Punjab Univ. J. Zool., Vol. 12, pp. 85-90, 1997

METABOLITES OF *DINOTHROMBIUM TINCTORIUM* (BIR BAHUTY), AS AN APHRODISIAC SUBSTANCE

FAZLI SUBHAN, SIKANDAR SULTAN, WAHEED ALAM, FAHEEM TAHIR AND ATHAR SAEED DIL

National Research Institute of Reproductive Physiology (FS, SS, WA, FT) and Public Health Division (ASD), National Institute of Health, Islamabad , Pakistan

Abstract: Metabolites of *Dinothrombium tinctorium* (Bir bahuty) were studied for their aphrodisiac potential, which deliberates upon the fertility enhancement potential of the rice mixed with metabolites of the mites, as administered in the Unani system of medicine. The results indicated significant increase in sperm count, weight of gonads and accessory glands, as well as the diameter of seminiferous tubules, spermatogonium, primary and secondary spermatocytes, spermatid and spermatozoa. The testosterone level in serum and the testicular tissue also increased significantly. The chemical pathology of the treated group was found to be within normal range.

Key words: *Dinothrombium tinctorium*, metabolites, aphrodisiac.

INTRODUCTION

Over 70-80 percent of population in the third world countries live in the rural areas and are often familiar with, and have faith in, the folk-lore and traditional systems of medicine prevailing in their areas. Fairly large number of medicinal plants (herbs), minerals and animal products are reported to have been used for fertility regulation in human beings.

In connection with our efforts to develop indigenous resources and establish their efficacy (through laboratory trials) for fertility regulation of male, we have evaluated a number of prescriptions and indigenous materials, aiming towards isolation of some natural material with potentials to regulate male fertility. *Dinothrombhium tinctorium* (Bir bahuty) is known in Unani System of Medicine for having aphrodisiac effect in males (Kabir, 1937; Board of Ayurvedic System of Medicine, 1971) and is administered both as total animal powder as well as ground rice mixed with the metabolic products of the mites.

Earlier studies in our laboratory have examined the aphrodisiac nature of the total animal powder of these mites (Subhan *et al.*, 1988, 1989a, 1989b, 1990a, 1990b, Subhan and Khan, 1991; Subhan, 1995). The hormonal profile analysis of the total animal powder of the mites has revealed significant levels of steroidal hormones (Subhan

0079-8045/97/0001-0083 $ 03.00/0

et al., 1995), and the total animal powder was also found to be rich in lipids, the main precursor of these androgenic hormones (Subhan and Tahir, 1996).

The present study is aimed at exploring the aphrodisiac potential of the metabolities of mites, as Shideler *et al.* (1993) have demonstrated that animal metabolites contain a reasonable amount of steroids.

MATERIALS AND METHODS

Animal trial

Adult male rats (Sprague dawley strain), were used in the study. The animals were divided into two groups as control and treated, each group containing 20 animals. Live Bir bahuty were placed on rice to collect their metabolites on rice. After thorough mixing, a dose of 100 mg finely ground rice and metabolite mixture was given orally to the treated animals for 8 weeks, in order to evaluate the effect on spermatogenesis. Food and water was available *ad libitum*. The parameters which were observed included semen analysis, weight of accessory glands, histological studies and testosterone level in serum and interstitial testosterone concentration.

Sperm count

Semen collected through electric ejaculation (Moore and Gallagham, 1930) was analysed for sperm concentration with the help of Mackler chamber. The sperms were counted from the whole cauda, which was put in a small test tube in 1 ml ptyroid's solution and cut with scissors into small pieces and then another 1 ml of ptyroid's solution was mixed, shaken and was placed for some time. The cauda pieces were removed with the forceps and the suspension was centrifuged for 30 minutes at 600 rpm. The pellet was washed with ptyroid's solution and then 2 ml solution was mixed and counted with a Neubauer counting chamber. The number of sperms were obtained by multiplying the sperm count in 10 squares of the grid or total number of sperms were counted (Parkashi *et al.*, 1985).

Histological study

One testis of each animal was subjected to histological process for observation of different stage of spermatogenesis, seminiferous tubules, interstitial cells and sertoli cells.

