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The Protective Effect of Garcinia on Cardiovascular System of Propylthiouracil Induced Hypothyroid Rat Model

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Abstract:
Background. *Hypothyroidism is considered as one of the most common of all endocrine diseases. The risk of cardiovascular disease is markedly increased with hypothyroidism. Garcinia cambojia (GC/HCA) is one of the commonly used natural products for losing weight and correcting lipid profile.*
Goals. *To investigate the efficacy and safety of GC/HCA in minimizing risk factors and histopathological changes of cardiovascular system related to hypothyroidism.*
Methods. *24 young adult male rats divided into 4 groups that received treatment orally daily for 10 weeks. (C): treated with D.W.; (HCA): treated with GC/HCA 500 mg/kg; (PTU): treated with PTU 5 mg/kg; (PTU+HCA): treated with PTU 5mg/kg + GC/HCA 500 mg/kg.*
Results*. Our results showed that GC/HCA was effective in reducing body weight (TBW), Triglycerides (TGs), total cholesterol (TC) and malondialdehyde (MDA) and increasing total antioxidants (TAO) induced by PTU administration. In addition, it caused marked improvement in cardiovascular histopathology associated with hypothyroidism. GC/HCA administration in healthy rats significantly decreased TBW, TC and LDL-c, however, it failed to cause significant decrease in C reactive protein (CRP) levels.*
Conclusion*. We suggested that the use of GC/HCA has promising prophylactic effect against disturbed lipid profile, oxidative stress and cardiovascular histopathology associated with hypothyroidism. Moreover, the results of this study showed the safety and efficiency of GC/HCA in reducing body weight in both hypothyroid rats and healthy controls.*
List of abbreviations:
Garcinia cambogia/ Hydroxycitric acid (GC/HCA), Total cholesterol (TC), Triglycerides (TG), Low density lipoprotein (LDL-c), High density lipoprotein (HDL-c), C-reactive protein (CRP), Control (c), Hydroxycitric acid (HCA), Propylthiouracil (PTU), Heart weight/body weight (HW/BW), Total body weight (TBW), Malondialdehyde (MDA), Total antioxidant capacity (TAO).
Key words: *Hypothyroidism, Propylthiouracil (PTU), Garcinia cambogia (GC), Cardiovascular system (CVS).*

I. Introduction

Thyroid gland is a small gland that plays pivotal role in the overall body function and homeostasis. Hypothyroidism is commonly associated with increased risk of cardiovascular disease mostly due to dyslipidemia, oxidative stress and increased levels of C- reactive protein (CRP).[1] Hypothyroidism increased the risk of atherosclerosis and coronary heart disease through endothelial dysfunction.[2] Adjustment of circulating thyroid hormone levels may reverse the symptoms in adults.[3] However, thyroid replacement therapy is not recommended by recent guidelines of major endocrine societies partly because of T_3:T_4 ratios, content adjustment and risk of over-replacement.[4] Therefore, development of safe dietary supplements or new modes of treatment for hypothyroidism may be of utmost importance.

Garcinia cambogia (GC) is a subtropical pumpkin shaped fruit from the native of Indonesia that comes from family Clusiaceae.[5] The main acid excreted from the dried fruit rinds of garcinia cambogia is hydroxycitric acid (HCA).[6] Human and rats previous studies proved that HCA had beneficial effect on improving lipid profile[7], decreasing CRP and reducing oxidative stress.[8] Therefore, HCA may play a protective role against atherosclerosis.[9] The main goal of this study is testing the effect of GC given orally for 10 weeks in minimizing cardiovascular complication in PTU-induced hypothyroid rat models. We investigated the effect of GC administration on TBW, HW/BW ratio, lipid profile, CRP, oxidative stress as well as changes in cardiovascular histopathology.

II. Materials and Methods

II.1 Drugs and chemicals

Propylthiouracil (PTU) was purchased from AMOUN pharmaceutical co. Batch No. 120113. Each tablet (50 mg) PTU was gridded and dissolved in 50 ml distilled water (D.W.) and given daily by gavage (5 mg/kg) for 10 weeks.[10] GC/HCA (Garcinia Cambogia Fruit Extract containing- NLT 50 % w/w of Hydroxycitric acid; 1,2-Dihydroxy-1, 2, 3-propanetricarboxylic acid and Chromium (as picolinate) 281.569 mcg equivalent to chromium 35 mcg). One capsule (500 mg) was dissolved in 10 ml D.W. (EVA Pharma for Pharmaceuticals & Medical Appliances Batch No. 405928) given at 500 mg/kg daily by gavage for 10 weeks.[11]

