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Therapeutic Potential of Ethanolic Extract of *Moringa Oleifera* Against Hypothyroidism-Induced Myocardial Ischemia in Male Rats

Article Info.

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Abstract

Moringa oleifera gained its importance as a natural antioxidant due to its high content of phenolic compounds, such as flavonoids and phenolic acids. The present experience was designed to assess the ability of the extract of *Moringa* leaves to prevent cardiac infarction in male rats with hypothyroidism. 30 male rats were distributed in 2 groups: a healthy group of 10 rats and a hypothyroid group of 20 rats. Hypothyroidism was induced by administering propylthiouracil (PTU) in drinking water for two months. After a confirmed thyroid deficiency induced by measuring thyroid function tests, showing low thyroid hormone levels and high TSH, animals were re-distributed equally into three groups of 10 rats each, as follows: the first group (intact animals) as a negative control, rats were administered (1 ml/kg) of normal solution; the second group (hypothyroidism untreated) as a positive control, rats were administered (1 ml/kg) of PTU; and the third group (HYPO-MOE treated), rats were treated with MOE at a dose of 300mg/kg. The treatment continued for one month. The oral administration of PTU resulted in a pronounced reduction $p \leq 0.05$ in the levels of thyroid hormones concomitantly with a significant elevation $p \leq 0.05$in TSH and lipid profile concurrently with a diminution $p \leq 0.05$ in the level of HDL. Also, results revealed an elevation in heart enzyme activity accompanied by an elevation of $p \leq 0.05$ of troponin I associated with histological changes in myocardial architecture, whereas the treatment with MOE resulted in an improvement in thyroid function, lipid profile, cardiac biomarkers and a restoration of cardiac architecture.

Keywords; Myocardial ischemia, *Moringa oleifera*, Hypothyroidism, lipid profile, troponin.

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Introduction

Hypothyroidism is one of the common hormonal disorders that affects all individuals across the world (1). It is characterized by the slowed-down metabolism of the body due to its inefficient or reduced production of thyroid hormones, triggering harmful effects (2). The causes of primary thyroiditis include the inability of the thyroid gland; secondary thyroiditis includes the lack of stimulation of the thyroid-stimulating hormone (TSH) secreted by the pituitary gland; and tertiary (central) thyroiditis is the lack of secretion of the hormone by the hypothalamus (3). The heart is affected by thyroid hormones (TH); heart structural and functional abnormalities can result from either hypothyroidism or hyperthyroidism by binding to its receptors in the nuclei of cardiomyocytes (4). Furthermore, thyroid hormones regulate the activation of many genes in contractile proteins, particularly myosin (5).

Although synthetic medications are used in modern treatment approaches, they usually have harmful effects, especially in association with their long-term use, and have high costs imposed on patients. This is why today natural products are successful in forming protective functions with the clinical therapeutic regimes towards a reduced rate of most widespread diseases in humans; it is due to their high phytonutrients and medicinal value. So, the tendency towards alternative and traditional treatments is increasing (6, 7). Among these plants, *Moringa oleifera*, Lam (commonly known as Sajna), is ubiquitous and belongs to the Moringaceae family; it is exceptionally nutritious for humans and livestock, as well as having a high economic value because of its medicinal uses. *Moringa oleifera* Lam. (family Moringaceae) is commonly identified by its tripinnate leaves, whitish-grey bark, and pods that resemble drumsticks, Herbarium ALAU, HNGB (M0066C): Identified by (2024) (8). *Moringa oleifera* possesses a rich chemical composition compared with other plants; this makes it most commonly used in several food industries. Also, *Moringa oleifera* is called the "miracle tree" because all its parts are widely used in traditional medicine and contain a desirable nutritional balance (9). *Moringa* leaf is a good source of bioactive phytochemicals, making them of biological importance as neuroprotective, hepatoprotective, anti-cancer, anti-inflammatory and antioxidant. Among the phytochemical compounds present in moringa are trace minerals, vitamins, polyphenols, saponins, proteins, amino acids, glycosides and alkaloids (10, 11). In addition, their leaves contain numerous antioxidant compounds which are used as antihypertensive, antimicrobial, antidiabetic, anti-hyperlipidemic, antineoplastic and cardioprotective activities, such as carotenoids, ascorbic acid, phenolic acids and flavonoids (12, 13). Nevertheless, few studies were done to demonstrate the harmful effect of thyroid disorder on heart function in humans and animals in Iraq. The current study was aimed to assess the impacts of hypothyroidism on biomarkers and histological alterations in the heart and the ameliorative effects of moringa extract on thyroid and heart functions and lipid criteria in male rats.

