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Cardiac toxicity in experimentally induced hypothyroid rats and alleviation by *Withania Somnifera* and Shilajit

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Abstract
96 female Sprague-drawly rats of 21 days old were divided into 8 groups with twelve rats in each group. Methimazole (0.02 %) was administered for induction of Hypthyroidism and its effects were ameliorated with Shilajt and *Withania somnifera* @100 mg /Kilo gram body wt. for 3 months. At the end of 3 months, lipid and thyroid profiles were analysed from serum. In each group 6 rats were sacrificed. Heart was collected for molecular studies and estimation of oxidative stress in homogenates. Rest, 48 female rats were mated with 24 euthyroid adult males and all the pregnant rats were administered with drugs as above. The rats after 21 days were then sacrificed and heart was collected for histopathological studies. The glutathione, serum T_3 and T_4 concentrations, in hypothyroid group were significantly (p<0.05) lower and total cholesterol, triglycerides, high density lipoproteins, thiobarbituric acid reacting substances and protein carbonyls and serum TSH was significantly (p<0.05) higher than other groups. In group II, section of heart in both parent stock and F_1 generation similar lesions with mild degree of variation were noted. The lesions observed were congestion, haemorrhages in endocardium and myocardium. Separation of muscle bundles and the pattern of arrangement varied. Edema, MNC infiltration and proliferation of fibroblasts were noted.

Keywords: Endocardium, hypothyroid, hormone, fibroblasts

Introduction
Thyroid hormones play a number of key roles in the regulation of cardiovascular performance. However, the possible contributions of thyroid dysregulation to the pathogenesis of heart failure remain insufficiently studied [1]. TThe classic serum biochemical abnormality is hypercholesterolemia, which occurs in ~80% of dogs with hypothyroidism. The thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), modulate several physiological processes in organisms and are critical in the growth, development, differentiation and maintenance of metabolic homoeostasis. The ability of thyroid hormones to induce anabolic and catabolic pathways, such as lipogenesis and lipolysis, contributes to thyroid hormone-induced increase in energy expenditure, besides several mechanisms involved in this effect, such as sympathetic activity modulation. Thyroid hormones stimulate lipid synthesis, mobilisation and degradation [2], The cardiovascular system is particularly sensitive to the effects of thyroid hormone. Increased or decreased concentrations of circulating thyroid hormone can produce clinically apparent changes in cardiovascular function. This article discusses the actions of thyroid hormone on the cardiovascular system and the clinical cardiac manifestations associated with thyroid dysfunction [3].
Herbal drugs have proven to be useful in number of diseases. Metabolic disorders are some disorders which progress at a slower rate but damage the whole functioning of the body. Conventional drugs available for these disorders cure symptomatically. Herbal drugs have the capacity to cure such metabolic disorders synergistically at different steps [4].
The objective of this study lies in usage of Withania and Shilajit for treating hypothyroidism and related disorders effectively.

Materials and Methods

Ninety six female Sprague-drawly rats of age 21 days, were bought from Mahaveeraa enterprises, Hyderabad. The study was conducted in the animal house of Veterinary Pharmacology & Toxicology Department, with appropriate dark and light cycle and temperature (25-27 °C). Polypropylene cages, mash feed and *ad libitum* water was arranged for rats. They were kept for a period of five days for acclimatisation. The study was carried out with acceptance of Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, Rajendranagar.

Experiment-1

The rats were sorted into eight groups with twelve rats in each group and the experiment was conducted for a period of 3 months with following drug schedule:

Group (n=6)	Treatment (3 months)
Group 1	Euthyroid
Group 2	Hypothyroidism with Methimazole @ 0.02%
Group 3	Hypothyroid + LT_4
Group 4	*Withania somnifera* root extract control @100 milli gram/ Kilogram body weight
Group 5	Hypothyroid +*Withania somnifera* root extract @100 milli gram/ Kilogram body weight
Group 6	*Shilajit* control @100 milli gram/ Kilogram body weight
Group 7	Hypothyroid + *Shilajit* @100 milli gram/ Kilogram body weight
Group 8	Hypothyroid +*Withania somnifera* root extract @ 100 milli gram/ Kilogram body weight. +*Shilajit* @ 100 milli gram/ Kilogram body weight

Blood samples were procured at monthly intervals from retro orbital puncture for estimation of lipid profile (CHOD / PAP, Peg / CHOD - PAP method for the determination of HDL cholesteroll in serum, GPO / PAPP method for the determination of triglycerides in serumm). At the end of third month, thyroidd Hormones (RIA, DiaSori kits, USA) were estimated from sera of blood. After treating for three months, from each group, six rats were sacrificed and heart, was collected for histopathological studies in suitable preservatives and further stored at -20 °C for analysis of GSH [5], TBARS [6] and protein carbonyls [7] in homogenates.