II.2 Animal preparation and experimental approach

Young adult male Wister albino rats (n = 24), weighed (150-200gm) were used in this study. The rats were housed in wire-meshed cages (30 x 25 x 13 cm) at 25˚C with normal humidity and 12 hours light-dark cycle. Rats left for accommodation and allowed free access for food and water one week before experimentation. This experiment was carried out according to the Local Ethical Committee Guidelines and Protocol of the Faculty of Medicine, Assiut University, Assuit, Egypt. Rats were divided into four groups; six animals each and administered treatment daily by gavage for 10 weeks; Control (C): received distilled water; Garcinia (HCA): received garcinia 500mg/kg. Propylthiouracil (PTU): received PTU 5mg/kg. Propylthiouracil and garcinia group (PTU+HCA): received both PTU (5 mg/kg) and garcinia (500 mg/kg).

II.3 Blood sampling and surgical techniques

Blood samples were collected from retro-orbital plexus of veins after 12 hours fasting. Serum was separated and stored at -20°C for further analysis. Rats were anesthetized by inhalation of diethyl ether (1.5 ml) and abdominal incision was immediately done. Hearts were removed, washed with ice-cold physiologic saline, dried using filter paper, and weighed. Heart and aorta samples were taken for histopathological evaluation.

II.4 Biochemical parameters

Parameters of thyroid function; FT_3, FT_4 and TSH plasma levels were estimated by using FT_3 ELISA kit (Cat. No. ENC-ERKR7013), FT_4 ELIZA kit (Cat. No. ENC-ERKR7014) and TSH ELISA kit (Cat. No. ENC-ERKP6015) supplied by Endocrine technologies Inc., 35325 Fircrest Street, Newark, CA 94560-1003, USA. TC was determined by enzymatic colorimetric method, by kits purchased from Centronic Gmbh Am klienfeld 11, 85456 Wartenberg/Germany (Ref: CF03000050). TG was determined by enzymatic colorimetric method GPO-POD kits for quantitative determination of triglycerides purchased from Spinreact, Ctra Santa Coloma, 7 E- 17176 Sant Esteve Spain (Ref: 1001310). HDL-c was determined by Stanbio HDL Cholesterol Procedure No. 0599 purchased from Stanbio laboratory, An EKT Diagnostic Company, 1261 North Main Street, Boerne, Texas 78006 RBR.0599.CE.EN.01. MDA was determined by colorimetric method by kits purchased from Bio-diagnostic, Diagnostic and research reagent; Catalog No. MD 25 29. TAC was determined by colorimetric method by kits purchased from Biodiagnostic, Diagnostic and research reagents, Cat. No. TA 25 13. CRP was determined by using Atlas C-Reactive protein latex reagent kits for qualitative and semi-quantitative measurement of CRP in serum, purchased from Atlas medical, William James House, Cambridge, CB4 4WX, UK.

II.5 Tissue sampling

Slices from left ventricle and thoracic aorta were fixed in 10% neutral buffered formalin for 24 h. After automated dehydration through aggraded alcohol series, the slices were embedded in paraffin, sectioned at 5 µm, and stained with H&E. Sections taken from aorta were stained with both Hematoxylin and Eosin (H&E) and Masson Trichrome before being evaluated using Leitz light research microscope (Leitz Wetzlar, Germany). Blind analysis of morphological changes was performed.

II.6 Equipment

Shimadzu UV spectrophotometer (2401/PC), ELx800 absorbance micro-plate reader from BioTek, Japan; Centrifuge, Biofuge, primo Heraeus, Germany; Electric balance, SARTORIUS AG, Germany; Water path, BW-10G, Korea; Leitz light research microscope (Leitz Wetzlar, Germany).

II.7 Statistical analysis

Graph Pad Prism V. 6.01 was used for analysis (GraphPad Software Inc., La Jolla, CA, USA). Data were presented as mean ± SD. Data were compared between the two groups using Unpaired Student Two Tailed "T"-test. Data were compared among the four groups using One Way ANOVA with Bonferroni Multiple Comparison test. A statistically significant difference was considered in (P) values less than 0.05.

III. Results

III.1 Thyroid stimulating hormone (TSH) levels after propylthiouracil (PTU) treatment in different rat groups

Highly significant rise in TSH levels after treatment with PTU was observed in PTU and PTU+HCA groups compared to control and HCA groups ($P<0.001$). Insignificant difference in TSH ($P> 0.05$) in HCA group compared to control group (Fig. 1).