Materials and Methods

Preparation of plant extraction:

Moringa oleifera (MO.) dried leaves were obtained in the local market of Basrah city. Iraq: the plant material was identified and authenticated by Prof. Sahar Malik Al-saadi at the College of Science/ University of Basrah. After cleaning, the leaves were dried in the shade, and then the dried leaves were ground into fine powder and stored in polythene bags in the refrigerator until the extraction. Using the Soxhlet apparatus method, we prepared the ethanolic extract by dissolving the powder (50 g) of *Moringa* leaves in 200 ml of 70% ethanol until the solvent loses its color; the extract was filtered and condensed by an evaporator under reduced pressure at a temperature of 45°C and then dried; the percentage yield was calculated and kept it in a tight closed container and stored it at 4°C until using it for animals in the current study(14).

Animals and their management

It was observed in thirty healthy male rats aged one month and ruling (55-65 gm) using experimentally observed animal standard laboratory conditions under a temperature of 22 ºC with 12 hr./day light and 12 hr. of darkness with the help of plain water in plastic bottles with stainless steel sipper tubes and a control diet on commercial ration in their rats under study period after 7 days of adaptation and then induction of hypothyroidism in rats. Rats were handled in accordance with the guidelines of the animals' care and the norms of the Ethical Committee of the Faculty of Veterinary Medicine- University of Basrah (UOB-VET.127/37/2026).

Induction of thyroid deficiency and protocol

30 male rats were divided randomly into 2 groups: a healthy group of 10 rats and a hypothyroid group of 20 rats. Hypothyroidism was induced by administering 1 mg/kg/ propylthiouracil (PTU) (manufactured by Quagen Pharmaceuticals, LLS, West Caldwell, NJ07006 Made in USA) in drinking water for two months following the protocol (15). After a confirmed thyroid deficiency was induced by measuring thyroid function tests, showing low thyroid hormone levels and high TSH above 2 mIU/L, indicating thyroid deficiency, the animals were re-divided into three sub-groups as follows: the normal control group received 1 ml of normal solution by gavage, the thyroid deficiency group received 1 ml of normal solution by gavage, and the thyroid deficiency-treated group received 300 mg/kg of *Moringa oleifera* ethanolic extract by gavage. The treatment continued for one month. After the end of the period, experience. Blood samples (5 mL) were taken from each rat through the heart (cardiac puncture). After isolation of serum, they were stored in the freezer at -20°C until used for measuring hormones and biochemical criteria, and then the animals were sacrificed, and the heart was removed for histological examination.

Hormonal and Biochemical Evaluation

The serum TSH and thyroid hormones (T4, T3) were measured by using the Rat ELISA kits (Cobas, Roche Diagnostics, Germany) technique according to the protocol of (16). The lipid profile (TC, TG, LDL-C, VLDL-C and HDL-C) was determined following the method of (17). The cardiac biomarkers (CTnI, CK MB, LDH, and AST) were estimated depending on the method described by (18).

