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Evaluation of 3, 5, 3'-triiodothyronine (T_3) Induced Changes in Clinical Chemistry, Histopathology and Cardiac Myosin Heavy Chain Gene Expression in Female Wistar Rat

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Abstract: Thyroid hormones specifically T_3, improves lipid and energy homeostasis along with significant effects on cardiac contractility through thyroid hormone receptor (TR) agonism. Such effects are amplified under hypothyroid animal models and have been routinely studied. To investigate similar effects in euthyroid rats, the present study was carried out using commercially available 3, 5, 3'-triiodothyronine (T_3) at the dose of 0.065, 0.26 and 0.39 mg/kg/day for 7 consecutive days. Clinical signs observed were hyperthermia, hyperesthesia, lethargy and increased salivation from 0.26mg/kg dose. Blood examination revealed marginally lower platelet count along with significant alterations in the levels of thyroxine, lipid profile, total protein, urea, creatinine, alkaline phosphatase, calcium and phosphorus. Histopathological changes were observed in heart, liver, kidney, thymus and thyroid tissues. Heart revealed multifocal myocardial degeneration with incidences of inflammatory cell infiltration from 0.26mg/kg dose. Quantitative estimation by the ratio of myosin heavy chain (MHC) α and β gene transcripts shown marked up-regulation in the cardiac tissues from the lowest dose of T_3. Altogether, molecular expression of myosin genes and histopathological changes in heart tissue including biochemical and hematological alteration can be used as a robust tool in assessing the cardiac risk factor of a compound targeting thyroid receptor during drug development.

Key words: Myosin heavy chain · 3 · 5 · 3'-triiodothyronine · Thyroid hormone receptor

INTRODUCTION

The thyroid gland is the source of three hormones, thyroxine (3, 5, 3', 5'-tetraiodothy-ronine, or T_4), triiodothyronine (3, 5, 3'-triiodothyronine, or T_3), and calcitonin. Thyroid hormones exert a wide variety of physiological actions through genomic and non genomic mechanisms and influence the metabolism of proteins, carbohydrates, and lipids cell morphology; membrane transport; ion homeostasis; oxygen consumption; heat production; and so on [1]. Relatively constant circulating concentrations of T_4 and T_3 are required for normal growth and development and the proper functioning of the neural, reproductive, cardiovascular, gastrointestinal, and hematopoietic systems [2].

The biologically active thyroid hormone T_3 affects cardiac contractility, heart rate (HR), diastolic function, and systemic vascular resistance through genomic and non-genomic mediated effects [3, 4]. Furthermore, congestive heart failure (CHF), which represents the final clinical event of the majority of cardiac diseases, can be associated with altered thyroid hormone economy.

Nuclear T_3 effects are mediated by the binding of T_3 to specific nuclear receptor proteins such as MHC (myosin heavy chain genes) [5]. There are two heavy myosin genes (α and β) whose products dimerize to form three different myosin chain isoenzymes: myosin V1 (α/α), myosin V2 (α/β), and myosin V3 (β/β). Thyroid hormone has been shown to regulate the expression of ventricular myosin isoenzymes by causing an accumulation of α-MHC mRNA and inhibiting expression of β-MHC mRNA [6, 7]. The mechanism of action of thyroid hormone on ventricular myosin isoenzymes has not been elucidated. Although the presence of nuclear binding sites for T_3 has been demonstrated in the heart, these may not be directly related to myosin since the

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hormone also regulates the Na'-K'- ATP_{ase} activity in this tissue.

The present study was undertaken to study the effect of T_3 on various organs system and to analyze variation in clinical chemistry. Alteration in the expression of myosin heavy chain in the cardiac tissues treated with T_3 were measured and co-related with the treatment dosage.

MATERIALS AND METHODS

Experimental Animals: Healthy young nulliparous female Wistar rats (130±15g) of 5-7 weeks, obtained from Animal Research Facility of Zydus Research Centre, Ahmedabad, and were housed in IVC (Individually Ventilated Cage) under standard laboratory conditions: temperature (25±3°C), relative humidity (30 to 70%), photoperiod (light and dark cycle of 12h each) with food and water provided *ad libitum*. This study was planned under IAEC (Institutional Animal Ethics Committee) approved protocol and all experiments involving animals, complies with the ethical standards of animal handling as per IAEC. All animals were acclimatised for five days prior to dosing.

Experimental Design: Three treatment groups comprising 5 female rats per group received a daily oral dose of 0.065, 0.26 and 0.39 mg/kg of T_3 for 7 consecutive days. An independent control group received only vehicle. Selected dose levels were 5X, 20X and 30X based on ED_{50} of cholesterol lowering effect of T_3 (20nmol/kg) estimated from in-house experimentation in cholesterol fed Sprague Dawley rats. The test item T_3 was formulated in 0.5% w/v DMSO, 1% w/v Hydroxyethylcellulose and 0.25% w/v Tween-80 in deionized water as suspension. A constant dose volume of 5-mL/kg body weight was maintained in this study.