III.2 Serum levels of free tri-iodothyronine (FT_3) and free tetra-iodothyronine (FT_4) after treatment with PTU in different rat groups

Significant decrease in FT_3 ($P<0.001$) and FT_4 ($P<0.001$) levels were found in PTU and PTU+HCA groups compared to control and HCA groups. Rat group taking HCA showed insignificant change ($P> 0.05$) in FT_3 and FT_4 compared to control group (Fig. 1).

III.3 Effect of treatment with GC/ HCA on lipid profile in hypothyroid rat model

Highly significant increase in TG, TC, LDL-c, and HDL-c levels in PTU group compared to both HCA and control groups ($P<0.001$). Co-administration of HCA decreased TG, TC, and HDL-c level significantly in PTU+HCA group compared to PTU group ($P<0.05$). However, insignificant change in LDL-c was observed in HCA+PTU compared to PTU group. Significant decrease in TC and LDL-c levels was observed in HCA group compared to control ($P<0.05$). No significant change was observed in TG and HDL-c levels in HCA group compared to control ($P>0.05$) (Fig. 2).

III.4 Changes in serum C reactive protein (CRP) with GC/ HCA treatment in rat model of hypothyroidism

Treatment with PTU caused significant increase in CRP in PTU group compared to control and HCA groups ($P<0.05$). CRP level was insignificantly decreased in PTU+HCA group ($P>0.05$) in comparison with PTU group. Insignificant difference in CRP level between PTU+HCA, HCA, and control groups ($P>0.05$) (Table 1).

III.5 Changes in serum malondiadehyde (MDA) and total antioxidant capacity (TAC) after GC/ HCA administration in rat model of hypothyroidism

MDA level was significantly increased in PTU group compared to control and HCA groups ($P<0.001$). Co-administration of GC/ HCA caused significant decrease in MDA level in PTU+HCA group as compared to PTU group ($P<0.001$). Values of MDA showed no significant change in HCA group compared to control group ($P>0.05$). Significant decrease in TAC in PTU group compared to control group ($P<0.01$) and HCA groups ($P<0.001$). Highly significant increase in TAC was observed in PTU+HCA compared to PTU group ($P<0.001$). No statistically significant difference in TAC was detected between HCA and control group ($P>0.05$) (Fig. 3).

III.6 Percentage changes in total body weight (%TBW) with GC/ HCA treatment in rat model of hypothyroidism

The difference in %TBW in PTU group was insignificant as compared to control group ($P>0.05$). However, highly significant decrease in %TBW was observed in HCA group as compared to control group ($P<0.001$). There was a significant decrease in %TBW in PTU+HCA group ($P<0.05$) as compared to PTU group (Table 2).

III.7 Changes in heart weight to body weight ratio (HW/BW) with GC/ HCA treatment

PTU caused highly significant decrease in absolute heart weight and HW/BW ($P<0.001$) compared to control group. Co-administration of HCA caused significant increase in absolute heart weight and HW/BW in PTU+HCA compared to PTU group ($P<0.01$). Insignificant change in HW/BW ratio in HCA group compared to control group. (Table 3).

III.8 Histopathological changes of the heart and blood vessels with GC/ HCA treatment in hypothyroid rat model

Left ventricular sections of different rat groups were stained with (H&E) and captured with high power field (HPF) of 400×. Histological sections of left ventricle of control group (Fig. 4- A & B) and HCA group (Fig. 4- C & D) showing normal structure with striations, branching fibers, continuity with adjacent fibrils, central nuclei and no degenerative changes .

Left ventricular sections of PTU group showed atrophic myocardium with marked edema (e) manifested by widely spaced myofibrils, discontinuous arrangement and lost striations. There is areas of focal

hemorrhage (h), increased nuclear number with other foci showing vacuolar degeneration (v) and congested vessels (c) (Fig.4-E, F, G).

Left ventricular sections of PTU+HCA group showed marked improvement with normal myofibrillar structure with striations, normal thickness, no edema or hemorrhage. Moreover, the branching appearance of cardiac muscle fibers, continuity with adjacent fibrils, and central location of the nuclei were preserved. Those sections showed no degenerative changes although nuclear numbers were increased (Fig. 4-H).

Sections from thoracic aorta of different rat groups stained with (H&E) stain and Masson Trichrome and captured with HPF of 400×. Sections of control and HCA groups (Fig.5-A, C) showing no pathological changes, intact endothelial lining (ti), elastic lamellae (e) and smooth muscle layer (s). Trichrome stained sections of control and HCA groups (Fig. 5-B, D) showed no change in collagen content (c) and identifying the regular arrangement of elastic lamellae.

