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1. Carter, S. K., *J. Natl. Cancer Inst.*, 1975, **55**, 1265.
2. Singal, P. K., Segstro, R. J., Singh, R. P., Kutryk, M. J., *Can. J. Cardiol.*, 1985, **1**, 139.
3. Thayer, William, S., *Biochem. Pharmacol.*, 1984, **33**, 2259.
4. Plaa, G. L. and Witschin, H., *Annu. Rev. Pharmacol. Toxicol.*, 1976, **16**, 125.
5. Halliwell, B. and Gutteridge, J. M. C., *TIBS*, 11, Sep. 1986, 372.
6. Halliwell, B., Gutteridge, J. M. C. and Blake, D., *Philos. Trans. R. Soc. London*, 1985, **311**, 659.
7. Adi, P., William, C. and John, M., *Toxicol. Lett.*, 1981, **7**, 379.
8. Beutler, E., Duron, O. and Kelly, B. M., *J. Lab. Clin. Med.*, 1967, **70**, 158.
9. Collier, M., *J. Med. Lab. Technol.*, 1970, **27**, 86.
10. Hendry, J. B., In: *Clinical Chemistry: Principles and Techniques*, Hoeber, Academic Press, New York, 1965, p. 503.
11. Oser, B. L., In: *Hawk's Physiological Chemistry*, (ed.) B. L. Oser, Tata–McGraw Hill, Bombay, 1965, 10407.
12. Bothwell, T. H., Carlton, F. W., Work, J. D. and Find, C. A., In: *Iron Metabolism in Man*, 1979.
13. Blackwell, L., *Br. J. Haematol.*, 1978, **38**, 281.
14. Yagi, K., *Methods Enzymol.*, 1984, **105**, 328.
15. Desai, D. I., *Methods Enzymol.*, 1984, **105**, 138.
16. Kyaw, A., *Clin. Chim. Acta*, 1978, **86**, 153.
17. Gutteridge, J. M. C. and Stocks, J., *CRC Crit. Rev. Clin. Lab. Sci.*, 1981, **14**, 257.
18. Davies, K. J. A., Sevanian, A., Muakkessah Kelly, S. F. and Hochstein, P., *Biochem. J.*, 1986, **235**, 747.
19. Geetha, A., Sankar, R. and Shyamala Devi, C. S., *Curr. Sci.*, 1988, **57**, 486.
20. Scarpan, Rigo, A., Maiorine, M., Ursini, F. and Gregolin, C., *Biochem. Biophys. Acta*, 1984, **801**, 215.
21. Thor, H., Smith, M. T., Hartzell, P., Bellomo, G., Jewell, S. A. and Orrhenius, S., *J. Biol. Chem.*, 1982, **257**, 12419.


## ANTIFERTILITY EFFECT OF *VINCA ROSEA* (LINN.) LEAF EXTRACT ON MALE ALBINO MICE—A SPERM PARAMETRIC STUDY

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*VINCA ROSEA* (Linn.) (*Catharanthus roseus*), of the family Apocynaeceae, with proven efficacy as anticarcinogenic[1], has also been shown to be antispermatogenic and antiandrogenic in rats[2,3]. A preliminary study[3] has shown that aqueous extract of leaves of this plant affects motility of rat sperm. The present paper reports the effect of aqueous extract of leaves of this plant on various sperm parameters of Swiss albino mice.

Male Swiss albino mice (8 weeks old, 30–38 g body weight), fed on standard mouse pellet diet (Hindustan Lever Ltd, Bombay) and water, were used. Fresh leaves of *V. rosea* were collected locally. Aqueous leaf extract was obtained according to Chinoy and Geetha Ranga[3]. Mice ($n=10$) in the experimental group received 10 mg equivalent of the dry leaf in 0.2 ml of water daily intraperitoneally for 15 days. Control mice ($n=10$) received physiological saline. The animals were sacrificed 24 h after the last injection by cervical dislocation and the cauda epididymides were removed. Modified Kreb's Ringer bicarbonate solution containing crystalline bovine serum albumin[4] was used as the medium for *in vitro* study of sperm. Cauda epididymis was placed in 200 $\mu$l of the buffer taken in an embryo cup. The cauda was punctured and minced using fine needles to release and disperse the sperm. Tissue debris was discarded, leaving a clear preparation for microscopic observation. Hanging drop preparations were made to assess sperm motility, relative percentage of live and dead sperm and sperm count; smears were prepared to assess relative proportions of normal and abnormal sperm[5].