Blood chemistry

Serum of the experimental animals was subjected to estimation of testosterone with RIA and biochemical test by the established methods for clinical chemistry.

RESULTS AND DISCUSSION

The treatment of rice containing metabolites of mites resulted in a significant increase in weight of the gonads and accessory gonads. As testosterone levels both in serum and testes (Table I), diameter of the histological sections of the testis (Table II) and higher sperm count/cauda epididymis (Table III). Blood chemistry analysis of the treated and control groups, except for serum and testes testosterone levels, show no significant differences (Table IV).

Table I: Effect of metabolites of mites on body weight, gonads weight and accessory gonads.

	Treated	Control
Initial weight of animals (g)	149.00±03.45[a]	153.00±02.550
Final weight of animals (g)	267.20±11.11	281.80±03.810
Weight of testes (g)	2.90±00.08	2.82±00.110
Weight of seminal vesicles (g)	1.95±00.09	1.13±00.090
Weight of prostrate (g)	0.91±00.05	0.48±00.050
Weight of epididymis (g)	0.82±00.04	0.38±00.010

[a] Mean ± SEM

Table II: Effect of metabolites of mites on morphometric observation of testicular section, with reference to diameter.

Parameters	Diamter (μm)	
	Treated	Control
Seminiferous tubules	196.24±2.71[a]	186.04±2.86
Leydig Cells	5.06±0.29	4.34±0.19
Sertoli Cells	7.29±0.22	6.35±0.32
Spermatogonia	5.07±0.10	4.32±0.14
Primary Spermatocytes	7.92±0.09	7.10±0.19
Secondary Spermatocytes	4.87±0.11	4.31±0.23
Spermatids	4.70±0.16	4.10±0.14
Spermatozoa	0.51±0.02	0.42±0.03

[a] Mean ± SEM

Table III: Effect of metabolites of mites on sperm motility and sperm count.

	Treated	Control
Sperm count (Mx10^6/cauda epididymus)	748.0±24.14	604.0±26.65
Motility		
Motile (%)	85.8±2.29	84.2±1.68
Non-Motile (%)	14.2±2.29	15.8±1.68
Motile/non-motile sperms	6.84	5.31

Table IV: Effect of metabolites of mites on the blood chemistry and gonads testosterone concentration.

	Treated	Control
Protein (mg/dl)	8.23±0.11[a]	8.17±0.27
Cholesterol (mg/dl)	73.08±4.57	76.77±3.22
Lipids (mg/dl)	130.40±6.89	134.97±5.79
AP (U/l)	96.70±8.96	94.40±9.12
SGOT (U/l)	25.90±1.04	26.12±0.90
SGPT (U/l)	23.70±1.08	22.43±1.14
Bilirubin (mg/dl)	0.38±0.01	0.36±0.01
Creatinine phosphate (mg/dl)	0.73±0.01	0.71±0.03
Glucose (mg/dl)	82.42±2.76	81.25±1.93
Urea (mg/dl)	28.30±1.65	26.35±1.48
Serum testosterone (ng/ml)	0.93±0.20	0.61±0.16
Testes testosterone (ng/mg)	0.20±0.04	0.11±0.004

[a] Mean ± SEM

Abbreviations: AP, alkaline phosphatase; SGOT, serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase.

The observations are in agreement with the reports of Kabir (1937) and the Board of Unani and Ayurvedic System of Medicine (1971) in having aphrodisiac effect of the rice having metabolites of mites.

The histological observation of the testicular section is suggestive of the fact that the metabolites of mites do posses some potent agent which has enhanced the process of spermatogenesis. The effect at the moment cannot be characterized due to the androgen or pituitary gonadotrophins, or due to nutritional factors. The normal level of blood chemistry, however, revealed a normal picture of the treated group.

The mode of action of the metabolites of mites, at hypothalamus, pituitary/or gonadal level, cannot be characterised on the basis of preliminary investigation. Further

experimentation to determine the serum luteinizing hormone releasing hormone (LHRH), luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels are under way. The increase in testosterone level in the treated group indicates the increased stimulation of leyding cells, probably due to LH.