Test Chemical: 3, 5, 3'- triiodothyronine (T_3) [Sigma-Aldrich, USA], Polyoxyethylene sorbitan monooleate (Tween 80) [Merck specialities private limited, Bombay, India], Dimethyl sulphoxide (DMSO) [Qualigens fine chemicals glaxosmithkline pharma limited, Bombay, India], TRIzol reagent [Invitrogen, Life Technologies, Carsbad, CA, USA].

Observations: Animals were observed for clinical signs and mortality daily. Moribund animals were isolated and/or euthanised and subjected to post-mortem examination, and organs were collected for histopathology. Body weights were recorded on day 1, 3 and 7. The feed leftover was measured on day 3 and 7.

Clinical Pathology: A detailed clinical pathology investigation was carried out in whole blood and serum samples for all groups at termination (Day 8) under overnight fasted conditions. Blood was collected from retro-orbital plexus.

Hematology: Whole blood was collected with an anticoagulant 2% di-potassium EDTA and analyzed by using Cell-Dyn 3700 hematology analyser (Abott laboratories, USA). Parameters evaluated were:

Total Leucocytes Count (TLC), Total Erythrocyte Count (TEC), Platelet Count (PLT), Haematocrit (HCT), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Differential Leukocyte Count (DLC).

Serum Biochemistry: Biochemical analysis was done using Daytona autoanalyser (Randox Laboratories, UK). Details of parameters evaluated and the methods used are as follows: Albumin (BCG), Total Bilirubin (TBil-DMSO Sulphuric acid), Calcium (Arsenazo), Total Cholesterol (TC-Cholesterol oxidase), High Density Lipoprotein (HDL-Direct clearance), Low Density Lipoprotein (LDL-Direct clearance), Creatinine (Alkaline picrate), Glucose (Glucose oxidase), Phosphorous (Phosphomolyb formation), Total Protein (TP-Biuret), Triglyceride (TG-Lip/GK colorimetric), Urea (Urease), Alanine aminotransferase (ALT-UV without p5p), Alkaline phosphatase (ALP-PNP AMP buffer), Aspartate aminotransferase (AST-UV) and Creatine Kinase (CK-UV: NAC activated).

Concentration of serum electrolytes such as Sodium, Potassium and Chloride were analyzed using Instalyte analyser (ERBA, Mumbai, India) by ion-selective method (ISE). Total T_3 (TT_3), Total thyroxine (TT_4) were estimated by ELISA method (CL, Biotech).

Gross and Histopathology: At terminal necropsy (Day 8), all survived animals were humanely euthanised by carbon dioxide asphyxiation and were subjected to complete gross examination. Heart, thyroid with parathyroid, brain, liver, kidneys, adrenals, ovaries, spleen and thymus were weighed for relative organ weight estimation.

The following principal tissues / organs were collected and fixed in 10% formal saline – heart, lungs, brain, pituitary, thyroid & parathyroid, liver, pancreas, spleen, thymus, lymph nodes (cervical and mesenteric), kidneys, adrenals, duodenum, jejunum, ileum, caecum, colon, rectum, skeletal muscle, ovaries, stomach and uterus with cervix and vagina.

Paraffin sections of the collected tissues were prepared and stained with hematoxylin -eosin for histopathological examination.

Gene Expression: Tissue samples from heart were dissected and snap-frozen in liquid nitrogen immediately at terminal necropsy and stored at –70±2°C till further analysis. Equal amount of frozen heart tissue and TRIzol reagent (1 ml /100 mg of tissue) was homogenized and total RNA (Ribonucleic acid) was isolated. Quantitation of total RNA was performed using Bio-photometer (Eppendorf, Germany) and the quality of RNA was ascertained by agarose gel electrophoresis. For gene expression of MHC-alpha, Myh6F (CACCCTGGAGGACCAGATTA)and Myh6R (TGGATCCTGATGAACTTCCC); and for MHC-beta, Myh7F (TGGCACCGTGGACTACAATA) and Myh7R (CTACAGGTGCATCAGCTCCA) specific RT-PCR (Real Time – Polymerase Chain Reaction) primers were used.

First strand cDNA (Complementary deoxyribonucleic acid) synthesis was achieved with 2µg of total RNA in a final volume of 20µl. About 2µl from this reaction cocktail was used directly to conduct PCR amplification in presence of SYBR-Green following real time RT-PCR using ABI-7300 system (Applied Biosystem, Singapore). Ribosomal Acidic Protein was used as internal control in this study. The level of expression observed in vehicle treated control sample was used as reference to determine the folds of gene expression in treatment groups.

Statistical Analysis: Quantitative data was analyzed by one-way ANOVA (Analysis of Variance) when the variances were considered homogeneous according to Bartlett test. Alternatively, if the variances are considered to be heterogeneous, a Non Parametric Kruskal-Wallis test was employed. Treated groups were compared with control group using Dunnett's test for significant ANOVA results and by the Dunn's test for significant Kruskal-Wallis results.

