Evaluation of Acid and Alkaline Phosphatase of Reproductive Organs of Male Albino Rats after Administration of Ethanolic Extract of Convolvlus Microphyllus

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Abstract

Oral administration of *Convolvulus microphyllus* (whole plant) EtOH (100, 200 and 300mg/day/rat) extract showed significant decrease in the acid and alkaline phosphatase (BU) of reproductive organs of male albino rats. There was a remarkable increase in the amount of cholesterol of testes was noticed.

The decline in acid phosphataise activity was possibly due to decline in the endogenous production of androgens. The decrease in ALP activity in reproductive organs could be correlated to interference in membrance transport. Increased level of cholesterol supports decreased synthesis of androgens. The androgen imbalance has inhibited the spermatogenic process and caused temporary infertility which was noticed in the histopathalogical study of treatments. Fluctuation in histological and biochemical parameters are all dose dependent which could be due to antiadrogenic nature of drug.

Key words:- Acid phosphatase (ACP) Alkaline Phosphatase (ALP), Cholesterol, testes, androgens, antiandrogenic, histopathology.

INTRODUCTION

The role of phosphatase in the testes is transport of substances across the cell membrances, its growth and differentiation (1). In the epididymis, alkaline phosphatase (ALP) is involved in the transport of organic molecules across the membrane and acid phosphatase (ACP) is implicated in scavenging activities (2). Rat testicular tissue contain four different acid phosphatase (3). Two of these enzymes (I & II) are mainly interstitial in location while acid phosphatase (III & IV) appeared to be prevalent in the tubule. The activity and localization of ACP of rat epididymis is under the control

of testosterone as suggested (4). ACP is present in all the germinal cells and increase with development of spermatogenesis and spermatids was shown by (5). According to (6) the changes in acid phosphatase characteristics respond to changes in endogenous androgen levels more rapidly than other parameters related to androgenic stimulation such as organ weight, total protein and DNA concentration. Secretory acid phosphatase is therefore a sensitive marker of prostatic secretory function.

A weakening of testicular alkaline phosphatase (ALP) after stilbesterol treatment in rabbit testes was observed by (7). Kalla & Bhasin (1977) (8) observed a decrease in ALP activity Cyproterone acetate treatment in the rat testes. Human males showed diminished, ALP activity in seminal plasma in case of oligozoospermia and azoospermia (9). The decrease in sperm number is also responsible for the decrease in ALP activity (10).

Salhanick and Terner 1979 (11 and 12, 13) suggested marked increase in cholesterol in testes implies inhibition of androgenesis and impairement of spermatogenesis. Dixit 1977 (14) and (15) have reported a significant increase in testicular cholesterol after administration of certain plant extracts.

Various plant extract and their active principle has been extensively tested for their impact on spermatogenic process and related physiology *Convolvulus microphyllus* sieb (Convolvulaceae) is commonly known as Shankhapushpi. Bindweed is reported to be a potent memory enhancing drug, which is used as a psycho-stimulant and tranquilizer. It also reduces mental stress. The ayurvedic pharmacopoeia of India documents the use of the plant for treating epilepsy. No work has been done on reversible antifertility activity of plant.

MATERIALS AND METHODS

Plant extract:-

Convolvulus microphyllus whole plant was collected from Jhalana Dungri, Jaipur. Shade dried plant was subjected to soxhalation in 50% ethanol.

Animal:-

Healthy male albino rats weighing about 200gms were used. Males were cohabited with proestrous females in the ratio of 1:3 only. The fertile males whose sire delivered average number of litter were used.

Experimental design:-

The males were grouped 5 in number, identified and kept in plastic cages. The daily dose of plant

extract was freshly prepared dissolved in 5ml of distilled water and administered every morning for 60 days.

Gr. I	Vehicle treated controls - The group received vehicle only i.e. distilled water.
Gr. II	100mg/day/rat alcoholic extract of *Convolvulus microphyllus* was orally fed to rats.
Gr. III	200mg/day/rat alcoholic extract of *Convolvulus microphyllus* was orally fed to rats.
Gr. IV	300mg/day/rat alcoholic extract of *Convolvulus microphyllus* was orally fed to rats.

The whole spermatogenic process requires 53 days in rats, out of which spermatozoa spend last 6-7 days in the final transit through epididymis (16). The plant extract was administered for one complete spermatogenic cycle.

Experimental Procedure:-

The male rats were kept in starved condition for 24hrs. after the last dose delivery, then weighed and sacrificed under ether anesthesia, autopsied. The reproductive organs i.e. testes, epididymis, seminal vesicle and ventral prostate were removed, cleared off fats and connective tissues weighed and kept at -20^{0}C.

