The Pharma Innovation Journal 2023; 12(8): 65-68

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(8): 65-68

www.thepharmajournal.com
Received: 19-06-2023
Accepted: 22-07-2023

Ramya B
Assistant Professor, Department of Veterinary Pathology, VCC, C.V.Sc, Mamnoor, PV Narasimha Rao Veterinary University, Telangana, India

Anand Kumar A
Professor and Head, Department of Veterinary Pathology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Madhuri D
Professor and University Head, Department of Veterinary Pathology, C.V.Sc, Rajendranagar, PV Narasimha Rao Veterinary University Hyderabad, Telangana, India

Gopala Reddy A
Professor (Veterinary Pharmacology and Toxicology), Controller of Examinations, PV Narasimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana, India

Shiva Kumar P
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, AHP, Mamnoor, PV Narasimha Rao Veterinary University, Telangana, India

Corresponding Author:
Ramya B
Assistant Professor, Department of Veterinary Pathology, VCC, C.V.Sc, Mamnoor, PV Narasimha Rao Veterinary University, Telangana, India

Experimental hypothyroidism induced molecular alterations in the liver of rats and alleviations with Shilajit and *Withania somnifera*

Ramya B, Anand Kumar A, Madhuri D, Gopala Reddy A and Shiva Kumar P

Abstract
The present experiment was conducted to evaluate aerobic and anaerobic carbohydrate metabolic enzymes and histopathological changes in liver of experimentally induced hypothyroidism in rats. The study conducted in 21 days female Sprague drawly rats of total 96 number for 3 months. These rats were separated into8 groups with equal rats in each group. They were administered with: Grou p (Gp) 1: Euthyroidd Group 2: Hypothyroidism (Methimazole @ 00.02%) Gp 3: Hypothyroid + Levothyroxine Gp 4: root extract of *Withania somnifera* control @ 100 milli gram/Kilo gram body weight Gp 5: Hypothyroid + root extract of *Withania somnifera* @ 100 milli gram/Kilo gram body weight Gp 6: *Shilajit* control @ 100 milli gram/Kilo gram body weight Gp 7: Hypothyroid + *a Shilajit* @ 100 milli gram/Kilo gram body weight Gp 8: Hypothyroid + *Withania somnifera* root extract @ 100 milli gram/Kilo gram body weight + *Shilajit* @ 100 milli gram/Kilo gram body weight. The mean serum T_3, T_4 concentrations, G6PD and glycogen synthase in Gp-2 rats were significantly (p<0.05) lower. Serum TSH in the hypothyroid group was significantly (p<0.05) higher. Histopathological section revealed fatty degeneration, sinusoidal dilation, congestion, aggregation of lymphoid and franc necrosis. Lesions in ameliorating agent administered groups were mild to moderate. The present study indicated presence of physiological and functional effects on liver of hypothyroid rats in experimental model and usage of herbal drugs may have beneficiary role in treating hypothyroidism.

Keywords: Methimazole, hypothyroidism, hepatotoxicity, *Withania somnifera*, Shilajit

Introduction
Thyroid has a vital function in modifiable cell metabolism via its hormones T_3 and thyroxin which are produced from tyrosine and iodine. Thyroid gland supervises usage off sources of energy, production of protein and manages the vulnerability of body to different hormones. These hormones are important in metabolic trails of fat, electrolyte, protein, sugar and mineral metabolisms [1]. Thyroid abnormalities are the foremost endocrinopathies both in human [2] and in veterinary medicine [3]. Reduced thyroid function is a due to insufficient fabrication or reduced action of either T_3 and/or T_4 thyroid hormones and raised plasma levels of TSH. It is well established that methimazole (0.02%) is administered orally in drinking water f or stimulation of experimental hypothyroidism [4] and was formerly used to bring on hypothyroidism [5].
'Ayurveda' is getting superior-class attentiveness as well as charisma in several areas of the globe due to its safety, ailment deterrent along with rejuvenative actions. Herbal and combination of herbs and mineral inventions in ayurveda are supposed to reinforce the body's defenses [6]. Despite the fact that various herbs are accessible for valuable thyroid hormone utility, current study was carried to uncover the efficacy of the herbs which control the thyroid hormones levels solely or in blend by using Withania somnifera and Shilajit.