Histopathological Investigation

After 24 hrs. of the last dose of treatment, the rats were sacrificed while still under anaesthetized ketamine and xylazine. The specimens of heart tissues were taken from all groups, and 10% buffered formalin-fixed thyroid and heart specimens were subsequently processed. The paraffin-embedded tissue blocks were made into sections with a thickness of 5 µm by using a microtome and dewaxed and stained routinely in H&E stain. Sections were examined to detect the histopathological alterations (19).

Statistical investigation:

The data from the present three investigations were analyzed with univariate analysis of variance (ANOVA) in the computerized SPSS (Statistical Packages for the Social Sciences) V.23 program. $P<0.05$ was considered statistically significant. The data were reported as mean ± standard deviation (SD). To compare groups.

Results

The outcomes seen in table (1) revealed a significantly elevated $p \leq 0.05$ TSH level concurrently with a marked decline $p \leq 0.05$ in thyroid hormone (T3, T4) levels in the hypothyroid group in comparison to the intact group, while the result exhibited a significantly decreased $p \leq 0.05$ TSH value accompanied by an increase $p \leq 0.05$in thyroid hormones (T3, T4) in groups treated with the ethanolic extraction of *Moringa oleifera*. Also, the finding illustrated in Table (2) indicates that there was a significant increase $p \leq 0.05$ in TC, TG, LDL-C and VLDL-C values in conjunction with a significant decrease $p \leq 0.05$ in HDL-C values in the positive group in comparison with the negative group. While it showed a noticeable decrease $p \leq 0.05$ in TC, TG, LDL-C, and VLDL-C values, it was accompanied by a marked increase $p \leq 0.05$ in HDL-C in treated groups with MOE in comparison to the hypothyroid group. As shown, outcomes in table (3) demonstrate that the administration of PTU caused a significant elevation $p \leq 0.05$ in cTn1, CK, LDH and AST values in comparison to intact animals, while the treatment with Moringa extract led to a remarkable decline in the above indices as compared to the hypothyroid group.

Histological findings

Histological alterations of the heart tissue were associated with changes in heart biomarkers in the hypothyroid group. These changes involved wavy cardiomyocytes (thin arrows) with cytoplasmic degeneration characterized by myofibrillar disorganization (thick arrows) and early nuclear condensation, consistent with early ischemic myocardial injury in Fig. 2 as a comparison to the normal section of heart in Fig. 1, while the heart section in the treated group with moringa extract appears to be completely repaired, live tissue (thin arrows) with a dramatic response, and the myocardial cells regain their normal architecture regarding the nuclei and cytoplasm. There is also interstitial tissue oedema and infiltration by inflammatory cells, reflecting good tissue response in fig. (3) as compared to the heart section in the hypothyroid group.

Table 1: Effect of moringa leaf extract on thyroid hormones concentration in experimental groups.

Parameters	1st group. N/S	2nd group.N/S	3rd group/ MOE
TSH µIU/L	1.20±0.27a	2.66 ±0.17b	1. 33±0.43a
T_4 mg/dl	3.11 ±0.23b	1.44 ±0.14a	2.86±0.84b
T_3 mg/dl	1.80±0.30b	0.56±0.17a	1.71±0.50b

groups ($p<0.05$). 1st group (intact as negative control/ normal solution). 2nd group (Hypothyroidism as Positive control/ normal solution). 3rd group (Hypothyroidism/ *Moringa oleifera* extract/300mg /kg)

Table 2: Effect of moringa leaf extract on lipid profile biomarkers in experimental groups

Parameters	1st group.N/S	2nd group. N/S	3rd group/ MOE
TC mg/dl	60.88±4.54a	154.47±3.47c	71.92±4.26b
TG mg/dl	55.82±3.52a	126.03±4.51d	68.73±3.54c
HDL-C mg/dl	39.99±2.5d	21.68±1.43a	30.51±1.4b
LDL-C mg/dl	20.17±1.4a	32.73±2.29c	27.45±3.6b
VLDL-C mg/dl	15.19±1.33a	23.87±2.74c	19.2±1.05b

