Assessment of Ameliorating Potential of Withania Somnifera in Goitrogen Concurrent Medication in Rats through Haematobiochemical Parameters

V.Shivakumar[*], S.K.Shukla and D.S.Kumar Wodeyar[1]

Assistant Professor, Department of Teaching Veterinary Clinical Complex, Veterinary College, Hassan

[1]Assistant Professor, Department of VPB, Veterinary College, Bangalore

Abstract

Present study has been carried out with an objective of eliciting ameliorating potential of Withania somnifera on haematobiochemical alterations in thiourea induced hypothyroidism. When rats were induced hypothyroidism using a thyroid depressant such as thiourea, it was found that thiourea treated group showed marked normocytic normochromic anaemia suggesting suppressive effect of thiourea on haemopoetic system. W. somnifera root extract (WRE) treated group showed significant increases in Hb, TEC, PCV suggesting WRE may have stimulatory effect on haemopoietic system. When WRE and Aqueous Iodine (Aq I2) were given with thiourea it revealed slight increases in Hb, PCV and TEC as compared to thiourea treated group suggesting WRE and Aq. I2 are counteracting the inhibitory effect of thiourea on haemopoietic system. Total protein, albumin and globulin decreased significantly and cholesterol level significantly increased in thiourea treated group when compared with the control group. WRE treated group revealed ability to decrease cholesterol level and significantly increases albumin level and A:G ratio. Experimental result revealed that W. somnifera to certain level nullified the depressing effect of thiourea on haemoatological and biochemical parameters suggesting its haemopoietic and anabolic effect but could not prevent thyroid depressing action of thiourea.

Key words: *Withania somnifera, thiourea, normocytic normochromic anemia, anabolic effect*

Introduction

Ashwgandha, *Withania sominifera* L., is an Ayurvedic medicinal plant used as a popular home remedy for several diseases (Patwardhan *et al.*, 1988). It is mentioned in Vedas as an herbal tonic and health food. It is an official drug and is mentioned in the Indian pharmacopoeia (1985). The chemical composition, pharmacological and therapeutic efficacy of *W. somnifera* has been established, it possess antioxidative, haemopoetic, immunomodulatory, anti-inflammatory, antitumour and rejuvenating properties to combat the deleterious effect of various toxicants (Mishra *et al.*, 2000). The principal bioactive compounds of *W. somnifera* are withanolides, (highly oxygenated C-28 steroid derivatives) and about 40 withanolides have been isolated and identified from *W. somnifera* has stimulatory effect on haemopoitic system. Present study has been carried out with an objective of eliciting ameliorating potential of *Withania somnifera* on hematobiochemical alterations in thiourea induced hypothyroidism.

** Part of M V Sc thesis*

Email Id: shiva.drvet@gmail.com

Materials and Methods

W. somnifera roots were procured locally and identified and authenticated from the Department of Biological Sciences of the GB Pant University, Uttarakhand. After cleaning and drying, the roots were grinded and stored in sealed plastic bags in dry place at room temperature till further use. The cold aqueous extract was prepared as per method of Singh *et al.* (2001). Totally 48 Albino rats of 2 to 2.5 months age weighing between 150 to 200 gm were procured from Experimental laboratory animal section, IVRI, Izatnagar. These were acclimatized for two weeks and randomly divided into six groups (I-VI). Group I served as healthy control and group II was given thiourea @ 0.5% in the feed (250mg/kg bwt). Rats of group III received aqueous extract of WRE @ 1.4 g/kg bwt.. In group IV, along with thiourea, aqueous iodine was given @ 0.25 mg/kg b wt. In group V, thiourea and WRE were given. Thiourea, WRE and Aq I2 were given in rats of group VI. On 30th day of treatment regime rats were scarified, blood was collected, serum separated and Total erythrocyte count, Packed Cell Volume and Haemoglobin were estimated using the method of Jain (1996). The serum obtained from the collected blood samples was used for the estimation of biochemical parameters (Total protein, Albumin, Globulin and Serum cholesterol) by specific diagnostic kits procured from ACE Diagnostics & Biotech Ltd., Gurgaon. The results were analysed by ANOVA technique as per method of Snedecor and Cochran (1975).