Materials and Methods
The experiment was done on 21 days old, Sprague drawly female rats of 96 number, obtained from Mahaveer enterprises, Hyderabad. They were retained in animal house ((Department of VPT) with round the clock dark and light cycle at a temperature of 25-27 °C. Rats were kept in polypropylene cages, set on marketable regular feed and *ad libitum* water. Rats were set aside for 5 days for adaptation former to begin study and was performed with acceptance of Institutional Animal Ethics Committee, CVSc., Rajendranagar (I/6/16/05-01-2016).

These rats were divided into 8 groups with 12 in each group. They were treated as: Gp 1: Euthyroid Gp 2: Hypothyroidism with Methimazole (0.02%) Gp 3: Hypothyroid + LT_4 Gp 4: Root extract of *Withania somnifera* control @ 100 milli gram/Kilo gram body weight Gp 5: Hypothyroid + root extract of *Withania somnifera* @ 100 milli gram/Kilo gram body weight Gp 6: *Shilajit* control @ 100 milli gram/Kilo gram body weight Gp 7: Hypothyroid + *Shilajit* @ 100 milli gram/Kilo gram body weight Gp 8: Hypothyroid + root extract of *Withania somnifera* @ 100 milli gram/Kilo gram body weight + *Shilajit* @ 100 milli gram/Kilo gram body weight for 3 months.

Blood samples were from retro orbital puncture and serum was separated. Thyroid profile was estimated from sera of blood collected by RIA by employing the Dia Sorin S.P.A. kits, USA. In thee last part of 3 months, subjects were sacrificed. Thyroid and liver was gathered in 10% neutral buffer formalin (NBF) for histopathological studies. The sections were stained with H and E stain [7, 8]. The tissues of liver stored at -20 °C for evaluation of the glycogen synthase [9] activity and (G6PD [10] in homogenates.

The experimental facts of every parameter was subjected to one way ANOVAA by using statistical package for social sciences (SPSS) version 15. 0. Differences between means were tested using Duncan's multiple comparison tests and significance level was set at 0.05.

Results and Discussion

Thyroid Profile

Serum T_3 and T_4concentrations

The serum T_3 and T_4 hormone concentration (ng/dl) of the group II was significantly (p<0.05) inferior than group I but the values of groups III, IV, V, VI and VIII were equivalent to group I. T_3 levels in group VII was significantly (p<0.05) inferior r than group I (Table 1).

Serum TSHH concentration

The serum Thyroid Stimulation Hormone concentration (µ I U/milli litre) of the group I was significantly (p<0.05) advanced than group 1. The serum TSH concentration in groups III to VIII was significantly (p<0.05) inferior than group II (Table 1).

These variation was because of thyroperoxidase enzyme inhibition by methimazole, that interfered in production of hormones by thyroid. Studies showed the decline in T_3 and T_4 via off-putting response mechanism might have motivated pituitary to discharge more TSH [11, 12].

Augment in thyroid hormone production in group V rats canbe due to adaptogenic character of Withania, which improved the hormones of the endocrine system and gets equilibrium to the thyroid profile. Immunomodulation effect of the herb has a stimulatory upshot on a lethargic thyroid and increased serum T_4 concentration [13]. *Shilajit* improved thyroid profile by organizing thyroid gland role through it's adaptogenic activity [14].