RESULTS

Clinical Signs and Mortality: No evidence of clinical signs was noticed in rat treated with 0.065mg/kg of T_3. Animals treated with 0.26 and 0.39mg/kg revealed hyperthermia, hyperaesthesia, lethargy and increased salivation from day 6 of treatment period. Two rats were subjected to moribund sacrifice on day 7 after treatment at 0.39mg/kg showing overt toxicity signs such as tremor and extreme lethargic condition.

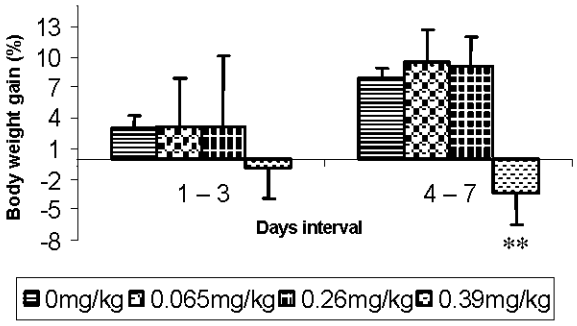

Fig. 1: Effect of T_3 on body weight gain (%)

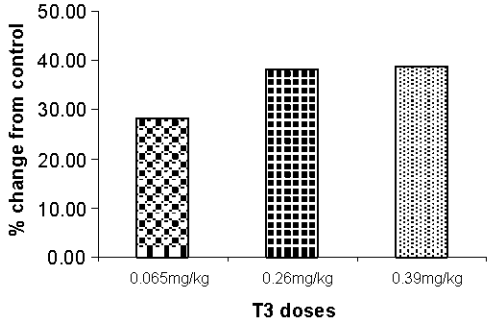

Fig. 2: Effect of T_3 on feed consumption

At the end of treatment period, during detailed veterinary examination the animals treated with 0.39mg/kg showing leanness and upon palpation around thoracic region clear buzzing sound from heart was evident in three rats.

Body Weight: No treatment related change in body weight and body weight gain was seen in animals treated with 0.065 and 0.26mg/kg. Reduction in body weight gain during day 1-3 interval and 4 -7 ($P<0.01$) was seen in rat treated with 0.39mg/kg in comparison with control (Fig. 1).

Feed Consumption: Dose dependent increase in feed consumption was found in rat treated with T_3 by 28.2%, 38.3% and 38.6% at 0.065, 0.26 and 0.39 mg/kg dose levels respectively in comparison with control (Fig. 2).

Hematology: Platelet count was low at all dose levels ($P<0.05$ at 0.065mg/kg, $P<0.01$ at 0.26 and 0.39mg/kg). Marginal reduction in monocyte and basophil count was found at all doses. Increased hemoglobin and haematocrit was noticed at 0.39mg/kg in comparison from control (Table 1).

Serum Biochemistry: Lipid profile revealed low level of triglyceride, total cholesterol ($P<0.05$ at 0.39mg/kg), low density lipoprotein ($P<0.01$ at 0.26 and 0.39mg/kg), which was more evident at higher doses.

Table 1: Summary of Hematology

Parameter	I / 0mg/kg	II / 0.065mg/kg	III / 0.26mg/kg	IV / 0.39mg/kg
TLC (10^3/µL)	4.37±0.77	5.40±1.39	3.74±1.24	4.28±1.29
TEC (10^6/µL)	6.88±0.16	6.59±0.72	7.07±0.23	7.57±0.58
Hb (g/dL)	13.72±0.40	13.18±1.29	13.68±0.53	15.05±0.64
HCT (%)	43.10±1.19	42.16±4.11	43.55±1.91	47.85±2.47
MCV (fL)	62.74±1.97	64.06±1.23	61.60±1.60	63.30±1.56
MCH (pg)	19.98±0.79	20.06±0.43	19.40±0.60	19.90±0.71
MCHC (g/dL)	31.88±0.41	31.28±0.16	31.45±0.35	31.45±0.35
PLT (10^3/µL)	888.20±57.82	762.80 *±72.05	652.50 **±30.29	669.00 **±134.35
Neutrophil (%)	21.04±4.03	14.18±6.44	18.13±3.38	19.65±5.44
Lymphocyte (%)	70.00±5.36	81.36±6.99	76.18±5.69	78.35±6.72
Monocyte (%)	5.97±1.55	2.60 **±0.75	3.81±1.87	0.80 **±0.81
Eosinophil (%)	1.33±0.71	0.74±0.34	0.90±0.90	0.68±0.64
Basophil (%)	1.63±0.49	1.11±0.26	1.00±0.45	0.52 **±0.21

Key: Values are Mean±SD, n = 5 for Group I, II, & III, n = 3 for Group IV (2 animals were moribund sacrificed on day 7).