Sections of thoracic aorta from PTU group stained with (H&E) showed marked thinning of aortic wall mainly in tunica media (tm) with ruptured elastic lamellae (thin arrow) , wide gapping (g) and decreased thickness of elastic lamellae, ulcerated endothelial lining (thick arrow) and vacuolar degeneration (v) (Fig. 5-E). Vascular sections of PTU group stained with Masson Trichrome showed increased collagen content of the tunica media (Fig. 5-F).

Sections of thoracic aorta from PTU+HCA group stained with (H&E) showed marked structural improvement with intact endothelial lining, elastic lamellae, smooth muscle layer, and restored number of elastic lamellae (Fig. 5-G). Sections of PTU+HCA group stained with Masson Trichrome showed marked decrease in collagen content and intact elastic lamella (Fig. 5-H).

IV. Discussion

Hypothyroidism is considered as one of the most common of all endocrine diseases. In the present study, rat model of hypothyroidism was established by PTU administration for 10 weeks as indicated by significant decrease in T3 and T4 as well as significant rise of TSH levels. The effectiveness of use of PTU in induction of hypothyroidism has been proved by previous studies.[10]

Dyslipidemia is strongly associated with hypothyroidism and is considered as important risk factor for cardiovascular diseases.[1] Our results showed significant rise in TC, TG and LDL-c levels in PTU group compared to control. In line with us, Laway et al found that dyslipidemia was associated with hypothyroidism.[12] Rise in TC, TG, and LDL-c can be explained by the effect of hypothyroidism in reducing fatty acid oxidation and decreasing the expression of hepatic LDL receptor-related protein 1 leading to reduction in clearance of circulating remnant lipoproteins.[13]

In addition, this study revealed significant rise in HDL-c levels in PTU group compared to control that is consistent with the results of Al-Noory et al who observed rise in HDL-c levels in rats taking PTU.[14] This rise in HDL-c can be explained by the effect of hypothyroidism on decreasing hepatic lipase and cholesteryl ester transfer protein that play an important role in HDL-c metabolism.[13] On the contrary, Gupta and his colleagues found significant decrease in HDL-c[15] while, Laway et al found no change in HDL-c with subclinical hypothyroidism.[12] This discrepancy in results could be explained on the basis of being studies done on subclinical not overt hypothyroid patients.

Co-administration of HCA caused significant decrease in TC, TG and HDL-c levels in PTU+HCA group compared to PTU group. This result is supported by the study of Amin et al who found that giving garcinia at 500 mg/kg (50% HCA) daily for 4 weeks decreased TC, TG, and LDL-c levels in high fat and high sucrose diet fed rats.[11] This effect of HCA may be explained by the inhibition of ATP-citrate lyase enzyme preventing the formation of acetyl CoA that is needed for fatty acid synthesis and denovo lipogenesis.[16] Moreover, HCA-induced inhibition of fatty acid synthase mRNA expression caused increased fat oxidation and degradation.[5] This study demonstrated significant decrease in TC and LDL-c levels in HCA group compared to control group. No significant deference in TG and HDL-c were found in HCA group compared to control group. Consistent with us Kim et al observed no change in TG and HDL-c after 10 weeks HCA supplementation (2 g/day) to overweight subjects compared to placebo.[17]

This study demonstrated significant rise in CRP in PTU group compared to control. Previous studies reported rise of CRP in hypothyroidism.[1] Rise in CRP levels is explained by reduction in thyroid hormone binding to thyroid hormone receptor α leading to cholesterol accumulation in aorta macrophages thus accelerating plaque appearance and elevation of inflammatory cytokines.[18] We found tendency toward decrease in CRP in PTU+HCA group compared to PTU group that does not reach the significance level. However, there was insignificant difference in CRP in PTU+HCA group compared to HCA group or compared to control group. HCA group showed insignificant difference in CRP levels compared to control group. This result is consistent with Bilal et al who observed insignificant change in CRP levels with daily administration of 65% HCA to rats fed with diet containing vegetable oil and cholesterol for 35 days.[19] On the other hand, Asghar et al observed significant decrease in CRP levels after Super CitriMax (HCA-SX) administration at 1500

mg/kg daily to obese rats for 7 weeks.[20] This conflict may be explained on the bases of different form, dose of GC given as well as duration.