Rest of 48 female rats, six from every group of the above experiment were mated with 24 male adult euthyroid rats of age above 3 months (procured from Mahaveera Enterprises, Hyderabad) and all the 48 pregnant rats were treated with methimazole and other treatments as mentioned above up to 17th day of gestation. Pups were reared till 21 days. The rats were then sacrificed and heart was collected in suitable preservatives for histopathology studies.

Results

Thyroid profile

The serum Triiodothyronine and Thyroxine hormone concentration (nano gram / deci liter) of the group 2 was significantly (p<0. 05) lesser than group 1 while the values in groups 3, 4, 5, 6 and 8 were comparable to group 1. Triiodothyronine concentration in group 7 was significantly (p<0.05) lesser than group 1. The serum Thyroid Stimulating Hormone concentration (µ IU / ml) of the group 2 was significantly (p<0.05) increased than group 1 and was significantly lesser in groups 3 to 8 than group 2 (Table: 1).

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

Groups (n = 6)	T_3 (nano gram / deci liter) concentration	T_4 (micro gram /deci liter) concentration	TSH (µ IU / milli liter) concentration
1	115. 68±4. 46 ^{b}	4. 71±0. 14 ^{b}	0.05±0. 01 ^{a}
2	101. 15±4. 06 ^{a}	3. 01±0. 24 ^{a}	5.91±0. 45 ^{c}
3	110. 73±4. 84 ^{b}	4. 36±0. 18 ^{b}	0. 87±0.03 ^{b}
4	118. 03±4. 69 ^{b}	4. 79±0. 17 ^{b}	0. 03±0.007 ^{a}
5	111. 74±4. 17 ^{b}	4. 24±0. 17 ^{b}	1. 04±0.03 ^{b}
6	116. 62±5. 02 ^{b}	4. 69±0. 19 ^{b}	0. 04±0. 009 ^{a}
7	106. 78±4. 71 ^{a}	3. 45±0. 12 ^{a}	1.91±0.16^{b}
8	113. 19±4. 76 ^{b}	4. 31±0. 21 ^{b}	0.51±0.12^{b}

Means with different superscripts differ significantly (p<00.05) One way ANOVA (SPSS: 15)

The alterations in thyroid profile of group 2 rats might be due to methimazole's inhibition of the enzyme thyroperoxidase, facilitating iodine's addition to tyrosine residues on the hormone precursor thyroglobulin. Thus the reduction in T_3 and T_4 through negative feedback mechanism could have stimulated pituitary to release more TSH [8]. Immunomodulation effect of Withania has a stimulatory effect on a sluggish thyroid and increased serum T_4 concentration [9]. Adaptogenic activity of *Shilajit* improved thyroid profile by controlling thyroid gland function [10].

Serum lipid profile

In serum lipid profilee, the concentration of total cholesteroll (mg/dl), high density lipoprotein cholesteroll (milli gram / deci liter) and triglycerides of the normal control groupp 1, *Withania* control groupp and *Shilajitt* control groupp was significantly (p<0.05) less than those of group 2. The groupss 3, 5 and 8 showed significant (p<0.05) decrease of total cholesterol, comparedd to group 2 while in group 7 concentration was significantly (p<0.05) higherr thann groupp 1 and the treatmentt groups 3, 5, 7 and 8 showed significant (p<0.05) reduction in triglycerides (Table:2).

Table 2: Serum lipid profile in different groups of rats (Parent stock)

Groups (n=6)	Total cholesterol (milli gram / deci liter)	Triglycerides ((milli gram / deci liter)	HDL ((milli gram / deci liter)
1	137. 05±7. 19 ^{a}	36. 47±2. 03 ^{ab}	33. 75±3. 47 ^{a}
2	172. 71±7. 29 ^{c}	49. 63±3. 01 ^{c}	51. 06±4. 08 ^{c}
3	149. 13±6. 72 ^{b}	37. 14±2. 87 ^{b}	41. 06±3. 72 ^{b}

4	127. 84±4. 01 [a]	32. 26±1. 92 [a]	28. 07±2. 07 [a]
5	152. 03±6. 64 [b]	41. 74±2. 41 [b]	38. 27±3. 19 [b]
6	134. 04±6. 71 [a]	34. 15±2. 04 [a]	31. 73±3. 26 [a]
7	162. 17±5. 08 [c]	41. 94±2. 76 [b]	43. 91±4. 01 [b]
8	143. 72±5. 03 [b]	38. 82±2. 56 [b]	36. 20±3. 61 [ab]