* = Significant from control group at 5% level (p<0.05), ** = Significant from control group at 1% level (p<0.01),

SD = Standard Deviation

Table 2: Summary Of Biochemistry

Parameter	I / 0mg/kg	II / 0.065mg/kg	III / 0.26mg/kg	IV / 0.39mg/kg
GLU (mg/dL)	81.16±6.97	111.92±32.20	82.60±7.14	73.10±0.90
TG (mg/dL)	49.04±16.69	30.89±15.87	36.42±15.51	29.45±6.25
TC (mg/dL)	60.26±17.16	45.84±9.12	44.13±7.45	36.85*±1.45
HDL-C (mg/dL)	19.66±3.68	18.94±3.10	23.10±9.05	16.50±1.50
LDL-C (mg/dL)	3.84±0.42	2.84±0.84	2.43 **±0.72	1.80**±0.10
ALP (U/L)	112.74±13.95	382.72 *±127.34	484.28 **±206.8	422.90*±81.70
AST (U/L)	208.16±22.76	200.74±32.68	247.48±58.64	210.6±8.36
ALT (U/L)	27.80±3.49	30.56±8.15	38.90±9.73	32.90±3.30
ALB (g/dL)	3.72±0.15	3.44±0.11	3.13 **±0.31	3.30*±0.10
TP (g/dL)	6.16±0.36	5.40**±0.12	4.73 **±0.50	5.05 **±0.15
Globulin (g/dL)	2.44±0.25	1.96 **±0.05	1.60 **±0.22	1.75 **±0.07
UREA (mg/dL)	52.16±12.00	43.08±9.03	49.65±13.08	37.45±4.45
TBIL (mg/dL)	0.14±0.03	0.11±0.11	0.18±0.11	0.19±0.03
CREA (mg/dL)	0.64±0.04	0.58±0.06	0.53*±0.08	0.45**±0.03
PHOS (mg/dL)	8.08±0.86	9.22±2.22	9.93±1.89	10.00±0.70
Ca (mg/dL)	8.84±0.21	10.34 **±0.34	9.58 **±0.29	8.20*±0.10
CK (U/L)	2379.80±693.45	1358.62 *±208.96	1298.57 *±565.73	979.80*±182.86
Na^+ (mmol/L)	142.96±0.73	142.56±1.47	140.18 **±1.28	141.90±0.28
K^+ (mmol/L)	3.42±0.32	4.06±0.41	3.42±0.57	3.26±0.08
Cl^- (mmol/L)	102.44±1.01	103.02±0.66	101.65±2.10	102.50±0.71
TT_3 (ng/ml)	0.355±0.167	0.787±0.418	1.570±0.958	3.335 **±1.401
TT_4 (mcg/dL)	2.756±0.914	0.558 **±0.353	0.245 **±0.138	0.553 **±0.038

Key: Values are Mean±SD, n = 5 for Group I, II, & III, n = 3 for Group IV (2 animals were moribund sacrificed on day 7).

* = Significant from control group at 5% level (p<0.05), ** = Significant from control group at 1% level (p<0.01),

SD = Standard Deviation

Total protein (P<0.01 at all dose), globulin (P<0.01 at all dose) and albumin level (P<0.01 at 0.26mg/kg and P< 0.05 at 0.39mg/kg) were found low in this study. Low levels of urea and creatinine(P<0.05 at 0.26 and P<0.01 at 0.39mg/kg). Elevation in alkaline phosphatase (P<0.05 at 0.065 & 0.39mg/kg, P< 0.01 at 0.26mg/kg), phosphorus and total T_3 (P<0.01 at 0.39mg/kg) at all dose levels. Reduction in total thyroxine (P<0.01 at all dose), creatinine kinase (P<0.01 at all dose), calcium was elevated at 0.065 and 0.26 mg/kg (P<0.01) and reduced at 0.39mg/kg (P<0.05), and sodium (P<0.01 at 0.26mg/kg) were observed (Fig. 3, 4, 5 & 6, Table 2).

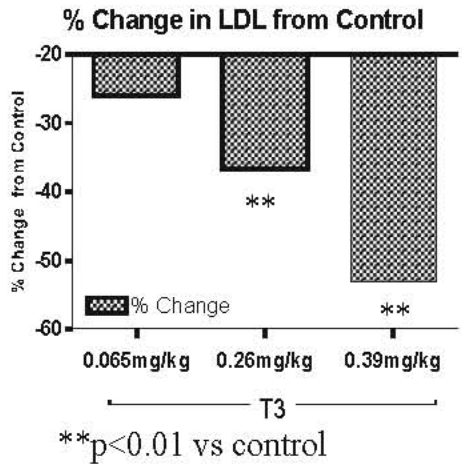

Fig. 3: Effect of T3 on LDL(mg/dL)

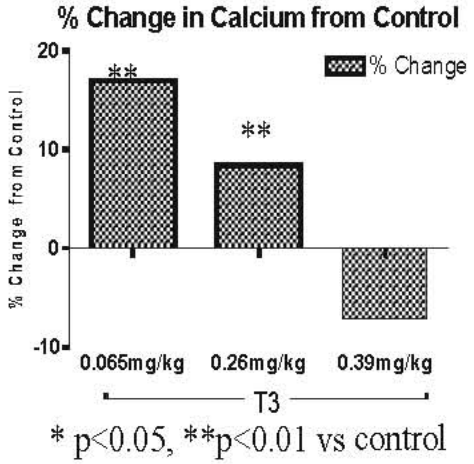

Fig. 6: Effect of T3 on calcium (mg/dL)

