HAYATI
Journal of Biosciences

Vol. 30 No. 6, November 2023 1054-1060
DOI:10.4308/hjb.30.6.1054-1060
ISSN: 1978-3019
EISSN: 2086-4094

Cardioprotective Activity of Costus Root Ethanol Extract in Experimentally-Induced Hypothyroidism in Female Albino Rats

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ARTICLE INFO

Article history:
Received June 30, 2022
Received in revised form April 15, 2023
Accepted July 3, 2023

KEYWORDS:
Hypothyroidism,
Costus,
Heart,
Propylthiouracil,
Albino rats,
anti-oxidant

ABSTRACT

Hypothyroidism is a popular endocrine disorder caused by a thyroid hormone deficit. Changes in the state of the thyroid gland significantly affect many organs, including the heart. This study examined the cardioprotective effects of an artificially induced hypothyroidism in female rats using an ethanol extract from the root plant Saussurea lappa (Costus). For the current study, 25 adult female albino rats were employed. They were split into five equal groups, including control, hypothyroid group, post-treatment group with costus extract, co-administered hypothyroid group with costus extract, and post-treatment group with levothyroxine. Thyroid hormones (T3, T4, and TSH), lipid profile, and oxidative stress indicators (catalase, superoxide dismutase, reduced glutathione, and lipid peroxidation levels) were also measured in the serum. The heart muscle was the subject of a histological research. The findings suggested that Costus root ethanol extract improved hypothyroidism in female rats, as proven by the reversal of many biochemical abnormalities and improvements in the heart's histology. Our study indicates that the root of Costus exhibited cardioprotection efficiency against hypothyroidism-induced serious effects on the heart, The antioxidant and radical-scavenging properties of its components may be responsible for this promising effect.

1. Introduction

Globally, cardiovascular disease (CVD) is recognized as a fatal illness issue with significant death and morbidity rates. According to a research by According to the World Health Organization, both developed and developing countries' primary cause of death for people between the ages of 40 and 50 is myocardial infarction (MI) (Kumar *et al.* 2011; Panwar *et al.* 2011; Saleem *et al.* 2012). Oxidative stress resulting from the massive production of free radicals and the concurrent reduction of antioxidant systems such as superoxide dismutase, catalase, and reduced glutathione (GSH) plays a significant role in MI (Saleem *et al.* 2012). Hypothyroidism is a common endocrine disorder characterized by reduced or insufficient synthesis of thyroid hormones, such as thyroxine (T4) and triiodothyronine (T3) (Ku and Lee 2010; Kim *et al.* 2012). Hypothyroidism that is left untreated may cause high blood pressure, dyslipidemia, infertility, cardiomyopathy, anemia, and neuromuscular dysfunction. (Vigneshwar *et al.* 2021). Numerous research have investigated the potential links between subclinical hypothyroidism and CVD (Leong *et al.* 2017; Inoue *et al.* 2020). Levothyroxine sodium is a widely used replacement medication for the treatment of hypothyroidism. Levothyroxine has therapeutic benefits, but it may also have unfavorable side effects, including behavioral issues, heat intolerance, tachycardia, irregular breathing, restlessness, insomnia, stomach pains, and weight loss (Katzung and Trevor 2015). Hence, there was a need to find an alternative herbal for treatment of hypothyroidism and improve related variables.

With over 1,620 genera and 23,000 species, the Asteraceae family of angiosperms is the largest and most important in terms of commerce (Amin *et al.* 2013) The indigenous population uses plants in the Asteraceae family, which has significant medicinal potential, to cure a wide range of ailments. 2018 (Alamgeer *et al.* 2018). The Asteraceae family contains the important medicinal plant Saussurea lappa

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(Costus). (Kumar *et al.* 2014; Huang *et al.* 2017). It is a perennial herbaceous plant that is used in many traditional medical systems around the world to treat things like diarrhea, tenesmus, dyspepsia, and vomiting (Tousson *et al.* 2019, 2020). Costus is rich of antioxidants and can protect against liver damage, diabetes, fungi, worms, tumors, inflammation, ulcers, microbes, and enhance the immune system (Nadda *et al.* 2020). The purpose of this study is to determine whether the root of the Costus guards against cardiac damage induced by PTU.