Table 1: Serum thyroid profile in different groups of rats (Parent stock)

Groups (Gp) (n = 6)	T_3 (ng/deci lt.) level	T_4 (µg/deci lt.) level	TSH (µ IU/milli lt.) level
Gp 1	115.68±4. 46^b	4.71±0.14^b	0.05±0.01^a
Gp 2	101.15±4. 06^a	3.01±0.24^a	5.91±0.45^c
Gp 3	110.73±4. 84^b	4.36±0.18^b	0.87±0.03^b
Gp 4	118.03±4. 69^b	4.79±0.17^b	0.03±0.007^a
Gp 5	111.74±4.17^b	4.24±0.17^b	1.04±0.03^b
Gp 6	116.62±5.02^b	4.69±0.19^b	0.04±0.009^a
Gp 7	106.78±4.71^a	3.45±0.12^a	1.91±0.16^b
Gp 8	113.19±4.76^b	4.31±0.21^b	0.51±0.12^b

Means with different superscripts different significantly (p<0.05)

One way ANOVA (SPSS:15)

Activity of enzyme in liver tissue

Glucose - 6 - phosphate dehydrogenase (G6PD) (m IU/milli gram protein)

The G6PD (m IU/milli gram protein) action was diminished significantly (p<0.05) in group 2 as measured up to 1st group. The groups 4 and 6 showed significant (p<0.05) rise as compared to group 2. The groups 3,, 5 and 8 showed an upgrading in the enzyme activity as compared to group 2, while in group 7 a significant (p<0.05) decline was noted as compared to group 1 (Table 2).

Glycogen Synthase (m IU/milli gram of protein)

The Glycogen Synthase (m IU/milli gram of protein) activity was dropped off significantly (p<0.05) in group 2 as compared to group 1. The glycogen synthase activity in groups 3 to 8 was significantly (p<0.05) higher than group 2 (Table 2).

The G6PD (m IU/milli gram protein) activity was reduced significantly (p<0.05) in group 2 in the current experiment could be due to reduced protein production and mRNA levels which effected glucose metabolism [15]. As there were precise receptors for enzyme and thyroid hormones at a particular site, modest quantitative and qualitative alter of thee thyroid hormone could have resulted in diminish enzyme activity [16].

The alterations in Glycogen Synthase (m IU/milli grm of protein) activity might be as a result of change in the concentrations of Adenosine Monophosphate and Adenosine Tri Phosphate that were potent modulators of enzymatic reactions [17]. Thee stimulatory effect of cortisol on the activities off the key gluconeogenicc enzymess was partial du e to partial glucose making in response to under nutrition [18].

Withania could have augmented glucose metabolism and increased T_4 concentration that amplified activities of G6PD and glycogen synthase [19]. *Shilajit* undo the effects due to Methimazole on G6PD and glycogen synthase by stimulating liver and managing dysfunction of thyroid gland. But the restructuring effect on G6PD was minimum [20].

Table 2: Activity of enzymes in various groups of rats

Groups (Gp) (n = 6)	Glucose – 6 - Phosphate dehydrogenase (m IU/milli gram of protein)	Glycogen synthase (m IU/milli gram of protein)
Gp 1	218.63±16. 04^{b}	6.97±1. 02^{c}
Gp 2	186.73±14. 65^{a}	2.72±0. 35^{a}
Gp 3	203.05±11. 28^{ab}	4.82±0. 68^{bc}
Gp 4	223.87±10. 16^{b}	6.77±0. 41^{c}
Gp 5	197.60±12. 55^{ab}	4.55±0. 75^{b}
Gp 6	217.09±9. 79^{b}	6.85±0. 56^{c}
Gp 7	193.49±8. 94^{a}	4.02±0. 38^{b}
Gp 8	200.66±11. 22^{ab}	4.31±0. 21^{b}

Means with different superscripts differ significantly (p<0.05)
One way ANOVA (SPSS: 15)

Histopathological alterations in hepatic tissue

In 1, 3, 4, and 6 groups no alterations of pathological importance were noted. In group 2, dilation of sinusoidal spaces, diffused fatty change shrunken hepatic cells, mild to moderate round cell infiltration, franc necrosis, severe congestion and nuclear changes viz: pyknosis, chromatin margination and aggregates of lymphoid were noted (Figs.1-3). In groups 5 and 7 mild to moderate fatty and other degeneration were seen (Figs. 4-5). The alterations in group 8 were almost comparable to control groups.