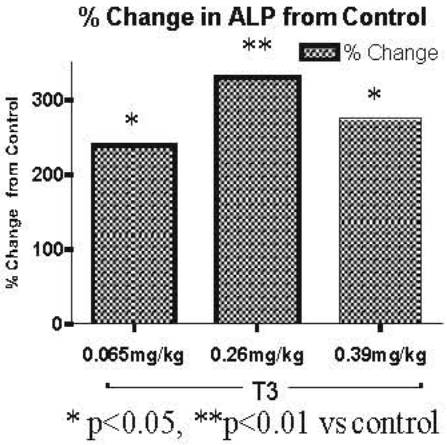

Fig. 4: Effect of T3 on ALP(U/L)

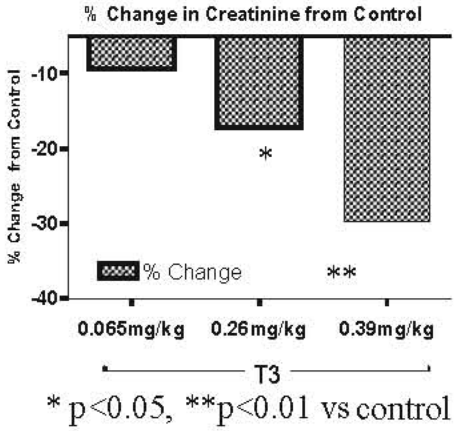

Fig. 5: Effect of T3 on Creatinine (mg/dL)

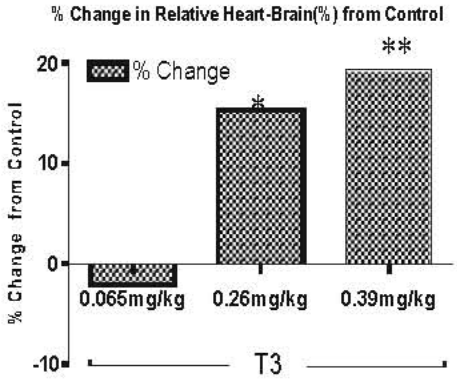

Fig. 7:

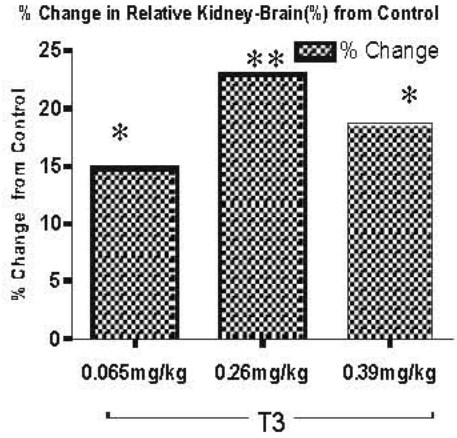

Fig. 8:

Organ Weight

Absolute Organ Weight: Higher weight of heart (P<0.01) at 0.39mg/kg and lower thymus weight (P<0.01) at all doses of T_3. Kidney and adrenal showed higher weights in comparison with control.

Relative Organ Brain Weight (%): Higher weight of heart (P<0.05 at 0.26mg/kg and P<0.01 at 0.39mg/kg), kidney (P<0.05 at 0.065 & 0.39mg/kg and P<0.01 at 0.26mg/kg) adrenals (P<0.05 at 0.26 & 0.39mg/kg and lower weight of thymus (P<0.01 at all dose) were noticed in relative organ weight percent (Fig. 7, 8 & 9, Table 3).

Table 3: Summary Of Organ To Brain Weight Percentage (%)

Organs	I / 0mg/kg	II / 0.065mg/kg	III / 0.26mg/kg	IV / 0.39mg/kg
Heart	34.024±2.939	33.315±2.018	39.227 *±2.139	40.607 **±2.228
Liver	307.470±35.405	349.296±42.342	371.179±41.590	368.531±51.445
Kidneys	73.041±4.960	83.867 *±3.916	89.850 **±6.920	86.695 *±8.710
Spleen	22.393±4.108	19.641±3.784	20.352±7.829	18.093±3.529
Adrenals	3.267±0.325	4.065±0.365	4.227 *±0.208	4.144 *±0.854
Ovaries	5.356±0.850	5.312±0.980	4.801±1.481	6.544±1.124
Thyroid+ Parathyroid	0.633±0.071	0.712±0.071	0.736±0.143	0.634±0.053
Thymus	26.356±2.201	20.061 **±2.672	17.692 **±2.390	17.785 **±3.525

Key: Values are Mean±SD (n=5),

* = Significant from control group at 5% level (p<0.05), ** = Significant from control group at 1% level (p<0.01), SD = Standard Deviation,

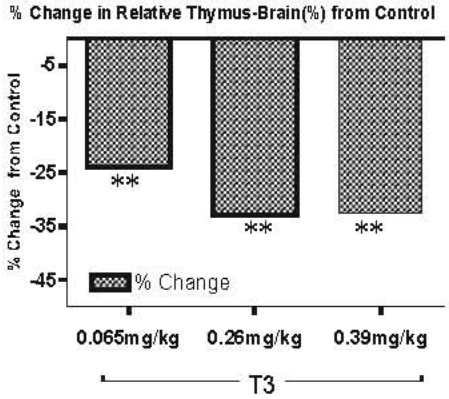

Fig. 9:

Histopathological finding

Thymus: Mild single cell necrosis of thymocyte in all animals treated with 0.26mg/kg and in three animals at 0.39mg/kg. In addition, three animal shown mild depletion of lymphocyte at 0.39mg/kg (Fig.15).

