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BIOCHEMICAL CHANGES IN EPIDIDYMIS FOLLOWING TREATMENT WITH COMBINED EXTRACTS OF *AMARANTHUS SPINOSUS* ROOTS AND *DOLICHOS BIFLORUS* SEEDS

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ABSTRACT: *Combined extracts of Amaranthus spinosus roots and Dolichos biflorus seeds at two different doses were administered to adult male rats to assess the structural and functional activity of epididymis. Biochemical studies were made throughout the experiment. Sperm motility was decreased markedly in the cauda epididymal fluid and the sperm counts were reduced in both caput and caudal segments. Epididymal weights, activities of acid and alkaline phosphatase and total LDH were decreased after high dose treatment in both the segments after 10 days of treatment. Protein concentrations were appreciably increased at both doses in the caput and at low dose only in the cauda segment. A reduction in sperm concentration was observed at both doses in cauda but only at high doses in caput. Thus, the herbal extracts impaired the structural and functional integrity of the epididymis.*

INTRODUCTION

Our preliminary studies with acetone dried extracts of *Amaranthus spinosus* roots (Amaranthaceae) and *Dolichos biflorus.* (Papillionaceae) seeds in female rats showed them to be uterotrophic and estrogenic in nature and a combination of these two herbal extracts was found to be highly effective[1]. Since estrogens are well-established for their anti-androgenic effects, we thought it worthwhile to study the effects of these two herbal extracts in combination in the male genital tissues. Our study has shown that the herbal extracts exerted an anti-spermatogenic and anti-androgenic effect in the testes[2]. The present study shows their adverse effect on epididymal structure and biochemical composition.

MATERIALS AND METHODS

A. spinosus roots (ASR) and *D. biflours* seeds (DBS) were procured locally and subjected to soxhlation with acetone separately. The syrupy mass obtained after evaporation of acetone of ASR and DBS was taken in equal quantities and then suspended in propylene glycol for treatment schedule (ASDB extract).

Adult male albino rats of Wistar strain (90-120 days old; 170-200gm body Wt.) were selected from the Institute's inbred colony. Animals were housed individually, maintained in natural laboratory conditions and were fed on pelleted diet and water was provided *ad libitum*. The animals were randomly allocated into the following 3 groups of 5 animals each.

Group I served as control and received vehicle only for 10 days; Group II received 25 mg of ASDB extract/day/rat/ for 10 days; Group III received 50 mg of ASDB extract/day/rat for 10 days.

All the animals were sacrificed for 24 hours after last injection schedule by cervical dislocation. Epididymis was dissected out, weighed and processed for biochemical studies.

Sperm motility and sperm count :

Immediately after sacrifice epididymal spermatozoa were quickly obtained by the puncture on the caput and cauda epididymis with a disposable hypodermic needle (gauge 21). Approximately 1 .1 of the fluid content form the epididymal lumen was collected in a microcapillary pipette and transferred into a sample vial containing 1 ml phosphate buffered saline (PBS) (for composition see reference)[3]. Motility of epididymal spermatozoa was determined in a haemocytometer chamber after 5 minutes incubation at room temperature as described by Pholpramool[4]. The number of spermataozoa was counted in a Neubauer haemocytometer as described by Biggers et al[5].

Biochemical and histological Investigations: Epididymis from one side was processed for estimation of protein[6], acid and alkaline phosphate[7], total **LDH**[8] and its isozymes, which were fractionated by PAGE[9] and quantified by densitometry. Students[8]'t' test was applied for statistical comparison of the difference in data between the two groups.

RESULTS

Sperm count and Motility: During low and high dose treatment with ASDB extracts, the quantitative and qualitative motility of sperms were impaired markedly with concurrent decrease in sperm count (Table 1).

Weight response: While no appreciable change in weight was observed in caput, a significant decrease was noticed in cauda at both the dosage of the plant extracts (Table 1).

Tissue biochemical study: Protein concentrations were increased both in caput and cauda at low doses. While at high dose, the same was increased only in caput (Table 2). The activities of acid and alkaline phosphate enzymes were markedly reduced in both the segments. While the total LDH activity was unaltered in caudal segment at high doses it was significantly decrease in the caput region at both dosages.

DISCUSSION

The epididymis plays an important role by providing suitable environment for sperm maturation and maintains a suitable milieu for morphological and biochemical changes in the spermatozoa[10]. It performs both secretory and absorptive functions[11].

Spermatozoa undergo a progressive morphological and physiological maturation during their passage through the epididymis and acquire motility and fertilizing capacity[12]. ASDB extracts administration rendered the sperm non-motile by altering the epididymal milieu and making it hostile to the motility of spermatozoa. Studies in hypophysectomized or castracted animals, receiving either no treatment or treated with androgen alone or in combination with antiandrogen[13] have shown that androgens are essential for maturation, motility and survival of spermatozoa in the epididymis. The decrease in sperm concentrations as well as the presence of immature spermatocytes in the epididymal plasma along with decrease in organ weights further indicate an anti-spermatogenic and antiandrogenic effect of the herbal extracts.