Data are represented as the mean ± SD: Small letters denote significant differences between groups ($p<0.05$). 1st group (intact as negative control/ normal solution). 2nd group (Hypothyroidism as Positive control/ normal solution). 3rd group (Hypothyroidism/ *Moringa oleifera* extract/300mg /kg)

Table 3: Effect of moringa leaf extract on heart biomarkers in experimental groups

Parameters	1st group. N/S	2nd group. N/S	3rd group/ MOE
cTnI pg/ml	0.43±0.14a	1.37 ±0.24c	0.67±0.06b
KC-MBU/L	105.85±6.24a	156.55±16.12c	124.06±3.01b
LDH U/L	30.01±4.89a	65.87 ± 6c	31.93±1.83b
AST U/L	48.38± 17. 11a	82.38±5.94c	67.32±3.50b

Data are represented as the mean ± SD: Small letters denote significant differences between groups ($p<0.05$). 1st group (intact as negative control/ normal solution). 2nd group (Hypothyroidism as Positive control/ normal solution). 3rd group (Hypothyroidism/ *Moringa oleifera* extract/300mg /kg)

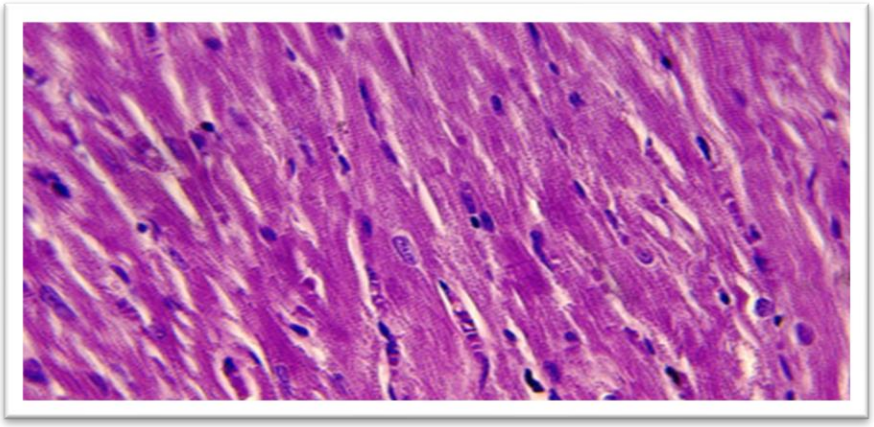

Fig. 1 Photomicrograph of control heart tissue (H and E, ×40) showing normal cytoarchitecture

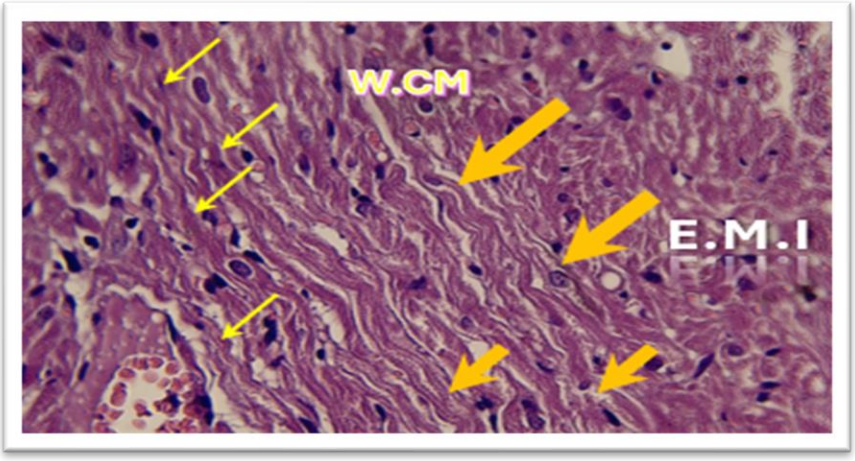

Fig. 2 Photomicrograph of heart hypothyroid group, showing wavy cardiomyocytes (thin arrows) with cytoplasmic degeneration characterized by myofibrillar disorganization (thick arrows) and early nuclear condensation, consistent with early ischemic myocardial injury, H&E, 40x

