EFFECTS OF COMBINED EXTRACTS OF DOLICHOS BIFLORUS SEEDS AND AMARANTHUS SPINOSUS ROOTS ON THE ACCESSORY SEX ORGANS OF MALE RATS

K. MURUGAN, G. VANITHAKUMARI and R. SAMPATHRAJ

Department of Zoology, Bharathiar University, Coimbatore – 641 046, India.

Received: 28 August, 1992  Accepted: 7 May, 1993

ABSTRACT: Biological effects of extracts of Dolichos biflorus seeds and Amaranthus spinosus roots administered at two different doses (25 mg of D.biflorus seeds and 25 mg of A.spinosus roots; 50 mg of D.biflorus seeds and 50 mg of A.spinosus roots) for 10 consecutive days were studied in the accessory sex organs of adult male rats. The observed adverse effect on organs weight, protein content and concentration as well as the enzymatic activities in the vas defers, seminal vesicle and ventral prostate indicates the probable antiandrogenic action of the drug in the male rats.

INTRODUCTION

Roots of Amaranthus spinosus (Family : Amaranthaceae) separately and in combination with other drugs are used in menorrhagia. Dolichos biflorus seed (Family : Papillionaceae) is implicated as an emmenogogue and abortifacient. Combination of these two extracts stimulate the flow of milk in cattle’s suggesting the galactogoucie property of herbs. Further the compounds like Spinosterol, β – Sisterol in the A. spinosus have been shown to possess estrogenic property. However, no report is available till date regarding the action of these extracts in combination of male animals. Hence, the present attempt was made to study the effects of these combined extracts at the different doses on the male accessory sex organs. Biochemical parameters that are under the influence of sex steroids were studied to know the probable antiandrogenic / estrogenic effect of the drug and also to determine the effective dose that would elicit the maximum response.

MATERIALS AND METHODS

Seeds of D.biflorus and roots of A.spinosus procured locally were shade dried and powdered. The powdered materials were extracted with acetone in a soxhlet apparatus. Acetone was allowed to evaporate. Then known quantity of the two extracts were dissolved in known volume of propylene glycol for intraperitoneal administration.

Adult male albino rats of Wistar strain (150 – 180 days old) weighing 150 – 200 gm body weight were maintained in a well ventilated animal house constant 10 hrs of darkness and 14 hrs of light schedule. The rats were given standard pellet diet (Hindustan Lever Ltd; India) with free access to water. The animals were divided into 3 groups with 5 animals in each groups.

Group – I: received propylene glycol intraperitoneally for 10 consecutive days.
Group – II & III : received the combined extract of *D. biflorus* seeds (25 mg) + *A. spinosus* roots (25 mg); and *D. Biflorus* seeds (50 mg) + *A. spinosus* roots (50 mg) respectively, intraperitoneal for 10 consecutive days.

The body weight of each animal was recorded before and after treatment schedule. 24 hrs after the last injection, the animals were sacrificed by depatitation. Vas deferens, seminal vesicle and ventral prostrate were dissected out cleared from the surroundings tissues and blood vessels, blotted on a filter paper and weighted accurately on a torision balance. The organ weights were expressed in terms of mg/100 gm body weight. The tissues were processed for biochemical studies. Quantitative examination of protein, acid, alkaline phosphates and prostatic acid phosphatase were carried out in a vas deferens, seminal vesicle and ventral prostate. The results were analyzed statistically using Students ‘t’ test.

**RESULT**

No significant change in the body weight was observed. However, the accessory sex organ weights were reduced in both the experimental groups. Table-1 denotes the unaltered protein content and concentration in vas deferens irrespective of the decrease in the organ weight. The activities of acid and alkaline phosphates enzymes were significantly decreased (P < 0.001) in the vas deferens of both the treated groups.

Table-2 depicts the decrease in the seminal vesicle weight and protein content in low dose group. The protein concentration was increased significantly (P < 0.05). The acid phosphates enzyme activity was markedly decreased (P < 0.001) in the drug treated groups. However, the activity of alkaline phosphates enzyme was not much changed in the treated groups.

