# SERUM TRI-IODOTHYRONINE AND THYROXINE PROFILE IN MURRAYA KOENIGII AND AEGLE MARMELOS COMBINATION TREATED ACYCLIC GOATS

RAVI DUTT<sup>\*</sup>, S. MEHROTRA, GYANENDRA SINGH and UMA SHANKAR Division of Animal Reproduction, Division of Veterinary Physiology & Climatology Indian Veterinary Research Institute, Izatnagar-243 122

## **ABSTRACT**

The study was conducted to examine the effect of *Murraya koenigii* and *Aegle marmelos* combination on serum tri-iodothyronine and thyroxine profile in acyclic goats. Effective 50% ethanolic extract doses of both the plants were extrapolated from rats (1000 mg/kg) to goats using dose equivalent system and converted into powder form based on percent yield. Final doses were prepared by mixing of half of the calculated dose of both the plants on body weight basis. Acyclic goats (n=14) were divided into two equal groups i.e. control (I) and treatment (II). Animals of group I were fed basal diet alone while those in group II were given leaves powder in basal diet for nine days. Animals of both groups were subjected to collection of blood on -8, -4, 0, 4, 8, 12, 16 and 20 days. Estimation of tri-iodothyronine (T3) and thyroxine (T4) was carried out using radioimmunoassay. Mean values of T3 were significantly higher on day 8, 12 and 16 i.e. during the period of follicular dominance in goats of group II as compared to group I. Mean values of T4 in group II did not differ significantly between and within group.

Key words: Murraya koenigii, Aegle marmelos, tri-iodothyronine, thyroxine, acyclic goats

In past, thyroid hormones were thought to influence mainly thermoregulation and homeostasis of energy and protein metabolism. Their involvement in regulation of certain ovarian functions in ruminants has also been reported (Huszenicza et al., 2002). Thyroid hormones play an important role in triggering resumption of the ovarian cycle postpartum (Reist et al., 2003) and regulate cyclic ovarian function through functional coordination of hypothalamic-pituitary-gonadal axis for secretion of gonadotropins (Stewart et al., 1994). Higher level of thyroxine during estrus is considered to cause a change in sensitivity of gonads to gonadotrophic hormones (Nalbandov, 1976). Deficiency of thyroid hormones has been reported to cause anoestrus, sub-estrus and delayed puberty by reducing gonadotropins secretion or decreasing responsiveness to ovaries to pituitary gonadotropins (Roberts, 1971). Existing hormonal therapy used for various reproductive ailments may cause harmful effects to animals besides having other limitations like high cost, non-availability of commercial preparations with ease, need of veterinary supervision etc.

Murraya koenigii (Curry leaf plant) and Aegle marmelos (Bel) have been documented in traditional

Indian practice to promote fertility in animals. Both the, *M. koenigii* (Mehrotra *et al.*, 2009) and *A. marmelos* (Kumar, 2008; Jondhale *et al.*, 2009) have been demonstrated individually to augment the reproductive function in laboratory rats, anoestrus goats and buffaloes. Combination of these two medicinal plants in anestrus goats (Dutt *et al.*, 2010) and buffaloes (Dutt *et al.*, 2011) led to favourable effects in restoration of fertility. Therefore, an attempt was made to examine the effect of combination of these two plants on thyroidal activity in acyclic goats.

## **MATERIALS AND METHODS**

The study was conducted on 14 non-descript adult acyclic goats of 3-4 years of age with fairly good body condition. The animals were maintained under uniform feeding and management conditions at Experimental Animal Shed of Animal Reproduction Division, Indian Veterinary Research Institute, Izatnagar (U.P.). The permission for experimentation on goats was taken from Institute Animal Ethics Committee of the institute. The average body weight of goats ranged between 19-25 kg. The goats were

confirmed acyclic based on absence of estrus through buck parading twice a day and through B-mode transrectal real time ultrasonography for three cyclic lengths. The animals were randomly divided into two groups i.e. control (I) and treatment (II) with equal number of goats in each. Mature, fresh and green leaves of the medicinal plants were collected subsequent to botanical identification at the National Botanical Research Institute, Lucknow as M. koenigii and A. marmelos of family Rutaceae. The leaves were shade dried, powdered in a mixer grinder and stored at room temperature. The 50% ethanolic extracts of M. koenigii (Mehrotra et al., 2004) and A. marmelos (Jondhale et al., 2009) have been examined per os @100, 300, 1000 mg/kg, respectively in rats and 1000 mg/kg dose was found effective in augmenting ovarian function. Powder dose extrapolation from rat (1000 mg/kg) to goats was done by the dose equivalent system described by Van Miert (1986), using Km factor. Km factor for rat and goat are 6 and 45.5, respectively (Van Miert, 1986). The dose of the extract was converted to powder form based on percent yield using the formula:

Dosage of the powder = Dose of extract  $\times$  100/Per cent yield

Per cent yield of *M. koenigii* and *A. marmelos* was 14.44 and 11.27, respectively (Personal communication). The per cent yield is the amount of 50% ethanolic extract recovered from the 100 gram of shade dried leaf powder which was done for laboratory animals. Leaf powder dose per kg body weight basis was worked out for *M. koenigii* and *A. marmelos* 

separately and halved. Final dose was obtained by mixing both the calculated doses, according to animal's body weight. It was administered through drenching after dissolving in 100- 150 ml water from day 0 (initiation) to day 8 on daily basis in goats of group II. All animals of both the groups were subjected to jugular venipuncture on day -8,-4, 0, 4, 8, 12, 16 and 20 to determine serum tri-iodothyronine (T3) and thyroxine (T4) profile using radioimmunoassay (RIA; I125 kits, Immunotech, France). Data obtained were statistically analysed using paired t-test and Student's t-test, respectively (Snedecor and Cochran, 1989).

### RESULTS AND DISCUSSION

Mean T3 values ranged between 1.03±0.15 to  $1.50\pm0.09$  and  $1.09\pm0.07$  to  $2.32\pm0.20$  ng/ml in groups I and II, respectively. In group II, the mean values of T3 were significantly (P<0.05) higher on day 8, 12 and 16 as compared to group I and day 0 value in the same group. Higher T3 concentration around estrus in group II on day 12 and 16 were in accordance with the findings of Patel et al. (1992) and Bhooshan and Kumar (2007). The higher values of T3 during estrus may possibly be associated with corresponding increase in estrogen activity in the body (Ingbar and Woebar, 1974; Bhattacharya et al., 1993) and due to stress of estrus leading to secretion of TSH from pituitary (Kesler et al., 1981). The T4 values ranged between 76.99±7.00 to 80.98±3.33 and 76.79±3.15 to 85.13±3.59 ng/ml in groups I and II, respectively. Mean concentration of T4 did not differ significantly between groups and also within group. There was no significant variation in T3

 $\label{thm:continuous} Table~1\\ Serum~tri-iodothyronine~and~thyroxine~concentration~(ng/ml)~in~goats~(mean\pm S.E)$ 

Experimental	Days of blood collection							
group								
	-8	-4	0	4	8	12	16	20
Serum tri-iodothyronine	(ng/ml)							
Group I (Control)	$1.35^{b}\pm0.14$	$1.50^{\text{b}} \pm 0.09$	$1.12^{b}\pm0.14$	$1.04^{b}\pm0.16$	$1.24^{b}\pm0.33$	$1.03^{\text{b}} \pm 0.15$	$1.09^{b}\pm0.20$	$1.13\pm0.24$
Group II (Treatment)	$1.49^{b}\pm0.14$	$1.69^{b}\pm0.13$	$1.09^{\text{bA}} \pm 0.07$	$1.12^{b}\pm0.10$	$1.78^{aB} \pm 0.06$	$2.32^{aB} \pm 0.20$	$2.10^{^{aB}}\pm0.28$	1.17±0.19
Serum thyroxine (ng/ml)	)							
Group I (Control)	$78.03\pm4.27$	$80.98 \pm 3.33$	$79.52 \pm 7.92$	$79.74 \pm 3.45$	$79.44 \pm 4.37$	$78.29 \pm 6.54$	76.99±7.00	78.68±7.59
Group II (Treatment)	77.08±3.09	76.79±3.15	78.91±4.59	79.93±3.55	79.83±5.00	85.13±3.59	$80.89\pm2.74$	77.56±1.71

Mean bearing superscripts (a, b) in a column differ significantly (P<0.05); Mean bearing superscripts (A, B) in a row differ significantly (P<0.05) in *Murraya koenigii* and *Aegle marmelos* combination treated group

and T4 concentrations among control acyclic goats on different experiment days which is in agreement with the observations of Kumar (2008) and Sarath *et al.* (2010).

Hypothyroid condition reduces the responsiveness of ovary to pituitary gonadotropins (Mudgal, 1992), hence lower level of thyroid hormones may play an indirect but important role in anoestrus condition (Sharma et al., 1999). Rhythmic alterations in the thyroid hormones activity during estrous cycle was probably due to changes in the level of gonadal hormones. From the present study, it might be concluded that treatment with M. koenigii and A. marmelos combination may modulate the thyroid function favourably through changes in gonadal hormones which might have future implications in acyclic goats. The active principles seem to operate either through mimicking gonadotrophins activity or stimulating the central mechanism for endogenous release of gonadotrophins along with possibility of local action, requiring further studies.