MDA is one of the products resulting from lipid peroxidation and is considered as marker of oxidative stress.[21] This study found significant rise in MDA level and significant decrease in total antioxidants in PTU group compared to control that is supported by previous studies.[1] Rise in MDA and lowering of antioxidant capacity in hypothyroidism may be explained by the positive correlation between hypercholesterolemia and oxidative stress due to hormonal change.[21]

The results of the present work showed that co-administration of HCA and PTU caused significant decrease of MDA and increased levels of TAC compared to PTU group. Previous studies observed decreased MDA in rat model of non-alcoholic steatohepatitis and in overweight subjects with HCA administration that was explained by inhibition of lipogenesis.[8] Kim et al reported that GC increases the expression of antioxidant enzymes mRNA in liver as a compensatory response to increased hepatic lipid peroxidation in obese rats fed with high fat diets.[16] Results of HCA group did not show any significant change in TAC and MDA levels compared to control group. In line with us, Surapaneni and Jainu observed insignificant change in MDA with HCA administration.[8] Mishra and his group showed positive antioxidant properties with HCA administration in rat liver mitochondria.[22]

HCA obtains its medical importance from being an anti-obesity agent.[5] This study showed significant decrease in TBW in HCA group compared to control. Moreover, TBW was significantly decreased in PTU+HCA treated rats as compared to PTU group. This beneficial effect of HCA in controlling TBW was reported by many previous studies that was explained by the antilipogenic effect of HCA.[15] Chuah and his group reported that HCA aids fat oxidation and degradation as well as regulation of serotonin level, glucose uptake, and decreasing appetite.[5] We found that PTU treatment caused insignificant change in TBW as compared to control. In line with us, several studies observed no change in TBW of hypothyroid subjects as compared to their controls.[23, 24] They explained their results by disruption in ghrelin/growth hormone secretagogue receptor (GHS-R) axis and growth hormone/insulin like growth factor-1 (GH/IGF-1) axis in hypothyroidism that was corrected by thyroid hormone administration.[23]

This study showed insignificant change in HW/BW in HCA group compared to control group. Previous studies revealed no change in HW/BW ratio with HCA-SX administration for 90 days to rats.[25] Our results demonstrated significant decrease in HW/BW in PTU group compared to control group that is supported by previous studies.[26] The decreased HW in hypothyroidism was explained by lytic and necrotic changes of cardiac muscle cells associated with hypothyroidism.[27] Co-administration of HCA and PTU significantly increased HW/BW compared to PTU group. Up to our knowledge, no previous study demonstrated the effect of co-administration of HCA and PTU on heart weight. We may explain this result by the ability of HCA to prevent cardiac atrophy by limiting lipid accumulation and necrosis of cardiomyocytes as indicated by the increase in heart weight. To investigate the previous explanation, we looked at cardiac histopathology.

Left ventricular sections of HCA and control groups showed normal structure with striations, branching fibers and centrally located nuclei. Shara et al observed no change on cardiac histology after 90 days of HCA administration to rats.[25] Left ventricular sections of PTU group showed degenerative and atrophic changes of cardiac muscle. In line with our finding, previous studies observed necrotic and lytic changes in cardiac muscle cells with hypothyroidism.[26]

This study showed marked improvement in cardiac architecture with GC/HCA co-administration, in PTU+HCA group compared to PTU group. No previous literature supporting the protective effect of GC on cardiac histopathology in hypothyroidism. However, previous studies reported the protective effect of falvonoids; α G-Rutin and luteolin on doxorubicin-induced cardiac toxicity in mice [28] and GC; the most potent flavnoid on ethanol-induced hepatic damage that was proved to be through its hypolipidemic and antioxidant effects.[29] Another study reported the protective effect of GC on renal oxidative damage caused by high fat and high sucrose diet.[11] Taken together, we suggested that the hypolipidemic and antioxidant effects of GC/HCA might be the cause behind the preserved cardiac architecture in PTU+HCA group compared to PTU group.

Sections of thoracic aorta stained with (H&E) and Masson Trichrome showed normal architecture in control and HCA groups. This result is supported by the study of Deshmukh et al who observed no change in vascular histology after HCA administration.[30] Sections of PTU group showed marked damage of the architecture of the aortic wall. In agreement with us, Moulakakis et al reported that hypothyroidism caused increased collagen tissue and decreased thickness of elastic fibers increasing stiffness and decreasing distensibility of the aorta.[31] In addition, Zaki and Youssef observed thinning and rupture of elastic lamina with intimal ulceration and aortic dissection in cases of hypothyroidism.[32] This result can be explained by raised inflammatory cytokines, disturbed lipid profile, and lowered cholesterol efflux accelerating plaques appearance.[19] HCA co-administration greatly improved the histopathological changes in thoracic aorta

associated with hypothyroidism. Koshy and Vijayalakshmi reported significant protective effect of flavonoid containing GC extract on experimentally induced vascular disease.[33]

V. Conclusion

The results of the present study suggested that the use of GC/HCA has promising prophylactic effect against disturbed lipid profile, oxidative stress and cardiovascular disease associated with hypothyroidism. Moreover, concerning cardiovascular system we demonstrated the safety of GC/HCA as anti-obesity agent. Future studies are needed to determine the efficiency and safety of GC/HCA in hypothyroid human subjects concerning not only the cardiovascular system but also all body systems.