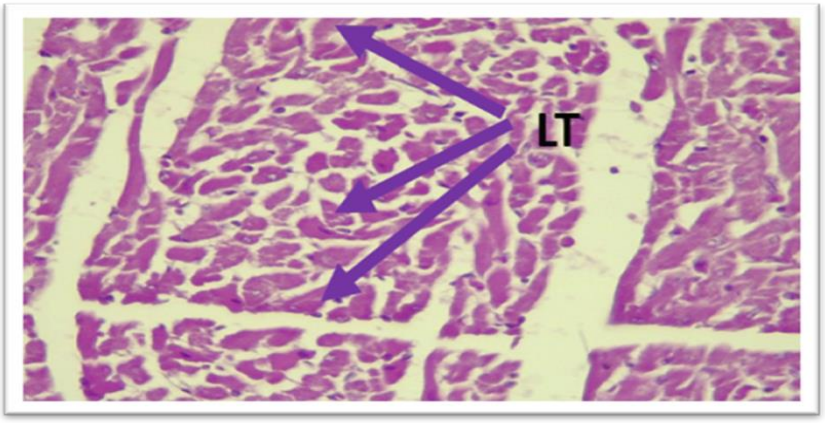

Fig. 3 Photomicrograph of the heart-treated group with Moringa extract showing complete repair, live tissue (thick arrows) with dramatic response, the myocardial cells regain their normal architecture, regarding the nuclei and cytoplasm. There is also interstitial tissue edema, and infiltration by inflammatory cells, reflecting good tissue response. 40X

Discussion

Hypothyroidism is a clinical syndrome resulting from a deficiency of thyroid hormones or, more rarely, from their impaired activity on the tissue (20). Thyroid hormones (THs) play fundamental roles in cardiovascular homeostasis; the changes in the levels of circulating THs may impair myocardial bioenergetic status with consequences on cardiac function, and thyroid dysfunction plays a central role in cardiac dysfunction and in the occurrence and progression of heart failure (21).

The current results indicated that administration of PTU caused a remarkable decline in thyroid hormone levels, accompanied by a more pronounced elevation in TSH levels. This may be due to PTU inhibiting both the synthesis of thyroid hormones in the thyroid gland by interfering with the action of thyroid peroxidase and suppressing the peripheral deiodinase, which transforms thyroxine (T4) to its active form, triiodothyronine (T3). This result agrees with the results found by (22, 23). Other studies mentioned that 6-n-propyl thiouracil is known as a thioamide-derived drug that treats hyperthyroidism by decreasing the secretion of thyroid hormones by the thyroid gland and inhibiting the 5' deiodinase enzyme, which converts T4 to active T3 (24-26). Other studies have shown that PTU causes primary hypothyroidism via the action of PTU as a substrate for the thyroid peroxidase (TPO) enzyme and inhibits its reduction and the incorporation of iodide into tyrosine molecules (27- 31). In addition, it inhibits the coupling of di-iodinated and mono-iodinated to form thyroid hormones; this action results in a significant decrease in the level of MIT, which are the main constituents in the production of T4 and T3. The above studies are in parallel with our study. The present study also agreed with the study carried out by (32). From the same

table, it was observed that M. oleifera ethanolic extract caused the return of thyroid hormone to limit normal values when compared with the control positive, this result matching the result found by (33).