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

The rise in low-density lipoprotein cholesterol and high-density lipoprotein cholesterol levels were due to decreased clearancee of chylomicron remnants in hypothyroidism. The rise in triglycerides and high-density lipoprotein cholesterol levels were due to decreased activity of lipoprotein lipase and facilitation of cholesterol transfer from these lipoproteins to HDL-C [11]. Withania due to it's hypotriglyceridaemic, hypo-cholesterolaemic and hypop-hospholipidaemic effects reduced total cholesterol and triglycerides levels in the present study [12]. *Shilajit* due to its direct action on lipid metabolic pathways, reduced total cholesterol and triglycerides markedly [13].

Oxidative stress parameters

TBARS and protein carbonyls (n moles/mg protein) revealed a significant (p<0.05) rise in group 2 as compared to group 1 while the groups 4 and 6 respectively showed a significant (p<0.05) decrease compared to group 2. The values in groups 3, 5, 7 and 8 are lower compared to group 2.

The concentration of GSH (n moles/mg protein) decreasedd significantly (p<0.05) in group 2 compared to group 1.The groups 3 to 8 showed a significant (p<0.05) raise in GSH concentrationn compared to group 2 (Table 3)

These alterations were due to hyperlipidemia in hypothyroidism that increased lipid peroxidation and hydrolyses of lipid peroxides. Also, increased aldose reductase activity reducess NADPH and there is raise in ROS [14]. DDecreased GSH levels in the present study might be due to reduced activity of G6PD that catalyses the NADPH and acts as a the important intracellular reductant in the cells which is needed for coupling of GSH [15]. Ashwagandha exhibits superoxide scavenging capacity and has ability to bleach the stable DPPHh, that indicated its antioxidant activity [16]. Antioxidant activity of shilajit might be due to presence of resonance stabilized soft-spin semiquinone free radicals, that produce free radical scavenging and antioxidant effects against paramagnetic NO, SO_3- and OH radicals [17].

Table 3: Oxidative stress parameters in heart of different groups of rats

Groups (n=6)	TBARS concentration (n moles of MDA released/milli gram protein)	GSH concentration (n moles/ milli gram protein)	Protein carbonyls concentration (n moles/ milli gram protein)
1	42.01±2.01 [a]	29.04±1.21 [bc]	1.06±0.09 [a]
2	49.78±2.31 [b]	20.13±1.06 [a]	3.26±0.12 [c]
3	45.74±2.87 [ab]	27.81±1.14 [b]	1.71±0.11 [a]
4	40.29±2.76 [a]	34.06±1.35 [c]	0.95±0.06 [a]
5	45.92±1.78 [ab]	26.76±1.57 [b]	1.91±0.13 [a]
6	41.66±2.51 [a]	31.07±1.26 [bc]	1.01±0.07 [a]
7	46.53±2.01[ab]	24.36±1.36 [b]	2.07±0.27 [b]
8	44.71±2.06 [ab]	28.71±1.64 [bc]	1.79±0.11 [a]

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

Histopathology (Parent stock)

No lesions of pathological significance were observed in group I, III, IV and VI both in parent and F1 generation.

In group II, section of heart showed separation of muscle bundles with moderate loss of branching pattern and striations and increase in interfibrillary space. Moderate congestion, proliferation of fibroblasts, mild to moderate mono nuclear cell infiltration, hemmorhages in endocardium, vacuolation and edema was noticed (Figs.1-2). In group V, mild congestion in the endocardium, loss of striations in muscle fibres and mild mono nuclear cell infiltration in comparison to group II (Fig.3). In group VII, sections showed discontinuity in muscle bundles with edema and mild congestion in the endocardium. (Fig.4). In group VIII, sections were similar to that of normal control groups (Fig.5).

F_1 Generation

Sections in group II showed marked congestion, haemorrhages in both endocardium and myocardium. In myocardium significant oedema, muscle bundles separation, MNC infiltration, degeneration, loss of striations and increased interfibrillar space was observed (Figs. 6-7). In group V, mild congestion in the endocardium, less separation of muscle bundles and loss of striations in muscle fibres was noted (Fig.8). In group VII mild changes like congestion, separation of muscle bundles were observed (Fig.9). In group VIII, sections were almost comparable to that of normal control groups (Fig.10).

The alterations noticed were due to disproportionate production of ROS that are correlated with the organ oxidative stress parameters [18].

The improved lesions in groups 5 and 7 and no lesions in group 8 are due to antistress, cardioprotective and immunomodulatory actions of Ashwagandha, *shilajit* and their combination [19].