Tissue Biochemistry:-

Acid and alkaline phosphatase were estimated in the reproductive organs viz testes, caput epididymis, cauda epididymis, seminal vesicle and ventral prostate (17). Cholesterol was estimated in testes (18).

Histological parameters:-

All reproductive organs testes, epididymis, seminal vesicle and ventral prostate were fixed in Bouins fixative. All the organs were cut into small pieces and processed. The paraffin embedding was followed by section cutting (3-4μ) and staining (Harris Hemotoxylin and Eosin).

Statistical Calculation:-

Values of tissue biochemistry were expressed in terms of mean value $\pm$ standard error. The different groups were compared among each other using student's t-test (19).

RESULT AND DISCUSSION

ACP in *Convolvulus microphyllus* treated rats were significantly reduced (Dia 3, 4) at all three doses.

The decline in acid phosphatase activity in ventral prostate which was possibly due to decline in the endogenous production was reported by (20). Androgen dependent ACP may be a precussor for secretory ACP and the biosynthetic interrelationship may involve controlling the state of glycosylation. Blackshaw and Massey 1978 (21) showed reduced acid phosphatase activity in cryporchid testes. Kaur and Mangat 1979 (22) also observed a decrease in testicular ACP activity after clomiphene citrate treatment in male rats. Girgis et at 1981 (9) noted a decreased level of ACP in human semen and suggested that it might be due to inhibition of secretory function of the prostate. Guraya and Gill 1977 (23) reported a decrease in cauda epididymal ACP following a low dose of α −Chlorhydrin and correlated it with the decrease of golgi cisternae as a result of reduction in the height of the cells. According to (24) the low ACP level in epididymis may in some way affect sperm maturation and hence fertility. Antiandrogenic effect of neem seed oil on the acid phsphatase activity of ventral prostate was suggested by (25). The acid phosphatase of the epididymal epithelial cells has been considered to be connected with the process of absorption (26).

The *Convolvulus microphyllus* treatment at all the dose levels has reduced the Alkaline phosphatase (ALP) contents of testes and epididymis (Dia 1, 2) in comparison to controls. This could be correlated with inhibition of spermatogenesis, since ALP helps in carbohydrate metabolism and synthesis of testicular hormones. Setty et at 1977b (27) observed a reduction in ALP activity after castration in epididymis and seminal vesicle which was restored to normal level by TP treatment.

Convolvulus microphyllus treatment increased the testicular cholesterol (Dia 5) at all the dose levels in dose dependent manner. Cholesterol is the most important precussor in the synthesis of steroid hormones and its level is immediately related to fertility and sperm output (28, 29, 30). Hall 1984 (31) emphasized the requirement of cholesterol for normal hormonal activity of testes. Since the androgen level is related to fertility and sperm output. The accumulation of cholesterol in testes form a direct evidence for antiandrogenic nature of plant extract. Mallinow et at 1980 (32) suggested the mechanism of hypolipidaemic action could be due to reduced intestinal absorption.

CONCLUSION

Significant fall in ACP of reproductive organs clearly show androgen dependence of ACP activity and antiandrogenic nature of plant. *Convolvulus microphyllu* treatment at all dose levels have remarkably changed the histoarchitecture of testes. The increased level of the testicular cholesterol was significant. *Convolvulus microphyllus* a well reputed plant in the ayurvedic system of medicine after screening in male albino rats are effective antispermatogenic and antiandrogenic agent, in a dose dependent manner.

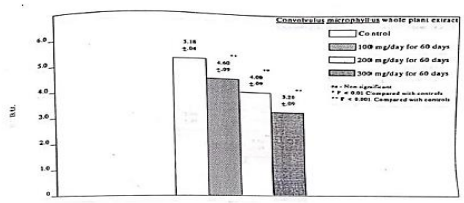
OBSERVATION:-

TABLE NO. 1 : TISSUE BIOCHEMISTRY OF REPRODUCTIVE ORGANS OF CONTROL AND CONVOLVULUS MICROPHYLLUS TREATED INTACT MALE RATS **(Mean ± SEM of 5 Animals)**