Conflict of interest:

No conflict of interest.

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VII. Tables

Table 1: Comparison of serum C-reactive protein (CRP) (mg/dl) levels in different studied groups

Measured Parameter	Control (n=6)	HCA (n=6)	PTU (n=6)	PTU+HCA (n=6)
CRP	2.00 ± 0.71	2.00 ± 1.00	8.00 ± 3.70 *#	6.40 ± 3.20

Hydroxycitric acid (HCA), propylthiouracil (PTU), milligram/deciliter (mg/dl), significance level (*) control group vs PTU group (* P<0.05), (#) HCA group vs PTU group (# P<0.05), n=number of rats/group. Data were expressed as mean ± SD.

Table 2: Comparison of percentage changes total body weight (TBW) in different studied groups

Measured parameter	Control (n=6)	HCA (n=6)	PTU (n=6)	PTU+HCA (n=6)
% changes in TBW	58.00 ± 3.80	-6.00 ± 18.00 ***	63.00 ± 14.00 ###	35.00 ± 12.00 ##~

Percentage increase or decrease in total body weight compared to original weight (% change in TBW) calculated by $[(TBW_{final} - TBW_{original})/ TBW_{original} * 100]$, hydroxycitric acid (HCA), propylthiouracil (PTU), significance level (*) control group vs HCA group (*** P<0.001), (#) HCA group vs PTU or PTU+HCA group (## P<0.01, ### P<0.001), (~) PTU vs PTU+HCA group (~ P<0.05), n=number of rats/group. Data were expressed as mean ± SD.

Table 3: Comparison of absolute heart weight and heart weight/body weight ratio in different studied groups

Measured parameters	Control (n=6)	HCA (n=6)	PTU (n=6)	PTU+HCA (n=6)
Absolute HW (mg)	1.20 ± 0.073	1.00 ± 0.05 **	0.70 ± 0.08 ***###	0.96 ± 0.09 **~ ~
HW/BW (mg/ gm)	5.00 ± 0.30	4.60 ± 0.18	2.90 ± 0.42 ***###	4.40 ± 0.25 *~ ~ ~

Hydroxycitric acid (HCA), propylthiouracil (PTU), milligram (mg), milligram/gram (mg/gm), significance level (*) control group vs any other group (* P<0.05, ** P<0.01, *** P<0.001), (#) HCA group vs PTU group (### P<0.001), (~) PTU vs PTU+HCA group (~~ P<0.01, ~~~ P<0.001), n=number of rats/group; HW: heart weight; HW/ BW: heart/ body weight. Data were expressed as mean ± SD.

VII. Figures

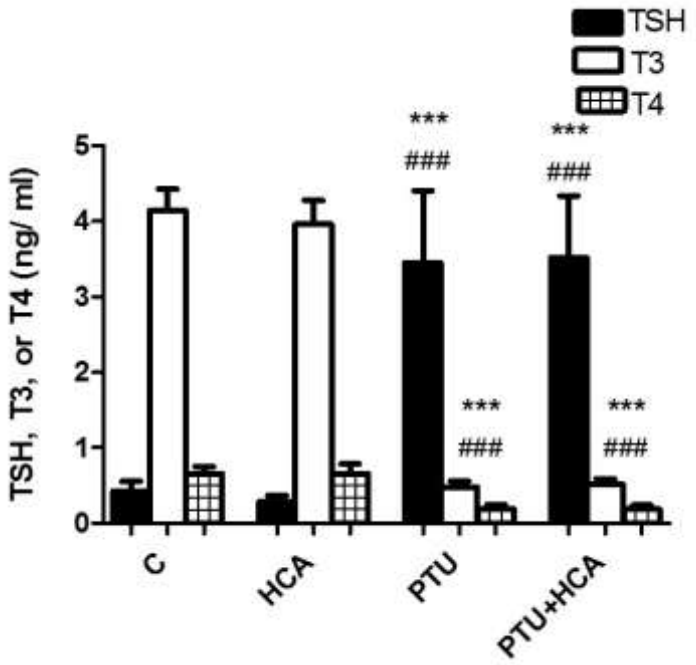

Figure (1): Comparison of serum levels of thyroid stimulation hormone (TSH), free tri-iodothyronine (FT_3)(ng/ml) and free tetra-iodothyronine (FT_4) between different studied groups. Control (c), hydroxycitric acid (HCA), propylthiouracil (PTU), nanogram/ml (ng/ ml). Data were expressed as mean ± SD. (*): significance of PTU vs control, (***P<0.001), (#): significance of PTU group vs HCA group, ($^{###}$P<0.001) (n = 6 in each group).