*V. rosea* leaf extract did not affect body weight of the mice. Sperm count of the treated mice decreased significantly to 67% of that of controls. The relative percentage of motile sperm decreased on treatment.

**For correspondence

**Table 1** *Effect of leaf extract of V. rosea on sperm parameters of male albino mice*

| Parameter | Control | Experimental |
|---|---|---|
| Sperm count ($\times 10^6$/ml) | 77.25 ± 9.09 (6) | 52.13 ± 3.96** (6) |
| Percentage of motile sperm | 71.67 ± 5.32 (6) | 57.17 ± 8.80** (6) |
| Duration of motility (min) | 92.83 ± 11.46 (6) | 61.67 ± 5.96** (6) |
| Normal sperm (%) | 85.33 ± 5.92 (6) | 69.50 ± 5.96* (6) |
| Abnormal sperm (%) | 14.67 ± 5.92 (6) | 30.50 ± 5.96** (6) |
| Live sperm (%) | 75.67 ± 5.57 (6) | 61.00 ± 9.53* (6) |
| Dead sperm (%) | 24.33 ± 5.57 (6) | 39.00 ± 9.53* (6) |

Values are mean ± SD.
*$P < 0.05$, **$P < 0.01$.

Duration of motility of the sperm of treated mice was 44% of that of controls. The relative percentage of abnormal sperm increased considerably. Among the abnormal sperm, categories like double-tailed, detached head, detached tail, mid-piece bending, irregular head and mid-piece loop formation were predominant. The relative percentage of live sperm decreased significantly (table 1).

Sperm count is considered to be one of the important parameters that affect fertility. Decrease in sperm count of *V. rosea* extract-treated mice opens up a promising avenue for further studies in antifertility methods.

Sperm have two principal attributes, namely motility and fertilizing ability[6]. Motility is an important prerequisite for fertilization in the case of internally fertilizing organisms. Any negative impact on motility would seriously affect fertilizing ability. In increasing the relative percentage of abnormal sperms and decreasing the relative percentage of live sperm, *V. rosea* appears to be deleterious at the level of cauda epididymis.

The authors thank Dr C. Nainar Mohamed, Principal, Jamal Mohamed College, for laboratory facilities and Prof. K. Shahul Hamid for technical assistance.

26 December 1988; Revised 20 February 1989

1. Chakraborti, S. K. and Mukerji, B., *J. Res. Indian Med.*, 1988, **3**, 70.
2. Joshi, M. S. and Ambage, R. Y., *Indian J. Exp. Biol.*, 1974, **6**, 256.
3. Chinoy, N. J. and Geetha Ranga, M., *Comp. Physiol. Ecol.*, 1983, **8**, 41.
4. Miyamoto, H., Toyoda, Y. and Chang, M. C., *Physiol. Reprod.*, 1974, **10**, 487.
5. Bauer, J. D., Ackerman, P. G. and Toro, G., *Clinical Laboratory Methods*, C. V. Mosby Co, St. Louis, 1974.
6. Timourian, H. and Watchmaker, G., *Dev. Biol.*, 1970, **21**, 62.

## QUANTITATIVE ANALYSIS OF THIOCYANATE IN URINE BY HEAD-SPACE GAS CHROMATOGRAPHY

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A widely used method for the determination of thiocyanate in biological fluids is the measurement of red colour of thiocyanate formed by adding ferric ion in acid medium. But it has its limitations because of interfering colours from phenols and keto acids, precipitation of some of the thiocyanate with protein by trichloroacetic acid[1, 2], conversion of thiocyanate to cyanide[3], and interferences due to various coloured complexes formed with ferric nitrate reagent in the urine samples of persons under treatment with medicines such as desprin and certain antibiotics.

Other prevailing methods also have limitations, such as, in the case of Aldridge's[4] method of pyridine–benzidine reagent[5], interference by amino acids such as tryptophan and by proteins. Monitoring of thiocyanate in altered thiocyanate metabolism is of great significance, such as during therapy in certain cases of poisoning. Therefore an attempt was made to overcome such interferences in the determination of urine thiocyanate using gas chromatography.

Gas chromatographic (GC) assay of thiocyanate in terms of cyanide was made by taking 10 ml of diluted or undiluted urine in a closed vial (15 ml)