#### **REFERENCES**

- Bhattacharya, N.K., Sanwal, P.C., Pandey, J.K. and Varshney, V.P. (1993). Plasma levels of sex steroid hormones during estrous cycle and pregnancy in Black Bengal goats. *J. Vet. Physiol. Allied Sci.* **12**: 33-37.
- Bhooshan, N. and Kumar, P. (2007). Profile of ovarian and thyroid hormones, alkaline phosphatase and cholesterol at estrous cycle, gestation and early lactation in blood plasma of Marwari goats. *Indian J. Anim. Sci.* 77: 1233-1237.
- Dutt, R., Mehrotra, S., Hoque, M., Shankar, U., Singh, G., Agarwal, S.K., Das, G.K. and Singh, S.K. (2010). Effect of *Murraya koenigii* and *Aegle marmelos* combination on resumption of fertility in anestrous goats. *J. Appl. Anim. Res.* 38: 249-252.
- Dutt, R., Mehrotra, S., Shanker, U. and Singh, G. (2011). Effect of Murraya koenigii and Aegle marmelos feeding on anestrus buffaloes. Indian J. Anim. Reprod. 32: 47-49.
- Huszenicza, G., Kulcsar, M. and Rudas, P. (2002). Clinical endocrinology of thyroid gland function in ruminants. Vet. Med. Czech. 47:199-210.
- Ingbar, H.S. and Woebar, A.K. (1974). The Thyroid Gland. In: Text Book of Endocrinology. William, H. and Roberts, W.B. (edts.), Saunders Company, Philadelphia.
- Jondhale, N.S., Shanker, U., Mehrotra, S., Agarwal, S.K., Singh, S.K., Das, G.K. and Deori, S. (2009). Effect of Aegle marmelos and Ficus religiosa leaves extracts on the ovarian

- function in rats. Indian Vet. J. 86: 1141-1144.
- Kesler, D.J., Johnson, H.D. and Garverick, H.A. (1981). Postpartum concentrations of thyroxine in plasma of dairy cows. J. Dairy Sci. 64: 1618-1620.
- Kumar, R. (2008). Effect of *Aegle marmelos* (Bel) and *Ficus religiosa* (Pipal) on the reproductive performance in anoestrus goat and buffalo. M.V.Sc. thesis, IVRI, Izatnagar, Bareilly.
- Mehrotra, S., Shankar, U., Hoque, M. and Agarwal, S.K. (2009). Follicular development and induction of estrus in anestrous goats by medicinal plants. *Indian Vet. J.* **86**: 527-528.
- Mehrotra, S., Shanker, U., Majumdar, A.C., Paliwal, O.P. and Agarwal, S.K. (2004). Effect of certain indigenous medicinal plants on follicular development and steroidogenesis in rats. *Indian J. Anim. Reprod.* **25**: 83-86.
- Mudgal, V.D. (1992). Reproduction in River Buffaloes. In: Buffalo Production. World Anim. Sci., Production Approach System 6, Chapter 8. Tuloh, N.M. and Holmes, J.H.G. (edts.), Elsevier, New York.
- Nalbandov, A.V. (1976). Reproductive Physiology of Mammals and Birds. (3<sup>rd</sup> edn.), W.H. Freman and Co., San Francis.
- Patel, A.V., Pathak, M.M., Mehta, V.M. and Janakiraman, K. (1992). Circulating levels of hormones in pregnant goats. Proceedings of 5<sup>th</sup> International Conference on Goats, Recent Advances in Goat Production, New Delhi.
- Reist, M., Erdin, D.K., Von Euw, D., Tschumperlin, K.M., Leuenberger, H., Hammon, H.M., Morel, C., Philipona, C., Zbinden, Y., Kunzi, N. and Blum, J.W. (2003). Postpartum reproductive function: association with energy, metabolism and endocrine status in high yielding dairy cows. *Theriogenol.* 59: 1707-1723.
- Roberts, S.J. (1971). Veterinary Obstetrics and Genital Diseases. (2<sup>nd</sup> edn.), CBS Publishers and Distributors. Darya Ganj, New Delhi.
- Sarath, T., Suguna, K., Mehrotra, S., Arunmozhi, N., Agarwal, S.K. and Shankar, U. (2010). Serum tri-iodothyronine and thyroxine profile in insulin treated acyclic goats. *Indian J. Anim. Reprod.* 31: 33-34.
- Sharma, K.B., Nayar, S., Malik, V.S., Singh, R., and Sodhi, S.P.S. (1999). Levels of hormones and minerals in cyclic, anestrus and subestrus buffalo heifers. *Indian J. Anim. Sci.* 69: 214-216.
- Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods. (8<sup>th</sup> edn.), Iowa State University Press, Ames, USA.
- Stewart, R.E., Stevenson, J.S. and Minton, J.E. (1994). Serum hormones in response to estradiol and (or) progesterone in ovariectomized cows after thyroidectomy. *Domestic Anim. Endocrinol.* **11**:13-24.
- Van Miert, A.S.J.P.A.M. (1986). The Use of Animals of Drug Licensed for Human Use Only. In: Comparative Veterinary Pharmacology, Toxicology and Therapy. Van Miert A.S.J.P.A.M., Bogaert, M.G. and Debackere, M. (edts.), Proc III Congress of European Association Vet. Pharmacology and Toxicology, Belgium, MTP Press Limited, Lancaster.