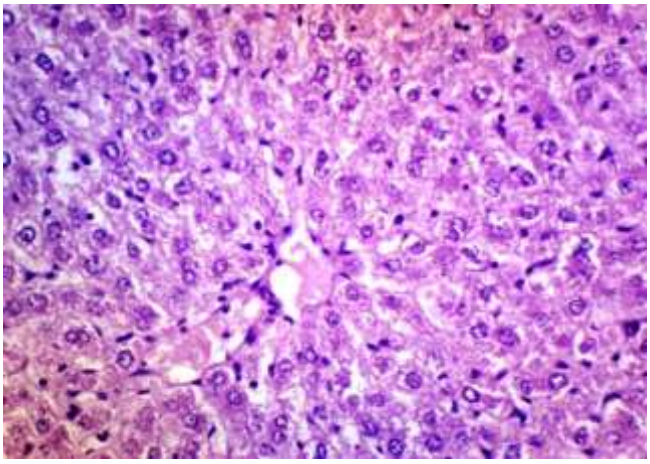

Fig 1: Hepatic section depicting severe congestion, fatty change, nuclei pyknosis and margination of chromatin in group i2. Hematoxylin & Eosin X 100

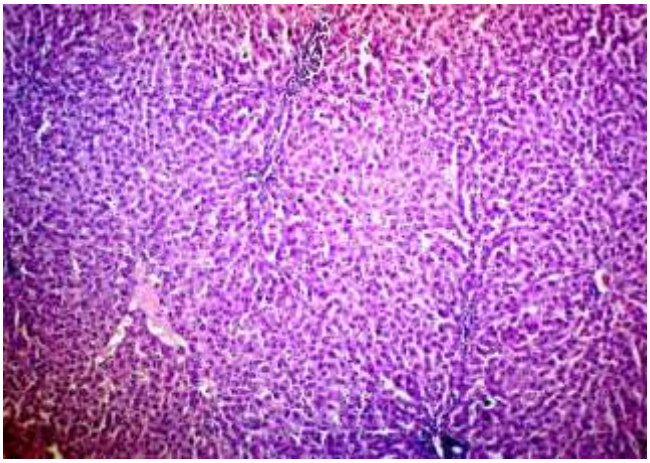

Fig 2: Hepatic section showing mild to moderate infiltration of mono nuclear cells congestion and little lymphoid aggregates in group 2. Hematoxylin & Eosin

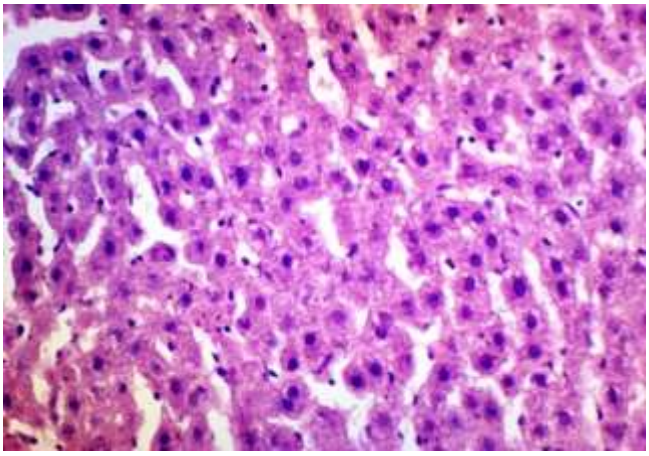

Fig 3: Hepatic section showing diffused fatty degeneration, sinusoidal dilation and shrunken hepatocyte in group 2. Hematoxylin & Eosin X 400

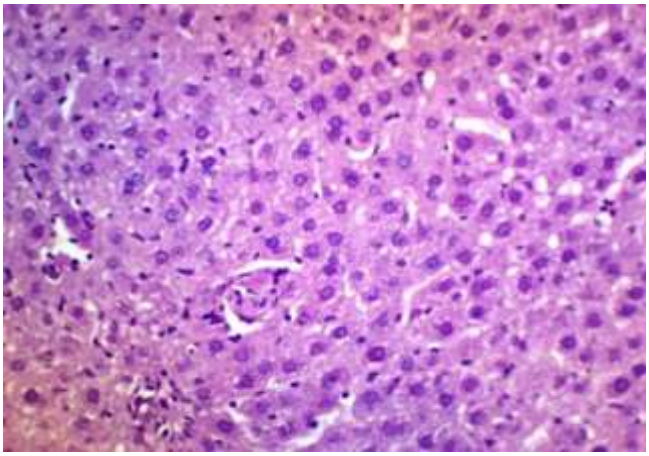

Fig 4: Hepatic section with fatty degeneration and round cell mild infiltration in group 5. Hematoxylin & Eosin X 400