It has been reported that using *Moringa oleifera* is likely to improve thyroid processes as it elevates the amount of serum T3 and serum T4 hormones. Increased thyroid hormones in this research may be attributed to the fact that some of the compounds in *Moringa oleifera* may increase the activity of the thyroid gland, which influences the physiology and functioning of the thyroid follicular cells. A recent study reported that heat stress in poultry causes a drop in the level of thyroid hormone (T3, T4) in the stress and stress-free groups after 21 days and 42 days, compared to the control group results (34). The MOEO supplementation against stress raises the development of thermoregulatory ability of the thyroid gland and may also be a regulatory factor of thyroid metabolism. The results are consistent with the results in the current study. Also, our study agreed with the study done by (33); according to the results, there was a significant increase in serum levels of lipids, including TC, TG, LDL-c and VLDL-c, accompanied by a decrease in HDL-c level following PTU administration, and this result was confirmed by (35), who found that administration of PTU for 14 days in rats produces a significant increase in lipid indices with a concomitant decrease in HDL level along with a substantial decline in T3 and T4 accompanied by increases in TSH levels. The explanation for this increase in the lipid picture may be due to a decrease in the number of hepatic LDL receptors, leading to the decreased clearance of circulating LDL, alongside hypothyroidism, producing a reduction in the cholesterol 7 alpha hydroxylase enzyme, thereby preventing the conversion of cholesterol into bile acid; this could contribute to an increase in LDL cholesterol levels (36). A previous study showed that hypothyroidism is associated with increased intestinal cholesterol absorption (37). Our results indicate that treatment with *Moringa oleifera* ethanolic extract causes a significant decrease in TG, TC, and LDL, along with an increase in HDL-C levels; these results agree with some studies (38-40). The beneficial effect of the leaves of *Moringa oleifera* may be attributed to the herb's being a rich source of flavonoids, phenolics, carotenoids, and vitamin C, which act as a good source of natural antioxidants.

The present findings exhibited a significant elevation in the levels of CK-MB, LDH, AST, and cardiac troponin I (cTn I) in rats that received PTU. These indices are considered the “gold standard” for the non-invasive diagnosis of myocardial injury in humans and animals, and an increase in plasma indicates acute heart injury and myocardial damage. In fact, cardiac dysfunction is a common finding in hypothyroidism, and this has been attributed to various changes that occur in the myocardium. This result was documented by those who found elevation in CK-MB (41); this elevation may be due to the occurrence of damage to cardiac muscle. Alongside this, hypothyroid-induced myopathy often presents with myalgia and a raised serum level of CK-MB.

This may occur as a complication of thyroid storm, which is precipitated by dehydration and electrolyte imbalance.

On the other hand, recent evidence indicates that THs must bind to thyroid hormone receptors. These receptors are intracellular DNA-binding proteins that bind hormone receptor complexes. The inhibition of thyroid receptor (TRα1) was shown to markedly depress post-ischemic cardiac function in mice after thyroid deficiency (42). These results are in accordance with results in our study. Also, the present study agreed with the study done by (43), whereas the results of the present study indicated that the treatment with MOE appears to improve heart biomarkers (CK-MB, LDH, and AST). This may be attributed to *Moringa oleifera* containing compounds with a potential cardioprotective role that reduce damage and improve function after a cardiac infarction through antioxidant, anti-inflammatory, and anti-apoptotic effects like flavonoids, phenolics, and vitamin E, which combat oxidative stress that damages heart tissue. These findings are consistent with results obtained by (44), who mentioned that administration of *Moringa oleifera* aqueous leaves extract (200 mg/kg BW) every day for 28 days for rats exposed to Doxorubicin once weekly for four (4) weeks results in the restoration of cardiac dysfunction. induced rats via the anti-inflammatory and antioxidant activities of the plant. Furthermore, cTn1 also improved in the group treated with *M. oleifera* ethanoic extract due to anti-inflammatory activity, and this result was in agreement with the result found by (45). Who found rats that were treated with *Moringa* after being exposed to diesel fumes for only five minutes for eight weeks revealed an increase in cardiac troponin I level after exposure to diesel, which might be due to the damage of cardiac muscle and myocardial hypoxia or ischemia, which leads to membrane disruption, causing troponin release, which is detected in serum.