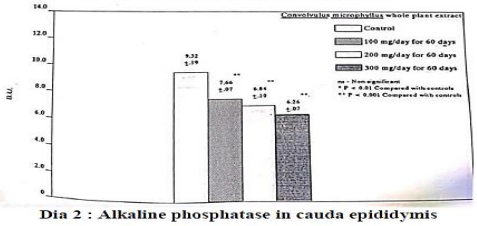
GROUPS	TREATMENT	ALKALINE PHOSPHATASE (B.U.)					ACID PHOSPHATASE (B.U.)					CHOLESTROL (mg/gm)
		Testes	Caput epididymides	Cauda epididymides	Seminal Vesicle	Ventral Prostate	Testes	Caput epididymides	Cauda epididymides	Seminal Vesicle	Ventral Prostate	Testes
I.	Control	5.18 ±0.04	7.89 ±0.06	9.32 ±0.19	4.72 ±0.04	5.17 ±0.09	2.86 ±0.18	3.80 ±0.23	4.20 ±0.02	2.47 ±0.18	2.74 ±0.18	4.51 ±0.11
II.	100mg/day for 60 days	4.60** ±0.09	6.48** ±0.13	7.66* ±0.07	3.60** ±0.10	4.34** ±0.10	2.2* ±0.09	3.28ns ±0.07	3.6** ±0.10	2.2ns ±0.09	2.26ns ±0.07	8.75** ±0.27
III.	200mg/day for 60 days	4.0** ±0.09	6.28** ±0.07	6.84** ±0.10	3.06** ±0.03	3.86** ±0.07	1.8** ±0.9	2.54* ±0.10	3.0** ±0.10	1.4** ±0.09	1.6** ±0.09	10.62** ±0.29
IV.	300mg/day for 60 days	3.2** ±2.09	5.54** ±0.10	6.26** ±0.07	2.4** ±0.10	3.14** ±0.10	1.46** ±0.07	2.26** ±0.07	2.4** ±0.10	1.2** ±0.09	1.4** ±0.09	11.87** ±0.29

ns = nonsignificant * = P 0.01 compared with control

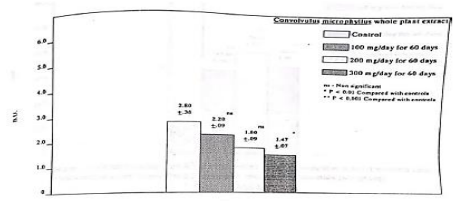
**** = P 0.001 compared with control**



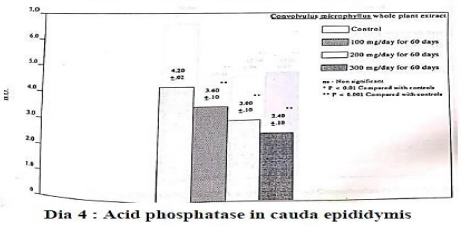
Dia 1 : Alkaline phosphatase in testes



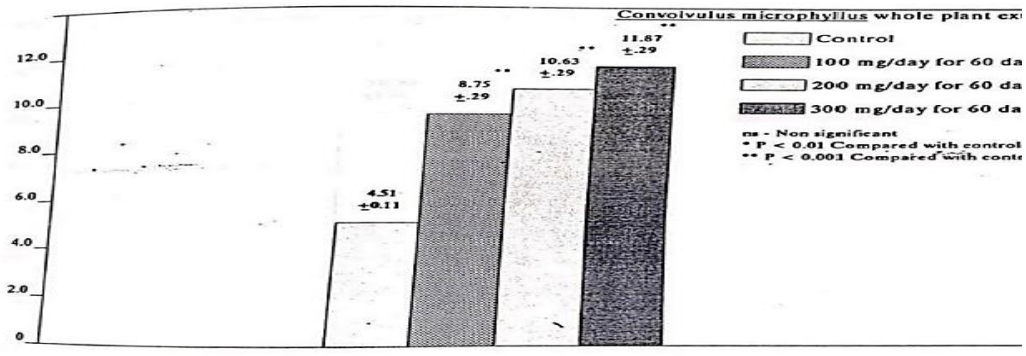
Dia 2 : Alkaline phosphatase in cauda epididymis



Dia 3 : Acid phosphatase in in testes



Dia 4 : Acid phosphatase in cauda epididymis



Dia 5 : Cholesterol in control and treated rats

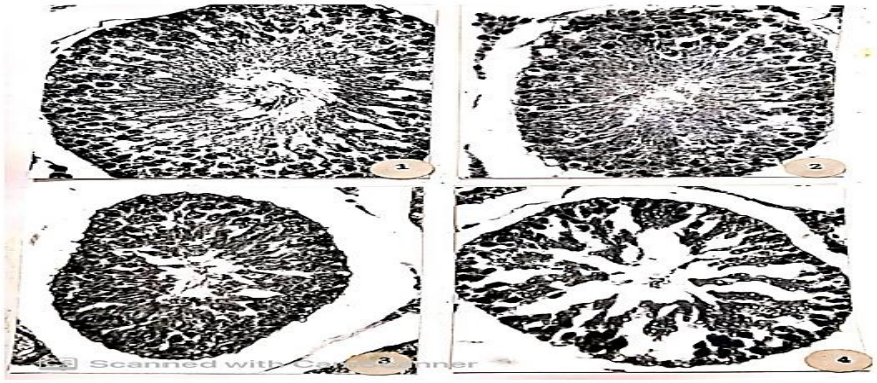


Fig 1 : Histological study of testes of control and treated rats

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