Heart: Focal minimal myocardial degeneration in two animals at 0.26 mg/kg and multifocal mild myocardial segmental degeneration & mild inflammatory cell infiltration in all animals treated with 0.39mg/kg (Fig.12).

Thyroid: Flattening of cuboidal epithelium and excessive accumulation of colloid in follicular lumen was observed in three animals treated with 0.39mg/kg (Fig.10&11).

Liver: Mild multifocal sinusoidal congestion, slight nuclear enlargement with condensation of chromatin and inconspicuous nucleoli and single cell necrosis were found in two and four animal treated with 0.26 and 0.39mg/kg respectively (Fig. 13&14).

Kidney: Mild multifocal capillary congestion in one animal at 5X. Mild multifocal renal tubular dilatation in two and three animal treated with 0.26 and 0.39mg/kg respectively (Fig.16).

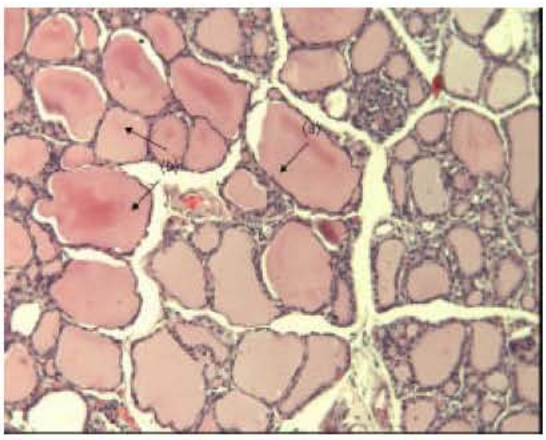

Fig. 10:

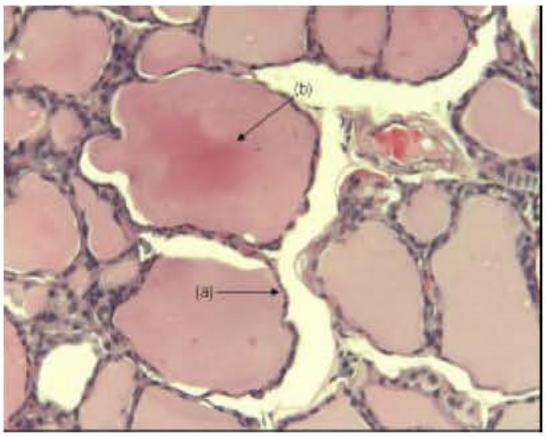

Fig. 11:

Flattening of lining epithelium (a), thyroid follicles distended with excessive accumulation of colloid material (b); H & E. 20x (1) and 40x (2).

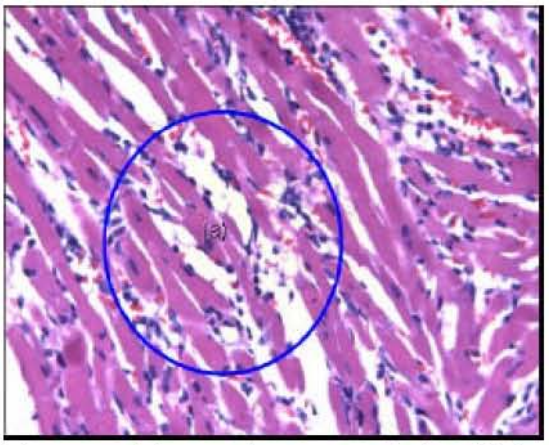

Fig. 12: Multifocal mild myocardial segmental degeneration & accumulation of inflammatory cells in the myofibres (a); H & E. 40x

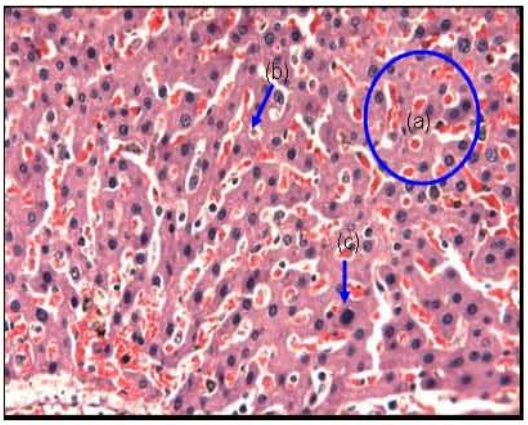

Fig. 13:

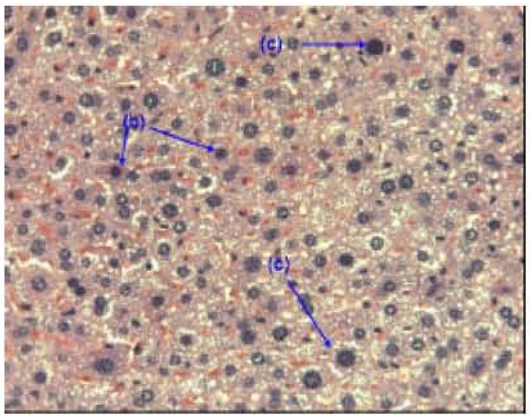

Fig. 14

Sinusoidal dilatation in the liver lobule (a), multifocal single cell necrosis (b), Nuclear enlargement at places with condensation of chromatin and inconspicuous nucleoli (c); H & E. 40x

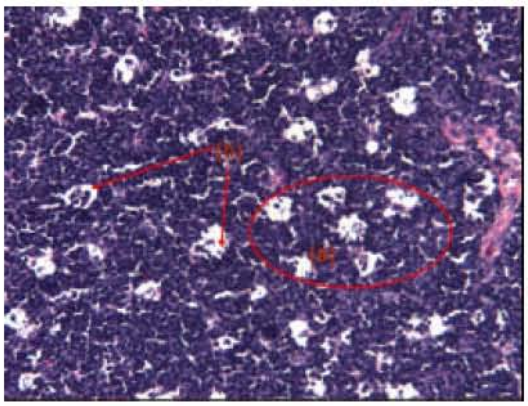

Fig. 15: Multifocal mild single cell necrosis of thymocytes (a),Cellular debris being engulfed by macrophages; H& E. 40x

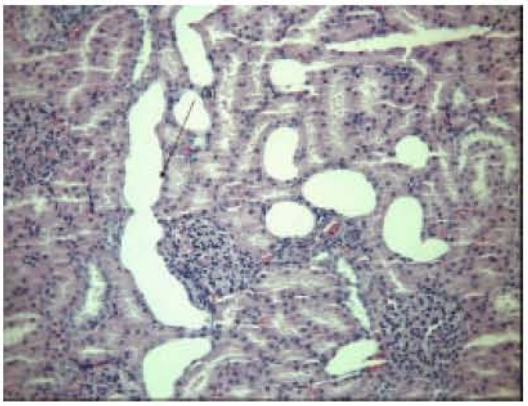

Fig. 16: Dilatation of renal tubules (a); H&E. 40x

Gene Expression: By real time RT-PCR analysis, gene expression studies of heart tissue samples shown a relative decline in both MHC-alpha [~35-42%] and beta [~94-96%] expression compared to the control samples and cumulative ratio of the level of MHC-alpha/beta transcripts was determined to be 10-18 after normalization

Table 4: Relative Expression Of MHC- α And MHC- β In The Heart Tissues

Treatment	MHC-α (% change from control)	MHC-β (% change from control)	Ratio of relative fold expression of MHC-α / β
Vehicle control	1.01±0.11	1.026±0.160	0.98
T_3 - 0.065mg/kg	0.65±0.06 (35.5%)	0.061±0.004 (94.05%)	17.45
T_3 - 0.26mg/kg	0.63±0.10 (37.62%)	0.032±0.027 (96.88%)	10.29
T_3 - 0.39mg/kg	0.58±0.07 (42.57%)	0.037±0.004 (96.30%)	18.23

Key: Values are Mean±SEM,

Values represent the relative change in expression of genes compare to vehicle treated control

(ΔΔCt = 0), SEM = Standard Error of Mean

with common internal control. The decline in MHC- beta is much higher as compared to MHC-alpha, which leads to markedly high MHC- alpha and beta ratio (Table 4).

DISCUSSION

Treatment related clinical signs such as hyperthermia, hyperesthesia, lethargy and increased salivation observed from 0.26 mg/kg. These effects were well reported with T_3 administration in various animal studies and may be result of increased basal metabolic rate which leads to enhancement of cellular metabolism [9-11].

During detailed veterinary examination, animals were found lean and upon palpation around thoracic region clear buzzing sound from heart was evident at the end of treatment period at 0.39 mg/kg. These alterations in heart may be due to positive inotropic and chronotropic changes on cardiac function and/or by increasing the sensitivity of myocardial cell to catecholamines and /or by indirect effect due to haemodynamic changes by T_3 [3, 4, 6, 12].

Decline in bodyweight gain (43.1%) in young rats at 0.39 mg/kg, may be an effect of increased breakdown of macronutrient via glycogenolysis and usually associated with mobilization and degradation of protein and lipids in tissues are well reported [13, 14].

Feed intake of T_3 treated groups was comparatively higher than control group by 28.2%, 38.3% and 38.6% at 0.065, 0.26 and 0.39 mg/kg dose levels respectively. The effects may be associated to substantiate the excess demand of energy towards increase in basal metabolic rate [15]. These hyperphagic effects were suggestive of augmentation of AMP–activated kinase activity in hypothalamus [16].