2. Materials and Methods

2.1. Plant and Preparation of Extract

Herb shop in Kirkuk, Iraq, provided the Costus roots then identified and authenticated by scientific botanist. Grinding and extraction of the Costus roots were carried out according to Mahmoud (2020). To summarize, 400 ml of 70 percent ethanol was used to extract 100 g of plant roots, which were macerated for 72 hours before being filtered 3 times and evaporated to produce a crude Costus extract.

2.2. Chemicals

Propylthiouracil made by the Italian company RECORDATI ILAC was used in the experiment. We also used the medication euthyroxin (100 µg), produced by Merck KGaA in Germany. Furthermore, the materials and reagents employed in this research were of the highest purity and analytical quality.

2.3. Determination of Total Phenolic and Flavonoid Contents

The total phenolic content was determined using a previously established colorimetric method (Siddiqui *et al.* 2017). Gallic acid (GAE) was used to create the standard curve. Total flavonoid levels were assessed using Choi *et al.* alapproach's (Choi *et al.* 2005), and quercetin equivalents were calculated using calibration curves generated with quercetin standard. GAE and quercetin equivalents/g-extract (QEs) were used to calculate total phenolic and flavonoid content.

2.4. Animal and Experimental Design

Used were 25 female Wistar albino rats weighing between 200 and 250 grams. Before beginning the experiment, the animals were acclimatized for two weeks in their individual cages under sanitary and regular climatic conditions (28±2°C, 60-70 percent relative humidity, and a 12-hour light/dark cycle), with access to feed and drink. Rats received complete care under standard managemental conditions guidelines. After becoming acclimated to the circumstances of the experimental room, the animals were randomly separated into five groups of five rats each:

- GI: Normal control included healthy animals receiving adlibitum feed and water for eight weeks.
- GII: Hypothyroid group, rats received 0.05 percent 6-n-propyl-2-thiouracil (PTU) orally every day for eight weeks.
- GIII: Post treated hypothyroid by Ethanol extract of Costus root (ECR), in which rats got 0.05 percent 6-n-propyl-2-thiouracil (PTU) daily oral gavage for consecutive 4 weeks to produce the hypothyroid condition and received Costus extract (300 mg/Kg) via oral gavage for additional 4 weeks (from 5^{th}-8^{th} week).
- GIV: Co-administration PTU-ERC (CO-PTU-ECR); rats got 0.5% 6-n-propyl-2-thiouracil (PTU) orally along with ECR (300 mg/Kg) for eight weeks.
- GV: Post treated hypothyroid by LT4 (P-T-LT4); in which rats were administered 0.05 percent 6-n-propyl-2-thiouracil (PTU) through orally administered for 4 weeks to induce hypothyroidism, and rats were administered Levothyroxin (LT4) (0.4 µg) via gavage for an additional 4 weeks (from 5^{th}-8^{th} week).

2.5. Phytochemical Analysis of Costus

A preliminary qualitative phytochemical analysis of the ethanolic root extract of Costus was carried out using the previously described standard procedures to determine the presence of active phytocompounds such as tannins, saponins, terpenoids, steroids, flavonoids, phenol, alkaloids, glycosides, and resins (Mathew *et al.* 2012). These reactions depended on a precipitate or a change in color caused by reagents that were specific to each family of active compounds.

2.6. Sampling

At the end of the experiment, rats were allowed to fast for 12 to 14 hours before blood samples were collected (Williams *et al.* 2020). Blood was spun for 10 minutes at 804.96 g to separate plasma samples, which were subsequently stored at -20 degrees Celsius for biochemical analysis.

2.7. Biochemical Blood Serum Assay

The effectiveness of lactate dehydrogenase (LDH) and creatinine kinase (CPK) enzymes in serum was determined using ELISA methods according to (Tietz 1999). Super oxide dismutase (SOD), catalase (CAT), total reduced glutathione (GSH), and lipid peroxidation (MDA) levels in the serum were measured spectrophotometrically in accordance with previously reported protocols (Misra and Fridovich 1972; Guidet and Shah 1989; Goth 1991; Al-Zamely *et al.* 2001). Using commercial kits from Linear, plasma levels of total cholesterol, triglycerides, and HDL cholesterol were measured as per the manufacturer's instructions. Using Friedewald's formulae, LDL- and VLDL-cholesterol levels were estimated analytically (Friedewald *et al.* 1972). Thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxin (T4) levels in plasma were also determined using commercial kits from Sigma (Sigma kit- SE120135, SE120121, SE120132) in line with the manufacturer's instructions.