Sperm maturation in the epididymis takes place with the proteins synthesized and secreted by the tissue[14]. These proteins are secreted mainly from the peripheral cells of the epithelial layer of the epididymis. In addition, the movement of large molecular proteins from blood into the epididymis is not restricted by barrier as present in tests[15]. ASDB extracts appear to have to adverse effect on protein levels in caput and cauda tissue. On the otherhand, it had induced an increase of the same, similar to the effects of other anti-androgenic compounds[10].

ACP and ALP serve as reliable markers for androgen action in the accessory sex organs of males[16], and are directly correlated with sperm count[17]. Hence, the observed reduction in ACP activity as well as the sperm counts suggest the probable decrease in androgen supply of the organ. Estradiol has been reported to cause a decrease in these enzymatic activities in other accessory sex organs[18]. Similarly, Malini et al[19] have shown a decrease in ACP and ALP activities in accessory sex organs in rats after administration of an estrogenic principles from Foeniculum vulgare seeds. Administration of Sapindus trifoliatus extracts has been shown to induce a marked decrease in ACP activity in epididymis of rats[20]. All these reports suggest degeneration of the tissues associated with the decreased phosphomonoesterases enzymatic activities.

In the present study ASDB extracts caused a significant decrease in the activity of NAD-LDH favouring the production of the substrate lactate, which clearly indicate a switchover from aerobic to anaerobic type of respiration. The consequent inhibition of pyruvate formation thus suggests abolition of citric acid cycle activity and thereby depletion of energy production. Since both glycolytic and pentose phosphate pathway enzymens are androgen dependent[22], a decrease in the glycolytic enzyme LDH in the present study may further confirm a probable deficiency of androgen supply to this organ.

Thus, the herbal extracts affected the epididymal biochemistry adversely.

Table 1

Effect of ASDB extracts on organ weights, sperm concentration and motility in the epididymis of rats

Parameter	Group I		Group II		Group III	
	Caput	Cauda	Caput	Cauda	Caput	Cauda
Organ Weight	148.00 .	299.00 .	155.00 .	220.00 .**	152.00 .	152.00 .**
(mg/100 g body Wt)	3.56 4.25	9.45	9.45	4.71	3.84	
Epididymal fluid:						
Sperm concentration	512.00 .	560.00 .	305.00 .***	320.00 .***	290.00 .***	300.00 .***
(Counts * 10/ml)	46.98	22.85	31.45	30.40	24.34	28.07
Sperm motility (FMI)	----	109.00 .	---	76.00 .***	---	50.00 .***
@		9.11		6.26		4.00
		(n = 5)		(n = 5)		(n = 5)

Each value is mean . S.E.M. of 5 animals

*P<0.05; **P<0.01; and ***P<0.001. Control V/s experimental

Group – I – Vehicle treated; Group II and Group III; received extracts of A.spinosus root and D.biflours seeds, for 10 days at the following ration : Group II 25 mg; Group III 50 mg, respectively.

@ Motility Vs the motility recorded after 5 minute, suspension of the caudal epididymal spermatozoa in phosphate-buffered saline (FMI = Forward motility index)

Table 2

Effect of combines extracts of ASDB on epidimal biochemistry in rats

Parameter	Group I		Group II		Group III	
	Caput	Cauda	Caput	Cauda	Caput	Cauda
Protein mg/100 mg tissue	6.91 . 0.45	5.76 . 0.48	14.76 .*** 1.06	8.83 .** 0.59	10.59** . 1.08	5.89 . 0.35
Acid phosphatase@	0.527 . 0.052	0.790 . 0.100	0.166 *** . 0.021	0.251***. 0.031	0.267***. 0.031	0.130 . 0.040
Alkaline phosphatase@	0.439 . 0.041	0.940 . 0.101	0.553 . 0.051	0.520 . 0.040	0.207**. 0.031	0.561** . 0.052
Total Lactate dehydrogenase m.I.U./min/mg protein	21.04 . 1.21	29.84 . 2.86	8.46 *** . 0.51	23.20* . 1.35	8.38 ***. 0.51	27.10 . 2.08

Each value is mean . S.E.M. of 5 animals

*P<0.05; **P<0.01; and ***P<0.001. Control V/s experimental

@ The values are expressed in . moles of p.nitrophenol formed/hr/mg/ protein.

Group – I – Vehicle treated; Group II and Group III; received extracts of A.spinosus root and D.biflours seeds, intrperitoneally for 10 days at the following ration : Group II 25 mg; Group III 50 mg, respectively.

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