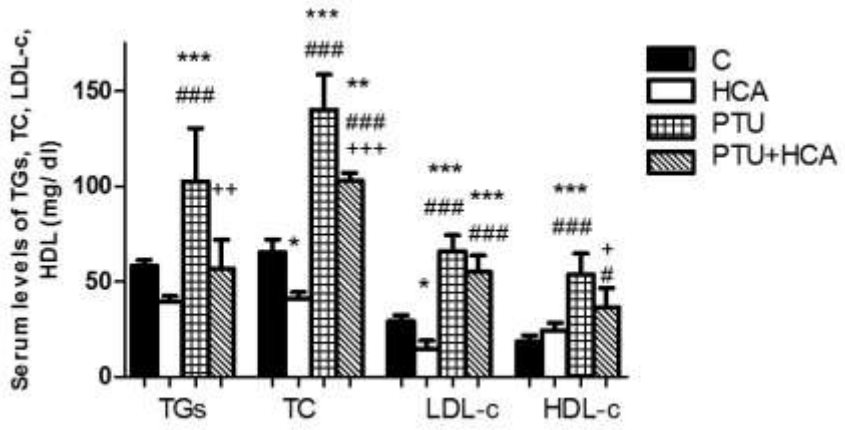

Figure (2): Comparison of triglycerides (TGs), total cholesterol (TC), low density lipoprotein (LDL-c), high density lipoproteins (HDL-c) serum levels (mg/dl) in different studied groups. Control (c), hydroxycitric acid (HCA), propylthiouracil (PTU), milligram/deciliter (mg/dl). Data were expressed as mean ± SD. (*): significance of PTU vs control, (*P<0.05, **P<0.01, ***P<0.001), (#): significance of PTU group vs HCA group, ($^{\#}$P<0.05, $^{\#\#\#}$P<0.001), (+): significance of PTU+HCA vs PTU group ($^{+}$P<0.05, $^{++}$P<0.01) (n = 6 in each group).

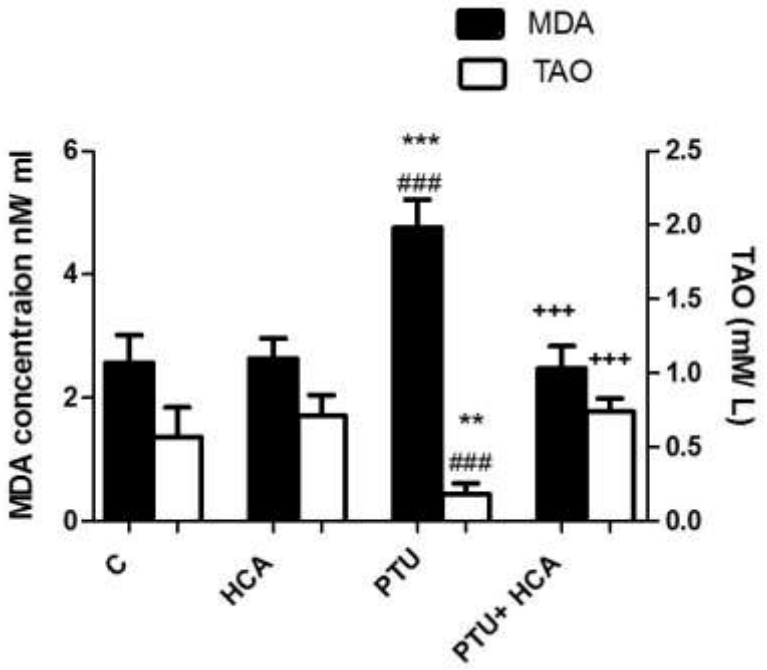

Figure (3): Comparison of serum malondialdehyde (MDA) (nmol/ml) and serum total antioxidant capacity (TAC) (mM/L) in different studied groups. Control (c), hydroxycitric acid (HCA), propylthiouracil (PTU), nanomol/milliliter (nmol/ml), mM/L= millimolar/litre. Data were expressed as mean ± SD. (*): significance of PTU vs control, (**P<0.01, ***P<0.001), (#): significance of PTU group vs HCA group, ($^{\#\#\#}$P<0.001), (+): significance of PTU+HCA vs PTU group, ($^{+++}$P<0.001), (n = 6 in each group).