Acknowledgements

The Authors wish to thank Mr. Jamil Akhtar (Laboratory Technician) and Mr. Nazar Hussain (Laboratory Attendant) of this Institute, for their hard work during the experimentation.

REFERENCES

BOARD OF UNANI AND AYURVEDIC SYSTEM OF MEDICINE, PAKISTAN, 1971. In *Tibbiu Pharmacopeia,* Nisar Art Press, Lahore, Pakistan.

KABIR, U., 1973. In *Kitabul Advia*, commonly known as Mukhzan-e-Almufardat, Shaukat Book Depot, Shaukat Bazar, Gujrat, Pakistan.

MOORE, C.R. AND GALLAGHAM, I.F., 1930. Seminal vesicle and prostrate function as a testis hormone indicator the electric ejaculation test. *Am. J. Anat.* **45**: 39-69.

PAKRASHI, A., SANYAL, S., BANERJEE, R. AND RUPAREL SEN, N., 1985. Effect of *Malvascus conzattii* flower extract on male fertility. *J. Contra.*, **31**: 101-108.

SHIDELER, S.E., ORTUNO, A.M., MORAN, F.M., MOORMAN, E.A. AND LASLEY, B.L., 1993. Simple extraction and enzyme immunoassays for estrogen and progesterone metabolites in the feces of *Macaca fascicularis* during non-conceptive and conceptive ovarian cycles. *Biol. Reprod.* **48**: 1290-1298.

SUBHAN, F. AND KHAN, Z.U., 1991. Effect of powdered *Dinothrombium tinctorium* on the testicular function of the male rat. *Proc. 14th Res. Sem., Pop. Welf., Nat. Res. Inst. Fertility Control*, Karachi, Pakistan. pp. 295-303.

SUBHAN, F. AND TAHIR, F., 1996. Metabolic parameters of *Dinothrombium tinctorium*, in relation to fertility regulation. *Hamdard Medicus.*, **39**(2): 77-78.

SUBHAN, F., 1995. Effect of *Dinothrombium tinctorium* on the reproductive function of immature male rat. *Hamdard Medicus.*, **38**(4): 79-85.

SUBHAN, F., ASHFAQ, G. AND KHAN, Z.U., 1989a. Effect of *Dinothrombium tinctorium* (Bir bahuty), on the reproductive functions male rat. *Proc. 2md Biennial Conf. Physiol.*, 22-23, February, 1989, at Army Medical College, Rawalpindi, Pakistan.

SUBHAN, F., ASHFAQ, G. AND KHAN, Z.U., 1989b. Effect of *Dinothrombium tinctorium* on male reproductive system. *Sym. on Contraception Res.*, 11-15 August 1989, Beijing, China.

SUBHAN, F., ASHFAQ, G. AND KHAN, Z.U., 1990a. *Dinothrombium tinctorium* (Bir bahuty), as a fertility regulation agent in male. First Pakistan Conference on Fertility and Sterility, 27-30 November 1990, Lahore, Pakistan.

SUBHAN, F., ASHFAQ, G. AND KHAN, Z.U., 1990b. Effect of *Dinothrombium tinctorium* (red velvet mites) on gonads and gonadal hormones (testosterone) in male rat (four

cycle treatment). *Proc. Pakistan Cong. Zool.,* **10**: 26-27 May 1990, Khanaspur, Murree, Pakistan.

SUBHAN, F., EMERY, M. AND SHAH, M.J., 1995. Hormonal profile of the extract of red velvet mite *Dinothrombium tinctorium. Hamdard Medicus.,* **38**(2): 87-95.

SUBHAN, F., KHAN, M.N., FATIMA, N., ASHFAQ, G. AND KHAN, Z.U., 1988. Effect of *Dinothrombium tinctorium* (Bir bahuty) on gonads and gonadal hormones (testosterone) in male rats (one cycle treatment). *Proc. 13th Res. Sem., Pop. Welf., Nat. Res. Inst. Fertility Control,* Karachi, Pakistan. pp. 103-112.

(Received: January 12, 1997)