Results

Haemoglobin, TEC, PCV and erythrocyte indices values in the rats of different groups are presented in Table 1. The Hb value was significantly decreased in group II (10.70 ± 0.34 g/dl) and non significantly increased in group III (14.49 ± 0.25 g/dl) as compared to control group (13.73 ± 0.22 g/dl). There was significant increase in Hb of group IV, V and VI as compared to group II but not much difference was noticed between group IV and V. However there was significant difference in Hb of group VI (14.08±0.18 g/dl) as compared to group IV (12.98±0.28 g/dl), V (12.76±0.52 g/dl). TEC values were significantly decreased in group II and significantly increased in group III when compared with control group. There was significant increase in TEC values of group IV, V and VI as compared to group II, but, not much difference was noticed between group IV and V. However there was significant difference in TEC of group VI when compared with group IV, V. The haematocrit value in group II (33.88±0.85%), IV (37.38±1.39%) and V (37.63±0.86%) was significantly lower than control group (41.75±1.10%). There was increased level of haematocrit in group III (44.25±0.86%) as compared to control group though, the increase was non significant. However there was significantly higher value of haematocrit in group VI (42.50±0.80%) as compared to group IV and V. Erythrocytic indices such as mean corpuscular concentration (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values are depicted

in Table 1. There was not much difference in the values of MCV, MCH and MCHC in between groups. However, MCHC value was significantly increased in group II (32.43 ± 0.62 g/dl) and there was also a significant decrease in group VI (25.58 ± 0.40 g/dl) as compared to control group (27.53 ± 0.41 g/dl). However the changes in the values of erythrocytic indices were well within the normal range.

Total protein profile in rats of different groups treated with WRE and thiourea is presented in Table 2. Total protein value was estimated to be 7.76 ± 0.03 g/dl in group I. There was a significant decrease in group II (7.17 ± 0.04 g/dl). Values of TP in group IV and group V were measured as 7.59 ± 0.05 and 7.23 ± 0.06 g/dl, respectively, which also showed significant decrease from control, but when compared to group II these values were found to be increased. The values of total protein in group III (7.79±0.07 g/dl) and VI (7.72 ±0.03 g/dl) were slightly varied as compared to group I, however, the difference was not significant. When groups IV and V were compared with group VI, it revealed that the effect of thiourea on protein was nullified to certain extent by giving both WRE and aqueous iodine together. There was significant decrease in albumin in group II (3.98 ± 0.02 g/dl) as compared to group I (4.34 ± 0.02 g/dl). Values of albumin were 4.49 ± 0.06 g/dl in group III which was significantly higher than control group. Group VI (4.38 ± 0.02 g/dl) showed significant difference as compared to group II and group V (4.08 ± 0.07 g/dl). The value of albumin in group VI (4.36 ± 0.03 g/dl) was increased as compared to groups II and V. There was significant decrease in globulin value in group II (3.19±0.05 g/dl), group IV (3.22±0.04 g/dl) and group V (3.15±0.05 g/dl) as compared to control group (3.42 ±0.02 g/dl). The values in groups III and VI were found to be 3.31±0.07 and 3.36±0.05 g/dl, respectively, which was slightly less than the values of control group, though the decrease was non significant. When groups IV and V were compared with group VI, it was found that use of iodine and *W. somnifera* were more helpful in combating the effect of thiourea than the either of drug alone. The A:G ratio in group I was 1.27±0.01 g/dl, which increased non significantly to 1.30±0.04 g/dl in thiourea control group. Its value significantly increased in group III (1.36±0.04 g/dl) and group IV (1.36±0.01 g/dl) as compared to control group and in group V (1.30±0.04 g/dl) and VI (1.29±0.03 g/dl), slight increase was noticed as compared to the control.

Cholesterol level in rats of different groups treated with *W. somnifera* root extract and thiourea is presented in Table 2. In group II (thiourea fed rats), its level (32.42±0.62 mg/dl) was significantly higher than control (27.53 ± 0.41 mg/dl) and in *W. somnifera* group (25.31 ±0.36 mg/dl), the level was significantly decreased than control. There was no significant difference in the values of cholesterol in between groups IV (26.93 ±0.25 mg/dl) and V (27.63 ±0.41 mg/dl). However, when groups IV and V were compared with group VI (25.58±0.40 mg/dl), it revealed that use of combination

of iodine and *W. somnifera* was more helpful in combating the effect of thiourea than either of the drug alone.