Hematological estimations revealed marginally higher haematocrit and hemoglobin levels at 0.39mg/kg. This finding is corroborative with the T_3 mediated clinical signs such excessive salivation and hyperthermia suggestive of decline in plasma volume and dehydrated condition of animals.

Platelets were marginally low at all dose levels by 14.1%, 26.5% and 24.7% at 0.065, 0.26 and 0.39mg/kg respectively [Table 1]. An undesired effect of T_3 on life span of platelet has been reported [17, 18].

Effects of T_3 on metabolic profile were evident through determination of various serum biochemical analytes in this study [Table 2]. Level of triglyceride was low at 0.26 and 0.39 mg/kg by 13.5% and 39.9% respectively. Marginal reduction of total cholesterol was noticed in this study. Similarly levels of LDL-cholesterol declined by 26%, 36.7% and 53.1% at 0.065, 0.26 and 0.39 mg/kg respectively from control group (Fig. 3). These effects may be due to rapid increase in hepatic LDL receptor mRNA to promote the LDL clearance process [19, 20]. These alterations by T_3 indicate the well known physiological actions of T_3 on lipid metabolism [15, 21, 22].

Total protein levels of T_3 treated rats were low with relative decline in albumin and globulin levels by 12.3%, 23.2% and 18% at 0.065, 0.26 and 0.39 mg/kg respectively. These effects may be due to marked protein catabolism resulting from excessive vigor followed by wasting of skeletal muscles under high T_3 concentration [23]. Moreover these effects conformity with low levels of serum urea and creatinine (Fig. 5) in our study [24, 25]. Levels of serum alkaline phosphatase were significantly high at all dose levels (Fig. 4), indicative of disturbances of bone metabolism and liver function of the test system [26, 27]. The levels of T_3 hormonal levels were high across the dose levels ensuring the absorption of exogenous T_3 in treated rats post administration. These alterations in T_3 levels had profound negative feed back suppressive effects on endogenous thyroxine by 78.7%, 91.1% and 79.9% at 0.065, 0.26 and 0.39 mg/kg. This is in conformity with our early histological changes in thyroid gland at 0.39 mg/kg through few incidences of flattening of cuboidal epithelium and excessive accumulation of colloid in follicular lumen (Fig. 10 & 11).

Organ weight estimations revealed a higher absolute and relative heart weights by 15.3%, 19.3% at 0.26 and 0.39 mg/kg respectively. Similar changes were evident in weights of kidneys [14.8%, 23% &18.7%] and adrenals

[24.4%, 29.4% & 26.8%] at 0.065, 0.26 and 0.39 mg/kg respectively. Thymus weight was low by 23.9%, 32.9% and 32.5% across the dose levels [Fig. 7, 8 & 9, Table 3].

Microscopic examination of heart tissue revealed multifocal mild myocardial degenerative changes with few incidences of mild inflammatory cell infiltration from 0.26mg/kg (Fig. 12). This is an expected exaggerated pharmacodynamic action of T3 on cardiomyocytes by altering the target genes and also through indirect hemodynamic effects. Liver showed (Fig. 13 & 14) mild sinusoidal dilatation, nuclear enlargement of hepatocytes with chromatin clumping & inconspicuous nucleoli and single cell necrosis from 0.26mg/kg [28]. Renal tubular dilatation was noticed at higher doses in this study (Fig. 16). These effects in liver and kideys are suggestive of multiple effects of T3 through TRβ mediated receptors in liver and kidneys. As a result of stress induced by excessive T3 adminstration exogenously in the test system, mild multifocal single cell necrosis and depletion of thymocytes was noticed in thynus gland at 0.26mg/kg and 0.39mg/kg (Fig. 15).

By real time RT-PCR analysis, gene expression studies of heart tissue samples shown a relative decline in both MHC-alpha [~35-42%] and beta [~94-96%] expression compared to the control samples and cumulative ratio of the level of MHC-alpha/beta transcripts was determined to be 10-18 after normalization with common internal control [Table 4].

Function of heart under the influence of T_3 is mainly regulated through TR-alpha receptors [7]. Decline in MHC-beta expression compared to MHC-alpha in this study confirms the perturbation of dynamics of formation of myosin forms. All these effects eventually results in abnormal contractility of heart as evident from clinical signs and changes in heart weight in this study. It has been reported that up regulation of MHC-alpha is an important factor in increasing the heart weight and other morphological and physiological changes in heart after T_3 administration in animal studies [6, 29, 30].

CONCLUSION

In conclusion, the influence of surplus T_3 in disturbing the general homeostatic mechanism was clearly evident in this study through manifestations of clinical signs, gravimetric changes, variations in biochemical analytes and hormones. Histopathological alterations noticed in heart, liver, kidneys, thymus and thyroid of this study clearly demonstrate the exaggerated physiological action on multiple targets after exogenous administration of T_3 hormone. Changes in MHC-alpha and beta expression even at the lowest dose studied give us an indication for the inclusion of this gene biomarker as a rapid tool for the early safety assessment of thyromimetics and drugs targeting cardiovascular system in drug discovery research.

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