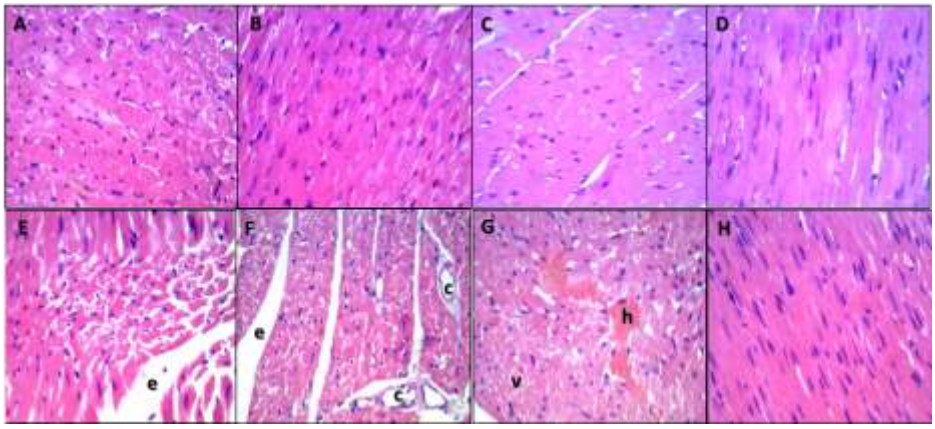

Figure (4). Left ventricular sections of control (A, B); HCA (C, D); PTU (E, F, G); PTU+HCA (H) groups stained with H&E. A, B, C, D: showing normal myofibrillar structure with striations, preserved branching appearance, continuity with adjacent fibrils, centrally located nuclei and no degenerative changes. E, F, G: showing atrophic myocardium, discontinuous arrangement, lost striations and increased nuclear number. E, F: showing edema (e); G: showing congested blood vessels (c); G: showing focal hemorrhage (h) and vacuolar degeneration (v); H: Showing normal myofibrillar structure with increased nuclear numbers (H&E, Magnification X 400).

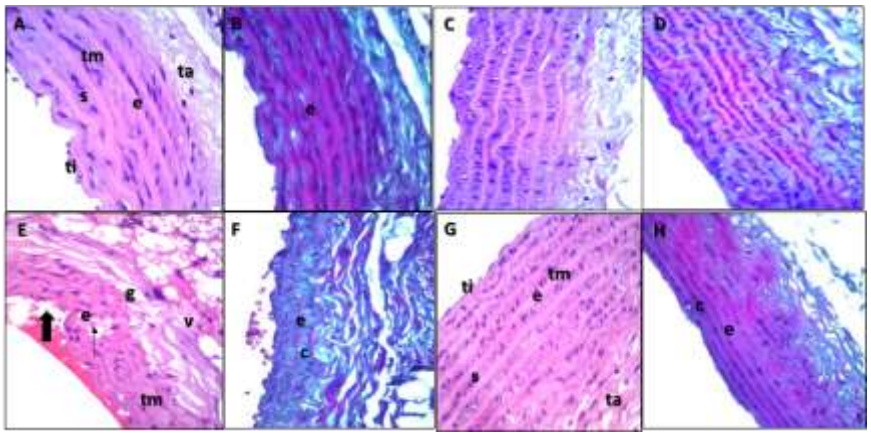

Figure (5). Structural changes in aorta of control (A, B) and HCA groups (C, D); PTU group (E-F); PTU+HCA group (G & H). A, B, C, & D sections of thoracic aorta; (A-D): showing intact tunica intima (ti), media (tm), & adventitia (ta); intact normally arranged elastic lamella (e); normal thickness of elastic lamellae (e) & smooth muscle cells (s). E & F: showing thinning of tunica media (tm), ruptured elastic lamellae (thin arrow), wide gapping (g) between elastic lamellae (e), ulcerated endothelial lining (thick arrow) and vacuolar degeneration (v); F: showing thinning of elastic lamella (e) and increased collagenous tissue (c) between the lamella. G: showing marked improvement with intact tunica intima (ti), elastic lamellae (e), smooth muscle layer (s), tunica media (tm). H: showing intact elastic lamella (e) and decreased collagen tissue (c). (A, C, E, G: stained with H&E; B, D, F, H: stained with Masson Trichrome; Magnification X 400).