Discussion

The thyroid gland is one of the most important regulators of body metabolism and its effect reaches throughout the body. It is in turn regulated by the two master glands of the endocrine system, the pituitary and the hypothalamus. Since the thyroid can affect practically every cell in the body, normal thyroid function is essential to health. In the present investigation, thiourea treated groups showed significant decrease in total erythrocyte count, non significant decrease in haemoglobin and haematocrit value suggesting that the thiourea treated groups were in anaemic condition. Similar results were also reported by many workers (Mak, 1988, Sokkar *et al.,* 2000). Reduced erythrocyte count could be due to reduced osmotic resistance of erythrocyte after thiourea treatment leading to rupture of the cells and subsequently anaemia (Hazleton, 1987). In *W. somnifera* treated group Hb, PCV and TEC were significantly increased. These findings suggest that, WRE has protective effect on the haemopoetic system. The haematopoiesis characterised by stem cell proliferation and increase in bone marrow cellularity was reported in mice by Aphale *et al.* (1998). When erythrocytic indices of present study were analysed, it revealed that rats were suffering from normocytic normochromic anaemia. Prasad *et al.* (1989) reported similar result in goats suffering from goitre. Anaemia in hypothyroidism was attributed to reduced rate of erythropoiesis rather than accelerated destruction (Cline and Berlin, 1963), hypocellularity of bone marrow (Axelrod and Barman, 1951), lack of direct action of sufficient thyroid hormone on erythroid precursor cells and decreased erythropoiesis (Adamson & Finch, 1966).

Hypoproteinaemia with hypoalbuminaemia noticed in thiourea treated group supports the contention of earlier workers. Hypoproteinaemia with hypoalbuminaemia observed in the present study may be attributable to hepatic dysfunction with decreased mitochondrial enzymes reported in rats with hypothyroidism (Ruzicka and Rose, 1981). In addition, reduced alimentary tract motility and absorption of nutrients in hypothyroidism of ruminants was also attributed to the development of hypoproteinaemia. However, *W. somnifera* treated group showed significant increase in albumin levels and A:G ratio than control groups with slight increase in total protein. Results are in contrast with the results of other workers. Samarth *et al.* (2002) reported increased level of total protein in *W. somnifera* treated broiler birds. Panda and Kar (1997), also reported similar significant increase in serum protein concentration following *W. somnifera* administration indicating the increased synthesis of protein in the body directly by the drug or indirectly through the increased thyroid hormone concentration, which are basically protein anabolic in nature (Turner and Bagnara, 1976)

Hypercholesterolemia was observed in thiourea treated groups which was in accordance with the observation of Nasseri and Prasad (1987). Higher cholesterol level are in agreement with earlier findings in spontaneous or induced hypothyroidism of different species of animals and man (Singh *et al.* 2002). Cholesterol synthesis is increased in presence of excess and reduced in thyroxine deficiency. These contradictory results have been attributed to decreased biliary excretion of cholesterol in hypothyroid animals causing increased cholesterol despite reduced synthesis (Dickson, 1993). In addition to that, increased TSH in hypothyroidism could account for hypercholesterolemia (Asboc-Hansen, 1958) and decreased lipoprotein lipolysis, reduced hepatic utilization and augmented hepatic production contribute significantly to increased cholesterol in blood (Weinberg, 1987). In *W. somnifera* treated group considerable decrease in the cholesterol level was seen, which is in accordance with the results of Dhirendra Kumar (2005). However when *W. somnifera* was given along with thiourea, there was not much of significant difference when compared to control but, the level of cholesterol was decreased when compared to thiourea treated group to a significant level. These observations suggest the protective role of *W. somnifera* on thiourea-induced alterations in the lipid metabolism.

Conclusion

So it can be concluded that, *W. somnifera* has stimulatory action on haemopoietic system. It, to certain extent, nullified the depressant action of thiourea. It has anabolic effect which was evident by increase in serum total protein and albumin It also has the ability to reduce serum cholesterol level and counteract increased serum cholesterol in thiourea treated groups.

References

Adamson, J.W. and Finch, G.A. 1966. Mechanism of Euthyroid marrow activation. *Mans. Ass. Am. Physns.* **73**: 419.

Aphale, A.A., Chhiba, A.D., Kumbhkaran, N.R., Mateenuddin, M. and Dahat, S.H. 1998. Subacute toxicity study of the combination of ginseng (*Panax ginseng*) and ashwagandha (*Withania somnifera*) in rats**:** a safety assessment. *Indian J. Physiol. Pharmacol.* **42**(2)**:** 299-302.