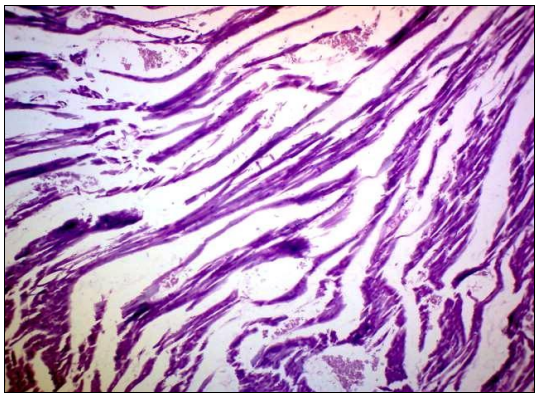

Fig 1: Heart section with moderate loss of branching pattern, separation of muscle bundles, striations and proliferation of fibroblasts Group II. H & E X 400

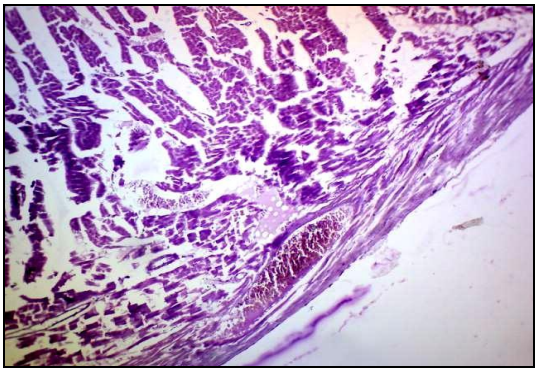

Fig 2: Heart section with mild haemorrhages in endocardium and oedema, moderate congestion Group II. H & E X100

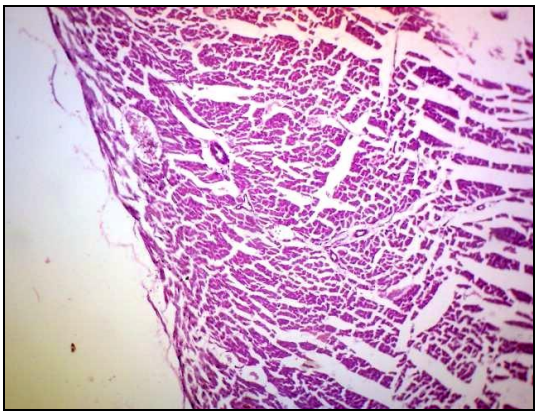

Fig 3: Heart section with less separation of muscle bundles and mild congestion in the endocardium Group V. H & E X100

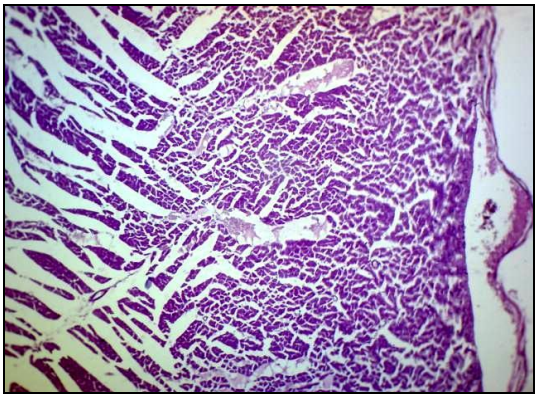

Fig 4: Heart section with discontinuity in muscle bundles with mild oedema and congestion in the endocardium Group VII. H & E X 100

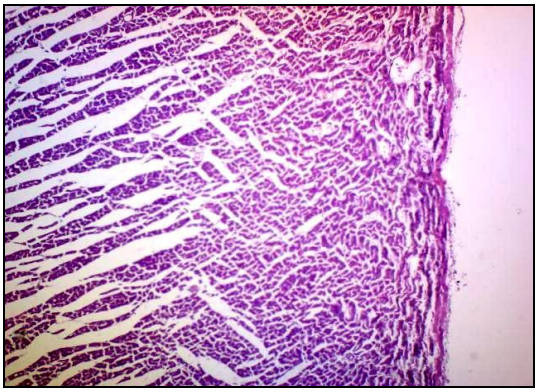

Fig 5: Heart section with no lesions of pathological significance Group VIII. H & E X 100