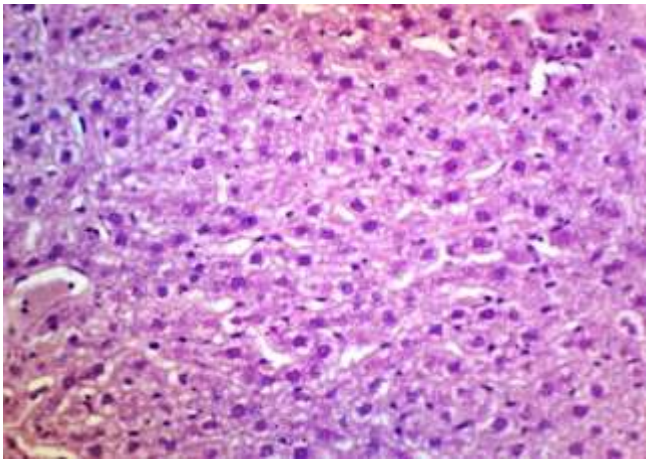

Fig 5: Hepatic section with mild to moderate fatty degeneration in group 7. Hematoxylin & Eosin X 400

The degenerations could be because of disturbed fabrication of poisonous oxygen species and reduced capability of defense mechanisms of antioxidants to hunt them resourcefully and stimulation of alteration of all major classes chemicals, change of structural and functional properties by cellular biomolecules. These attributes can be connected with the result of lipid profile, G6PD, Glycogen Synthase activity and organ oxidative stress factors of the present study [15, 21, 22].

The improved lesions in groups 5 and 7 and nearly zero alterations in group 8 could be due to reverse action of oxidative stress properties and immune protecting of *Withania somnifera*, *shilajit* and their combination [23, 24].

Conclusion

Biochemical and histopathological deviations in the experiment indicated hepatotoxicity at the molecular level. Alleviating actions of *Shilajit* only were fewer noteworthy than *Withania somnifera* only and amalgamation of thee herbs. Treating the rats with *Withania somnifera* and *Shilajit* independently and pooled eased the harmful effects on liver caused by hypothyroidism. Additive action of these herbs in alleviating the consequences of hypothyroidism is superior to administration of herbs seperately.

Acknowledgements

Authors thank DST (Inspire Fellowship) for funding the research and PV Narsimha Rao Veterinary University for providing the infrastructure for the research. I am also thankful to the subjects of my research for sacrificing their lives for a noble cause.