Asbboc-Hansen, G. 1958. Hypothyroidism in sheep. *Physiol. Rev.* **38**: 466

Axelrod, A.R. and Barman, L. 1951**.** Blood, **6**: 436. (c.f. Horton, L., Coburn, R.J., England, J.M. and Himsworth, R.L. The hematology of hypothyroidism. *Quat. J. Med.*, New series. XLV, **177**: 101-124)

Cline, M.J. and Berlin, N.I. 1963. Erythropoiesis and red cell survival in hypothyroid dog. *Am. J. Phys.* **204:** 415-418.

Dhirendra Kumar, 2005. Potential of *Withania somnifera* to combat acute

and subacute lead toxicity in broiler chickens. M.V.Sc. thesis., G.B. Pant University of Agriculture and Technology, Pantnagar, India.

Dickson, W.M. 1977. Endocrinology, reproduction and lactation. In: Duke's Physiology of Domestic animals. Swenson, *M.J. 9th edn.* pp. 731-771.

Hazleton, 1987. *13-week drinking water study in rats with thiourea.*. Hazleton Laboratories America Inc. for SKW Trostberg AG, Trostberg, pp. 1–360. (HLA Study No. 2319-119).

Indian pharmacopoeia, 1985. Controller of publications, *3rd ed*, vol. II. Government of India, New Delhi.

Jain, N.C. 1996. Schalm's Veterinary Haematology. 5th Ed. Philadeiphia, Lea and Febringer.

MAK, 1988. Thiourea. In: Henschler D, ed. *Occupational toxicants: Critical data evaluation for MAK values and classification of carcinogens. Volume 1*. Deutsche Forschungsgemeinschaft (DFG); Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission). Weinheim, VCH Verlagsgesellschaft mbH, pp. 301–312.

Mishra, L.C., Singh, B.B. and Dagenias, S. 2000. Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A review. *Alt. Med. Rev*. **5(4):** 334-346.

Nasseri, A. and Prasad, M.C. 1987. Effects of hypothyroidism on reproductive behaviour in female sheep: clinical studies. *Indian Veterinary Medicine Journal*. **11:** 191–199.

Panda, S. and Kar, A. 1997. Evidence for free radical scavenging activity of Ashwagandha root powder in mice. *Indian J Physiol Pharmacol*. **41:** 424-426.

Patwardhan, B., Panes, G.T. and Kulakarni, P.H. 1988. Ashwagandha (*Withania somnifera*): a review. *Journal of National integrated Medical association*. **30**: 7-11.

Prasad, M.C., Sharma, A.K. and Ramkumar. 1989. Final report on foetal pathology in hypothyroid goats. Division of Pathology, IVRI, Izatnagar.

Ruzicka, F.J. and Rose, D.P. 1981. The influence of thyroidal status on rat hepatic mitochondrial NADH duroquinone reductase. *Endocrinology*. **81**: 664-667.

Samarth, V.R., Jagtap, D.G., Dakshinkar, N.P., Nimbulkar, M.V. and Kothekar, M.D. 2002. Effect of Ashwagandha root powder (*Withania somnifera)* on growth performance of broiler. *Indian Vet. J.* **79(7):** 733-734

Singh, B., Saxena, A.K., Chandan, B.K., Gupta, D.K., Bhutani, K.K. and Anand K.K. 2001. Adaptogenic activity of a novel, with anolide-free aqueous

fraction from the roots of *Withania somnifera*. Dun. *Phytother. Res.* **15**(4)**:** 311-318.

Singh, J.L., Sharma, M.C., Maheshkumar, Rastogi, S.K., Gupta, G.C., Singh, S.P., Sharma, L.D., Gandhi, V.K. and Kalicharan. 2002. Assesment of therapy in goitrous goats through some cardiac function test. *Small Ruminent Research*. **44:** 119-124.

Snedecor, G.W. and Cochran, W.G. 1975. Statistical methods, 7th Edn. Oxford an Indian Book House, New Delhi.

Sokkar, S., Soror, A., Ahmed, Y., Ezzo, O. and Hamouda, M. 2000. Pathological and biochemical studies on experimental hypothyroidism in growing lambs. *Journal of Veterinary Medicine, Series B,* **47(9):** 641–652.

Turner, C. and Bangara, T. 1976. General endocrinology. W.B sanders Company, Philadelphia. Landon. Toranto. pp. 178-221.

Weinberg, R.B. 1987. Lipoprotein metabolism: hormonal regulation. Hosp. Practice. 223-243.