Results presented in Table-3 indicate the reduced prostatic weight accompanied by decreased protein content as well as concentration under the drug treatment. The alkaline phosphates activity was reduced markedly (P < 0.001) in the low dose treated group. Whereas no significant change was observed in the acid phosphates enzyme activities. The prostatic specific acid phosphates appears to be increased in low dose treated groups.

**DISCUSSION**

In the present study, the combined extracts of *D. biflorus* seeds and *A. spinosus* roots seems to reduce the Vas deferens weight similar to the castration effect. The unaltered protein content and concentration in the Vas deferens irrespective of the decrease in organ weight indicates mainly the reduction in secretory products in the human rather than the decrease in structural protein. The growth and metabolic activities of Vas deferens are known to be under androgenic control.

The reduction in the activity of alkaline phosphatase may indicate the possible inhibition of hydrolysis and transport mechanisms in the tissue as seen after the removal of androgen source. The decrease in acid phosphatase activity under the influence of the combined extracts stimulates the changes observed after castration and antiandrogen treatment.

Testosterone is known to stimulate the growth and maintains the seminal vesicle and prostate. The majority of proteins in the seminal vesicular fluid are synthesized by the gland that secretes them and are
under the androgenic steroid control\textsuperscript{13}. Therefore, the present result indicate the probable adverse effect of the extracts on the functional integrity of the tissue.

Alkaline phosphates of rat and mouse seminal vesicle belong to the category of stromal rather than epithelial secretory enzymes. In seminal vesicle it has been noted that the muscular coat is mainly responsive to estrogenic stimulation and epithelial tissue to androgen hormone. This indicates that the different cellular layers respond differently to castration or any treatment. In the present study the combined extracts appear to maintain the alkaline phosphates activity. Acid phosphates serves as a reliable marker enzyme for androgen action in the seminal vesicle and prostate of rats. In the present study, demonstration of decreased activity of acid phosphates under both high and low dose administration of the combined extracts suggests the probable adverse effect of the drug similar to that induced by estrogens. Dixit and Gupta\textsuperscript{14} have also reported a reduction in the acid phosphates activity in the seminal vesicle of gerbils administrated with fruits of Sapindus trifolialus.

The present observation (Table-3) of reduced prostatic weight accompanied by decreased protein content as well as concentration under the drug treatment may indicate the lowered androgen supply to this organ. The prostatic involution may be the result of cessation of the anabolic activities in the prostatic tissue in the absence of testicular hormones\textsuperscript{15}. As a consequence, bio-chemical constituents such as nucleic acids and protein contents might have decreased rapidly in the regressing prostate.

In prostate, alkaline phosphatase has widespread distribution in the granular epithelium and in the interstitial connective tissue. Part of the enzyme is secretory in nature and could be detected birth in the lumen and in secretory granules\textsuperscript{17}. Since, alkaline phosphatase activity is known to be linked with the cleavage of phosphate esters of monosaccharide, it might be likely that the prostate gland is involved in the active accumulation of monosaccharide and incidentally the liberated phosphorous might be taken for enzyme metabolism.

Combined extracts of \textit{D.biflorus} seeds and \textit{A.spinosus} roots caused a reduction in alkaline phosphatase activity in ventral prostate similar to the adverse effects exerted by castration and antiandrogens suggesting the probable inhibition of secretory processes and also slowing down of the cleavage of monosaccharide for the energy metabolism. The decreased prostate weight, protein contents and concentration as well as alkaline phosphatase activity all indicate the deleterious effect of the drug in the prostate of rats.

The presence of separate lysosomal and secretory acid phosphatase in the prostate has been demonstrated\textsuperscript{18}. Acid phosphatase in secretary granule is hormone dependent\textsuperscript{19} whereas lysosomal acid phosphatase is hormonally independent and increased its activity after castration or estrogen treatment\textsuperscript{20}. Phosphorycholine secreted by the seminal vesicles is rapidly dephosphorylated to free choline and orthophosphate by the acid phosphatase activity in prostate indicating its active participation in the formation of seminal plasma since, both fructose and inorganic phosphorus are essential components of this fluid\textsuperscript{21}.