References

1. Murgod R, Soans G. Changes in electrolyte and lipid profile in hypothyroidism. Life Science Biology and Chemistry. 2012;2(3):185.
2. Shi Y, Ritchie JWA, Taylor PM. Complex regulation of t hyroid hormone action: multiple oportunities for pharmac ological intervention. Pharma and Therapeutics 2002;94:235-251.
3. Rijnberk A, Kooistra HS, Jan Mol A. Endocrine diseases in dogs and cats: Similarities and differences with endocrine diseases in humans. Growth Hormone and Research. 2003;13:S158-S164.
4. Zhou XL, Han Y, Mail WJ, Liu J, Wang H, Feng L, *et al*. Different doses and routes of administration of methimazole affect thyroid status in methimazole induced hypothyroidism in rats. The West Indian Mediterranean Journal, 2015, 65(1).
5. Farrokhi E, Hosseini M, Beheshti F, Vafaee F, Al-Reza Hadjzadeh M, Dastgheib SS. Brain Tissues Oxidative Damage as A Possible Mechanism of Deleterious Effects of Propylthiouracil-Induced Hypothyroidism on Learning and Memory in Neonatal and Juvenile Growth in Rats. Neurosciencience, 2014, 5(4).
6. Udupa KN, Singh RH. Clinical & Experimental Studies on Rasayana Drugs and Panchkarma Therapy (monograph), Central Council for Research in Ayurveda and Siddha, New Delhi, India; c1993.
7. Clayden EC. Practical section cutting and staining, 4th edition; c1962. p. 137-139.
8. Culling CFA. Hand book of histopathological technique; c1974. p. 421.
9. Danforth WH. Journal of Biologiy and Chemistry. 1965;240:588.
10. Noltmann EA, Gubler CJ, Kuby SA. Glucose-6-phosphate dehydrogenase. Journal of Biology and Chemistry. 1961;236:1225-1230.
11. Calvo R, Obregon MJ, Ruiz de Onha C, Escobar del Rey F, Morreale de Escobar G. Congenital Hypothyroidism, as studied in rats crucial role of maternal thyroxine but not of 3,5,3'-triiodothyronine in the protection of the fetal brain. Journal of Clinical Investigation. 1990;86:889-899.
12. Chakrabarthi S, Guria S, Samantha I, Das M. Thyroid dys function modulates glucoregulatory mechanism in rats. Indian Journal of Experimental Biolology. 2007;5:549-553.
13. Bhattacharya SK, Muruganandam AV. Adaptogenic activity of *Withania somnifera*: An experimental study using a rat model of chronic stress. Pharmacology Biochemistry and Behaviour. 2003;75:547-555.
14. Meena H, Pandey HK, Arya MC, Ahmed Z. Shilajit: A panacea for high-altitude problems. International Journal of Ayurvedic Research. 2010;1(1):37-40.
15. Lombardi A, Beneduce L, Moreno M, Diano S, Colantuoni V, Ursini MV, *et al*. 3,5 Diiodo L Thyronine regulates Glucose 6 Phosp-hate Dehydrogenase activity in the rat. Endocrinology, 2000, 141(5).
16. Haghighi B, Mesripour A, Mesripour M. The Effects of Hypo and Hyperthyroidism on Glucose 6-Phosphate Dehydrogenase Activities in Regions of Rat Brain. Iranian Biomedical Journal. 2005;9(2):63-66.
17. Takahashi T, Suzuki M. Effects of various thyroid states on the metabolism of adenine nucleotides and of glycogen in rat liver Febs Letters, 1971, 12(4).
18. Fowden AL, Mapstone J, Forhead AJ. Regulation of glucogenesis by thyroid hormones in fetal sheep during late gestation. Journal of Endocrinology. 2001;170:461-469.
19. Muller MJ, Acheson KJ, Jequier E, Burger AG. Effects of thyroid hormones on oxidative and nonoxidative glucose metabolism in mice. American Journal of Physiology. 1988;255:E146-E152.
20. Wilson E, Rajamanickam GV, Dubey GP. Review on shilajit used in traditional Indian medicine. Journal of Ethno pharmacology. 2011;136(1):1-9.
21. Udayakumar R, Sampath K, Mariashibu TS, Rajesh M, Anbazhagan, VR, Chang SK, *et al*. Hypoglycaemic and Hypolipidaemic Effects of *Withania somnifera* Root and Leaf Extracts on Alloxan Induced Diabetic Rats. International Journal of Molecular Sciences. 2009;10(5):2367–2382.
22. Hafez ME, Sahar IY, Rahman SAM. Parenchymatous Toxicity of Tramadol: Histopathological and Biochemical Study. Journal of Alcoholism and Drug Dependence. 2015;3(5):1-6
23. Ghosal S, Lal J, Jaiswal AK, Bhattacharya SK. Effects of Shilajit and its Active Constituents on Learning and Memory in Rats? Phytotherapy Research. 1993;7:29-34.
24. Bhattacharya A, Ghosal S, Bhattacharya SK. Antioxidant effect of *Withania somnifera* glycowithanolides in chronic foot shock stress induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. Journal off Ethno pharmacology. 2001;74:1-6.