2.8. Histological Techniques

The heart was eliminated, fixed in 10% buffered formalin, dehydrated with increasing amounts of ethanol, alcohol, and xylene, paraffin embedded, sectioned at a thickness of 5 µ, stained with hematoxylin and eosin (H and E), and examined under a microscope (Luna 1968).

2.9. Statistical Analysis

Values are given as mean+ standard deviation (SD) and evaluated using SPSS Version 21.2 using ANOVA and Duncan multiple comparison test. The threshold for significance was fixed at ($p \leq 0.05$).

3. Results

3.1. Phytochemical Analysis of Costus:

The following Table 1 presents the phytochemical characteristics of ethanolic extract of Costus Positive for alkaloids, glycosides, flavonoids, tannins, phenols, steroids, and resins, but negative for terpenoids and saponins.

3.2. Total Phenolic and Flavonoid Contents

The ethanolic extract of the root of the Costus plant contains a large concentration of phenols and flavonoids, according to the results of this study. The amount of phenols was 27,298 mg/g GAE whereas the number of flavonoids was 53,312 mg/g QE.

Table 1. Initial phytochemical screening of costus ethanol root extract

Constituents	Results
Alkaloids	+
Glycosides	+
Flavonoids	+
Tannins	+
Phenolic	+
Saponins	-
Steroids	+
Resins	+
terpenoids	-

Presence = +, non-presence = -

3.3. Heart Enzymes (CPK and LDH)

A significant ($p \leq 0.05$) increase in serum CPK and LDH activities was noticed in hypothyroid group (GII) when compared to control group (G1) as shown in Table 2. All the other groups (GIII, GVI and GV) showed a significant reduction ($p \leq 0.05$) in serum CPK and LDH activities compared to hypothyroid group (GII).

3.4. Oxidative–Antioxidants Parameters

Table 2 displays the values of the oxidative stress indicators. Hypothyroid rats displayed significantly lower levels of SOD, GSH, and CAT activity compared to control rats, and greater levels of MDA. When compared to the hypothyroid G (II), treatments with Costus ethanol root extract significantly improved the levels of CAT, SOD, and GSH and lowered MDA in animals G III and G IV Furthermore, the G (V) that got levothyroxine differed significantly from the control G (I).

3.5. Lipid Profile

Compared to the control group, the blood levels of total cholesterol (TC), triglycerides (TG), LDL cholesterol, and VLDL cholesterol increased significantly ($p \leq 0.05$) in the hypothyroid group (II), whereas the HDL cholesterol level decreased significantly ($p \leq 0.05$) (Table 3). All the groups (III, IV, and V) demonstrated a substantial decrease in blood lipid profile in comparison to the hypothyroid group (II).

3.6. Thyroid Function Test

Table 4 showed the effect of ethanol root extract of Costus on thyroid hormones PTU-induced hypothyroidism in female rats. Hypothyroid group (II) showed a significantly decrease ($p \leq 0.05$) in T3 and T4 levels and a very marked increase in TSH

Table 2. Effect of ECR on serum CPK and LDH activities and liver antioxidants profile and MDA

Group	I	II	III	IV	V
LDH IU/L	349.75±38.353^{c}	561.75±65.830^{a}	463.25±47.738^{b}	436.50±35.940^{b}	358.25±20.532^{c}
CPK IU/L	35.75±3.700^{d}	63.29±5.488^{a}	46.47±4.717^{c}	45.36±4.744^{c}	54.93±4.082^{c}
MDA mmol/ml	1.59±0.039^{c}	2.41±0.257^{a}	2.14±0.186^{b}	1.97±0.069^{b}	1.49±0.047^{c}
SOD mmol/ml	0.82±0.063^{a}	0.43±0.019^{c}	0.62±0.132^{b}	0.56±0.057^{b}	0.83±0.015^{a}
GSH mmol/ml	0.42±0.045^{bc}	0.28±0.021^{f}	0.37±0.031^{cd}	0.34±0.019d	0.45±0.029^{ab}
CAT mmol/ml	1.58±0.176^{a}	1.01±0.053^{c}	1.35±0.174^{b}	1.20±0.054^{b}	1.61±0.079^{a}