In the present study, the total acid phosphatase appears not to be affected by the treatment however, the prostatic specific acid phosphatase appears to be increased in
low dose level suggesting its source probably to be lysosomal, which is not under any hormonal control. It is known that in the rat the acid phosphatase exhibits very low activity\(^2^2\) and an increase in this enzyme seems to be no conducive for the proper functioning of the sperms or it may cause an adverse effect.

From the foregoing results it is evident that the combination of \textit{A.spinosa}s roots and \textit{D.biflora}s seed extracts have definite adverse effects on the reproductive physiology of the accessory organs like Vas deferens, Seminal vesicle and ventral prostate. The pronounced differences observed in the rate of regression in weights of these accessory sex organs and the reduction in the enzymatic activities might be dependent on the rate, sensitivity or threshold or degree of the responses to the endogenous androgens by these accessory sex organs following the administration of the plant drug.

**TABLE –I**

\textbf{Effect of combined extracts of \textit{Dolichos biflorus} seeds and \textit{Amaranthus spinosus} roots on Vas deferens of adult rats.\(^{19}\)}

| Parameters                        | Group-I       | %   | Group-II       | %     | Group-III      | %     |
|-----------------------------------|---------------|-----|----------------|-------|----------------|-------|
| Organ weight (mg/100 g of body weight) | 68.8 ± 2.07   | 100 | 59.8 ± 1.53**  | 86.91 | 60.0 ± 2.99    | 87.20 |
| Protein Concentration (mg/100mg tissue) | 6.29 ± 0.22   | 100 | 5.36 ± 0.38    | 85.21 | 6.27 ± 0.39    | 99.68 |
| Protein content (mg/organ whole wt.) | 4.01 ± 0.33   | 100 | 3.47 ± 0.34    | 86.53 | 3.75 ± 0.30    | 93.51 |
| Alkaline Phosphatase (b)          | 3.49 ± 0.18   | 100 | 2.23 ± 0.14**  | 63.90 | 2.83 ± 0.13*   | 81.00 |
| Acid Phosphatase (b)              | 1.93 ± 0.09   | 100 | 1.42 ± 0.06*** | 74.00 | 1.34 ± 0.07*** | 70.00 |

(a) Each value is mean ± SEM of 5 experiments  
(b) Enzyme activity is expressed as µ moles of p-nitriphenol formed/hr/mg protein.

GROUP I, II and III as described in the text.  
\(*P < 0.05; \ **P < 0.01; \ ***P < 0.001\) Group – I vs others.
TABLE –II
Effect of combined extracts of *Dolichos biflorus* seeds and *Amaranthus spinosus* roots on seminal vesicle of adult rats.a

| Parameters                          | Group-I     | %  | Group-II         | %  | Group-III      | %  |
|------------------------------------|-------------|----|------------------|----|----------------|----|
| Organ weight (mg/100 g of body weight) | 226 ± 9.26  | 100| 147 ± 18.71**   | 65.04| 137 ± 9.55*** | 60.61 |
| Protein Concentration (mg/100mg tissue) | 8.70 ± 0.22 | 100| 8.26 ± 0.27     | 94.94| 10.60 ± 0.49* | 99.68 |
| Protein content (mg/organ whole wt.) | 19.63 ± 0.95 | 100| 11.84 ± 1.63**  | 60.31| 15.17 ± 1.19** | 82.08 |
| Alkaline Phosphatase (b)            | 1.19 ± 0.08 | 100| 0.94 ± 0.07**   | 80.00| 1.05 ± 0.08*  | 88.23 |
| Phosphatase (b)                     | 0.759 ± 0.04 | 100| 0.256 ± 0.03*** | 33.72| 0.148 ± 0.02*** | 19.49 |

(a) Each value is mean ± SEM of 5 experiments
(b) Enzyme activity is expressed as µ moles of p-nitrophenol formed/hr/mg protein.

GROUP I, II and III as described in the text.
*P < 0.05; **P < 0.01; *** P< 0.001 Group – I vs others.