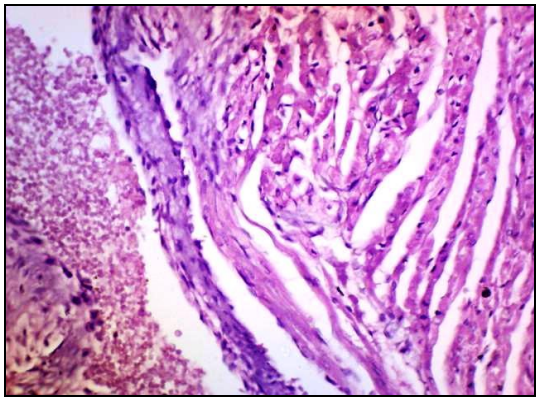

Fig 6: Heart section with severe congestion haemorrhages in both endocardium and myocardium Group II. H & E X 400

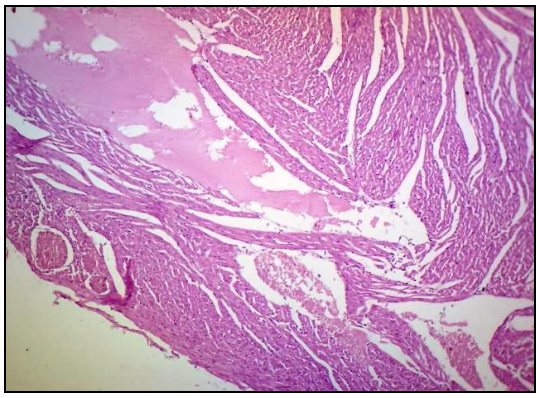

Fig 7: Section of heart showing severe oedema in myocardium, separation of muscle bundles, degeneration, MNC infiltration, loss of striations and increased interfibrillar space Group II. H & E X100

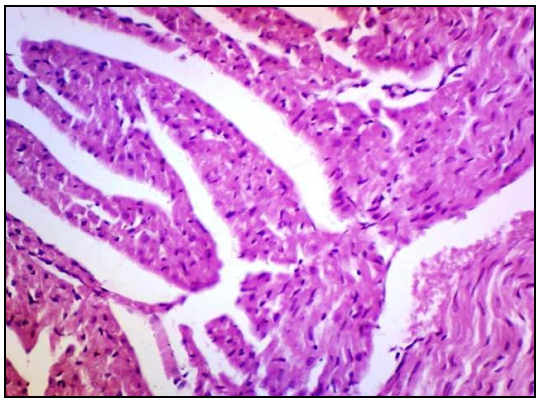

Fig 8: Section of heart showing mild congestion in the endocardium, loss of striations in muscle fibres and less separation of muscle bundles Group V. H & E X400

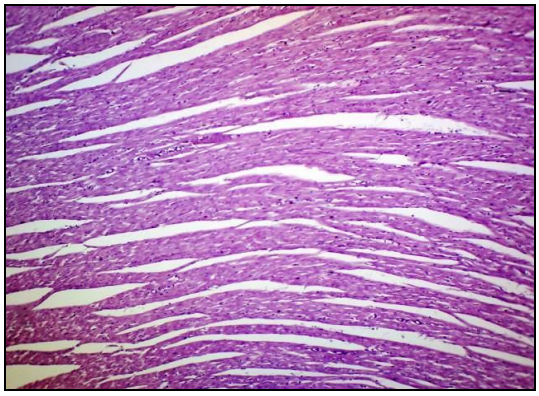

Fig 9: Heart section with mild changes like congestion and separation of muscle bundles Group VII. H & E X 100

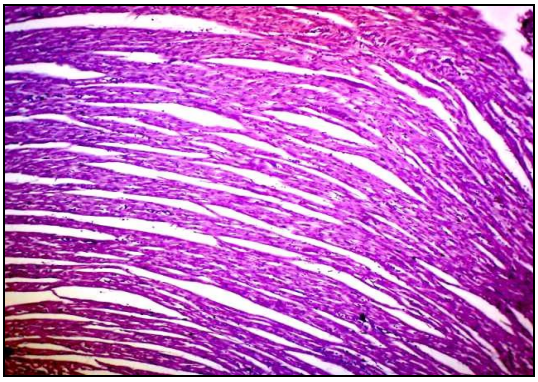

Fig 10 Heart section with lesions comparable to that of normal control groups Group VIII. H & E X100

Conclusion

The present study indicated that, biochemical and histopathological alterations in the study indicated cardiotoxicity at molecular level. The organ damage might be due to thyroid hormones deficiency that leads to free radicals release. Administration of *Withania somnifera* and *Shilajit* individually and combined alleviated the deleterious effects caused by hypothyroidism due to their antioxidant and endocrine stimulant properties. Synergistic action of herbs in alleviating the effects of hypothyroidism is superior to administration of herbs individually.

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