degenerated and the interstitium atrophied remarkably in comparison to control groups (Fig. 1 and Fig. 2). Histochemical preparation showed $\Delta^3\beta$ HSD activities in the interstitium, thecal cells and corpus luteum. Following treatment with the drug $\Delta^3\beta$ HSD diminished

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TABLE I

Weights of ovary, uterus and total concentration of ovarian ascorbic acid and cholesterol after treatment with Pinus l.
(M±SE)

| Treatment | Wt. of ovary mg/100 g B.W. | Wt. of uterus mg/100 g B.W. | Ascorbic acid mg/100 mg | Cholesterol mg/100 mg |
|-----------------|----------------------------------|-----------------------------------|----------------------------|--------------------------|
| 98% alcohol (8) | 50.56± .31 | 157.76± .84 | 240.54± .82 | 1.31± .02 |
| Pinus l. (8) | 39.10± .31 | 106.75± .93 | 288.33± 2.27 | 1.71± .01 |
| P-value | <0.001 | <0.001 | <0.001 | <0.001 |

Figure in the parentheses indicates number of animals.

significantly in all the above mentioned compartments of ovaries (Fig. 3 and Fig. 4) along with accumulation of ascorbic acid and cholesterol (Table 1). No notable alterations were observed between the alcohol and distilled water treated controls.

DISCUSSION

It has been accepted that the sequential changes of the vaginal smear in the different phases of estrous cycle are closely associated with secretion of gonadal steroids. Knorr *et al*⁵ successfully established that $\Delta^3\beta$ HSD is an important enzyme in the production of steroid hormones. Chatterton *et al*⁶ observed that maximum synthesis and secretion of ovarian steroids take place in proestrous and estrous stages. The present study demonstrates that $\Delta^3\beta$ HSD of the different steroidogenic compartments (Fig. 3 and Fig. 4) of the ovary is depressed following administration of Pinus l. along with



Fig. 1: Histology of ovary of control rats (×120).

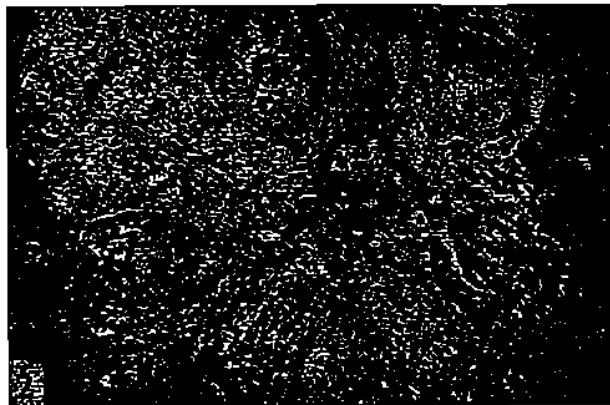


Fig. 2: Histology of ovary of Pinus l. treated rats. Showing degeneration of graafian follicles and atrophies interstitium ($\times 120$).



Fig. 3: The $\Delta^3\beta$ -HSD activity of ovary of control rats ($\times 120$).

significant reduction in the weights of ovary and uterus (Table 1). The important role of $\Delta^3\beta$ HSD in the biogenesis of ovarian steroids have been established earlier and it is also well documented that the weight of accessory sex glands largely depend upon the status suggested, that treatment with Pinus l. causes a reduction in the production of ovarian steroids. Histological examination of ovary (Fig. 1 and Fig. 2), also lead to suggest the altered steroidogenic activities of ovary in this condition.

The role of cholesterol⁷ and of ascorbic acid⁸ in the process of gonadal steroidogenesis is well established. Dey *et al*⁹ noticed an increase in concen-



Fig. 4: Decreased in $\Delta^53\beta$ -HSD activity in the ovary of Pinus l. treated rats ($\times 120$).

tration of cholesterol and ascorbic acid in the hypofunctioning ovaries. From this point of view the accumulation of cholesterol and ascorbic acid in the ovaries in Pinus l. treated rats gives additional support to the depressed ovarian steroidogenic activity and hypofunctioning of the gland as a result of the above treatment. Therefore, on consideration of our accumulated data it may be concluded that arrest of estrous cycle following administration of Pinus l.¹ is possibly due to the reduction in ovarian steroidogenesis.

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Editorial comment: The studies carried out by the learned authors of the paper of the effects of Pinus lambertiana, a homoeopathic drug, on ovarian activity of rats are no doubt, very interesting. But it is difficult to comprehend how they become useful for homoeopathic prescribing. Homoeopathy studies dynamic action of drugs on human beings and in therapeutics.

matches them with the dynamic action of the disease expressed through signs and symptoms, both subjective and objective. The pharmacognostic studies of massive doses of drugs and their capacity to bring about pathophysiological and histo-pathological changes in animals is the style of research beneficial to the therapeutic science belonging to the modern system of medicine. Homoeopathic research should be directed by the principles of drug proving towards ascertaining the pathogenetic action *Pinus lambertiana* is capable of bringing in the human beings.