TABLE –III
Effect of combined extracts of *Dolichos biflorus* seeds and *Amaranthus spinosus* roots on ventral prostate of adult rats.a

| Parameters                          | Group-I     | %  | Group-II         | %  | Group-III      | %  |
|------------------------------------|-------------|----|------------------|----|----------------|----|
| Organ weight (mg/100 g of body weight) | 228 ± 8.40  | 100| 125 ± 2.72***   | 54.82| 122 ± 6.42*** | 53.50 |
| Protein Concentration (mg/100mg tissue) | 7.65 ± 0.41 | 100| 3.90 ± 0.22***  | 50.98| 3.71 ± 0.23*** | 48.49 |
| Protein content (mg/organ whole wt.) | 19.09 ± 1.99 | 100| 8.40 ± 0.24***  | 49.24| 9.24 ± 0.56*** | 48.61 |
| Alkaline Phosphatase (b)            | 8.50 ± 0.38 | 100| 3.27 ± 0.17**   | 38.47| 3.36 ± 0.13*** | 39.53 |
| Acid Phosphatase (b)                | 0.272 ± 0.05 | 100| 0.252 ± 0.02    | 33.72| 0.169 ± 0.02*** | 62.13 |
| Prostatic acid                      | 0.261 ± 0.02 | 100| 0.415 ± 0.03*** | 159 | 0.273 ± 0.03   | 104.5 |

(a) Each value is mean ± SEM of 5 experiments
(b) Enzyme activity is expressed as µ moles of p-nitrophenol formed/hr/mg protein.

GROUP I, II and III as described in the text.
*P < 0.05; **P < 0.01; *** P< 0.001 Group – I vs others.
REFERENCES

1. Dymock, W., Warden, C.J.H. and Hooper, D., Pharmacographica India, (Eds) Bisswn Singh and Mahendra Pal Singh, Vo.II, P.124 (1976).

2. Nadkarni, R.M., In : Indian Material Medica (ed) Nadkarni, A.K. Vol. I, Third edition, p.294 (1976).

3. Ingham, J.L. Keen, N.T. and Markham, K.R. Phytochemistry, 20 : 807 (1981).

4. Fernando Judur, Bean George., J. Am. Oil. Chem. Soc., 62 : 89 (1985).

5. Bauminger, S. Londner, H.R. Parel, E and Arnon, R.J. Endocrinol., 44 : 567 (1969).

6. Lowry, C.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J.J. Biol. Chem., 193 : 265 (1951).

7. Anderseh, M.A. and Szczpinske, A.J., Am. J. Clin. Pathol., 17:571 (1947).

8. Arora, R., Dinakar, N., and Prasad, M.R.N. Indian J. Exp. Biol., 15: 829 (1977).

9. Dass, R.P. Indian J. Exp. Biol., 15:16 (1977).

10. Guraya, S.S., and Kaur, A.J., Proc. Indian Acad. Sci. Anim. Sci., 90 : 496 (1981).

11. Kumar, P., Rashawa, D.S., and Dani, H.M. Horm. Metab. Res., 10:300 (1978).

12. Nazhian, S.J. and Mahesh, V.B. Arch. Androl., 4:283 (1980).

13. Ostrowaski, M.C., Kistler, M.C., and Kistler, W.S. J. Biol. Chem., 254 : 38 (1979).

14. Dixit, V.P., and Gupta, R.S. Med. Plant. Res. Planta. Medica., 16 : 242 (1982).

15. Lee, C. In: The prostatic cell : Structure and function. (ed) Lee, C. Part – A, pp. 145 – 159 (1981).

16. Umapathy, E., and Rai, U.C. Indian J. Exp. Biol., 18:1090 (1980).

17. Bern, H.A. Am.J. Anat., 84:231 (1949).

18. Vanha – Pertula,T., Niemi, R., and Helminen, H.J. Invest. Urol., 9 : 245 (1972).
19. Rosenkrantz, H. Ann. N.Y. Acad. Sci., 166:466 (1969).

20. Tenniswood, M., Bird, C.E., and Clark, A.F. Can. J. Biochem., 54:350 (1976).

21. Mann, T., and Lutwak – Mann, C. Physiol. Reviews, 31 : 27 (1951).

22. Huggins, C., and Webster, W.D.J. Urol. 59: 258 (1948).