The multidirectional effect of the use of PTU on the histological structure of the myocardium is the formation of degenerative changes in the myocardium, which is reflected in the reduction of the size of the cytoplasm of cardiomyocytes, pyknosis of their nuclei, loss of cardiomyocytes, and the formation of fibrotic changes in agreement with (46), who concluded that the emergence of fibrotic changes in the cytoplasm of cardiomyocytes in response to intake of PTU caused loss of cardiomyocytes. Myocardial architecture changes are found with the restoration being achieved in the treatment with the moringa extract. These results are in concurrence with findings by (47).

Conclusion

Conclusion: This work exhibited that *Moringa oleifera* extract may be considered a natural product that has anti-hypothyroidism and cardio-protectant properties.

Conflicts of Interest

The authors declare that there are no conflicts of interest associated with this manuscript.

Ethical approval

This research was approved by the veterinary medicine department at the University of Basra approved ID 127/37/2026

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الإمكانات العلاجية للمستخلص الايثانولي لنبات المورينغا اوليفيرا ضد نقص تروية القلب الناجم عن قصور الغدة الدرقية في ذكور الجرذان

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الخلاصة

اكتسبت المرينغا اوليفيرا أهميتها كمضاد اكسدة طبيعي بفضل محتواها العالي من المركبات الفينولية مثل الفلافونويد وحمض الفينول صممت هذه التجربة لتقييم قدرة مستخلص أوراق المورينغا على الوقاية من احتشاء عضلة القلب لدى ذكور الجرذان المصابة بقصور الغدة الدرقية: تم توزيع 30 ذكرا على مجموعتين: مجموعة سليمة مكونة من 10 جرذان, ومجموعة مصابة بقصور الغدة الدرقية مكونة من 10 جرذان, تم استحداث قصور الغدة الدرقية عن طريق إعطاء بروبيل ثايويوراسيل في مياه الشرب لمدة شهرين بعد تأكيد قصور الغدة الدرقية عن طريق قياس وظائف الغدة الدرقية الذي اظهر الذي اظهر انخفاض مستويات هرمونات الغدة الدرقية وارتفاع الهرمون المحفز للغده الدرقية, تماعادة توزيع الحيوانات بالتساوي على 3 مجموعات, كل مجموعة تضم 10 جرذان على النحو التالي: المجموعة الأولى: حيوانات سليمة كمجموعة ضابطة سلبية , تم إعطاء الجرذان 1 مل من المحلول الطبيعي المجموعة الثانية(المصابة بقصور الدرقية الغير المعالجة) تم إعطاء الجرذان 1 ملغم/كغم من البوربيل ثايويوراسيل المجموعة الثالثة(المصابة بقصور الغدة المعالجة بمستخلص المورينغا) تمت معالجة الجرذان بمستخلص المورينغا 300 ملغم/ كغم واستمر العلاج لمده شهر واحد: أدى تناول البروبيل فمويا الى انخفاض معنوي في مستويات هرمونات الغدة الدرقية, مصحوبا بارتفاع كبير في هرمون المحفز للغدة الدرقية ومستويات الدهون في الدم, بالتزامن مع انخفاض في مستوى الكولسترول عالي الكثافة كما كشفت النتائج عن ارتفاع في نشاط انزيمات القلب مصحوبا بارتفاع التروبونين والمرتبط بتغييرات نسيجيه في عضلة القلب, في حين أدى العلاج بمستخلص المورينغا الى تحسين وظيفة الغدة الدرقية, ومستوى الدهون, والمؤشرات الحيوية القلبية, واستعادة عضلة القلب

الكلمات المفتاحية: نقص تروية عضلة القلب، المورينجا اوليفيرا، قصور الغدة الدرقية، مستوى الدهون في الدم، التروبونين