*There is no statistically significant between letters that are similar to one another. ($p \leq 0.05$, n = 5). *Values are expressed as mean ± SD.*I = control, II = hypothyroid, III = P-T-ECR, IV =CO-PTU-ECR, V = P-T-LT4

Table 3. Effect of ECR on lipid profile

Group	I	II	III	IV	V
TC mg/dl	99.53±5.909^{cd}	218.16±20.43^{a}	142.68±17.84^{b}	165.12±9.451^{b}	90.71±2.004^{cd}
TG mg/dl	58.46±5.470^{bc}	106.26±5.167^{a}	48.95±4.408^{c}	54.98±7.656^{bc}	67.28±2.320^{b}
HDL mg/dl	32.92±5.706^{a}	19.62±5.589^{c}	28.38±2.112^{ab}	34.79±2.821^{a}	24.52±3.736^{bc}
LDL mg/dl	54.91±9.349^{c}	177.29±22.806^{a}	104.50±16.495^{b}	119.33±12.806^{b}	55.80±2.823^{c}
VLDL mg/dl	11.69±1.094^{bc}	21.25±1.033^{a}	9.79±0.881^{c}	10.99±1.531^{bc}	13.45±0.464^{b}

*There is no statistically significant between letters that are similar to one another. ($p \leq 0.05$, n = 5). *Values are expressed as mean ± SD.*I = control, II = hypothyroid, III = P-T-ECR, IV =CO-PTU-ECR, V = P-T-LT4

Table 4. Effect of ECR on serum thyroid hormones level

Group	I	II	III	IV	V
TSH (mlu/l)	0.08±0.003^{c}	3.41±0.325^{a}	2.71±0.266^{b}	2.57±0.369^{b}	0.08±0.001^{c}
T4 (ng/ml)	5.37±0.507^{a}	2.24±0.688^{c}	3.00±0.157^{b}	2.99±0.487^{b}	5.75±0.134^{a}
T3 (ng/ml)	3.37±0.304^{a}	1.49±0.317^{c}	2.15±0.224^{b}	2.01±0.157^{bc}	3.47±0.531^{a}

*There is no statistically significant between letters that are similar to one another. ($p \leq 0.05$, n = 5). *Values are expressed as mean ± SD.*I = control, II = hypothyroid, III = P-T-ECR, IV =CO-PTU-ECR, V = P-T-LT4

levels when compared to the control group (I). The results showed that ethanol extract of Costus root indicated a significant increase in T3 and T4 level and decrease in TSH level in groups (III, IV) when compared to other groups. There was no statistically significant difference between the control group (I) and the levothyroxine-treated group (V). In addition, there was no significant difference for the effect of the treatment with the alcoholic extract of Costus root on the level of thyroid hormones in animal groups III and IV.

3.7. Histopathology

Slices of Group I tissue under light microscopy showed that the cytoarchitecture of the heart tissue was normal (Figure 1, section 1). GII revealed numerous histological changes in the heart tissue, including severe thickening of the coronary artery wall (TW), severe congestion (CON), severe lymphocyte infiltration (LI), mild degeneration (D), and mild hemorrhaging. It is also noted that there are traces of amyloid protein (Am) in section (2-A,B). Comparatively to G-II, tissue slices from both groups III-IV-V showed regenerative changes in cardiac tissue architecture. The outcomes are displayed in sections (3A, B), (4A, B), and (5A, B) of Figure 1.

4. Discussion

In hypothyroid individuals, an increase in LDH and CPK and a reduction in T4 have been found, indicating that they may be utilized as screening measures for hypothyroid and to a lesser degree hyperthyroid patients (McGrowder *et al.* 2011). Cytosolic enzymes including LDH, and CPK are diagnostic indicators of cardiac tissue injury. Heart enzyme levels indicate plasma membrane integrity and/or permeability (Thippeswamy *et al.* 2009). Our results show the rats with hypothyroidism have altered levels of the cardiac markers LDH and CPK in their blood (GII). These results are comparable to what Mahmoud *et al.* (2016) observed namely that oral administration of PTU 0.05% led to a significant increase in the concentration of the previously stated cardiac enzymes, they observed that hypothyroidism is associated with elevated levels of inflammatory markers and dyslipidaemia, which may contribute to the development of cardiovascular risk in the

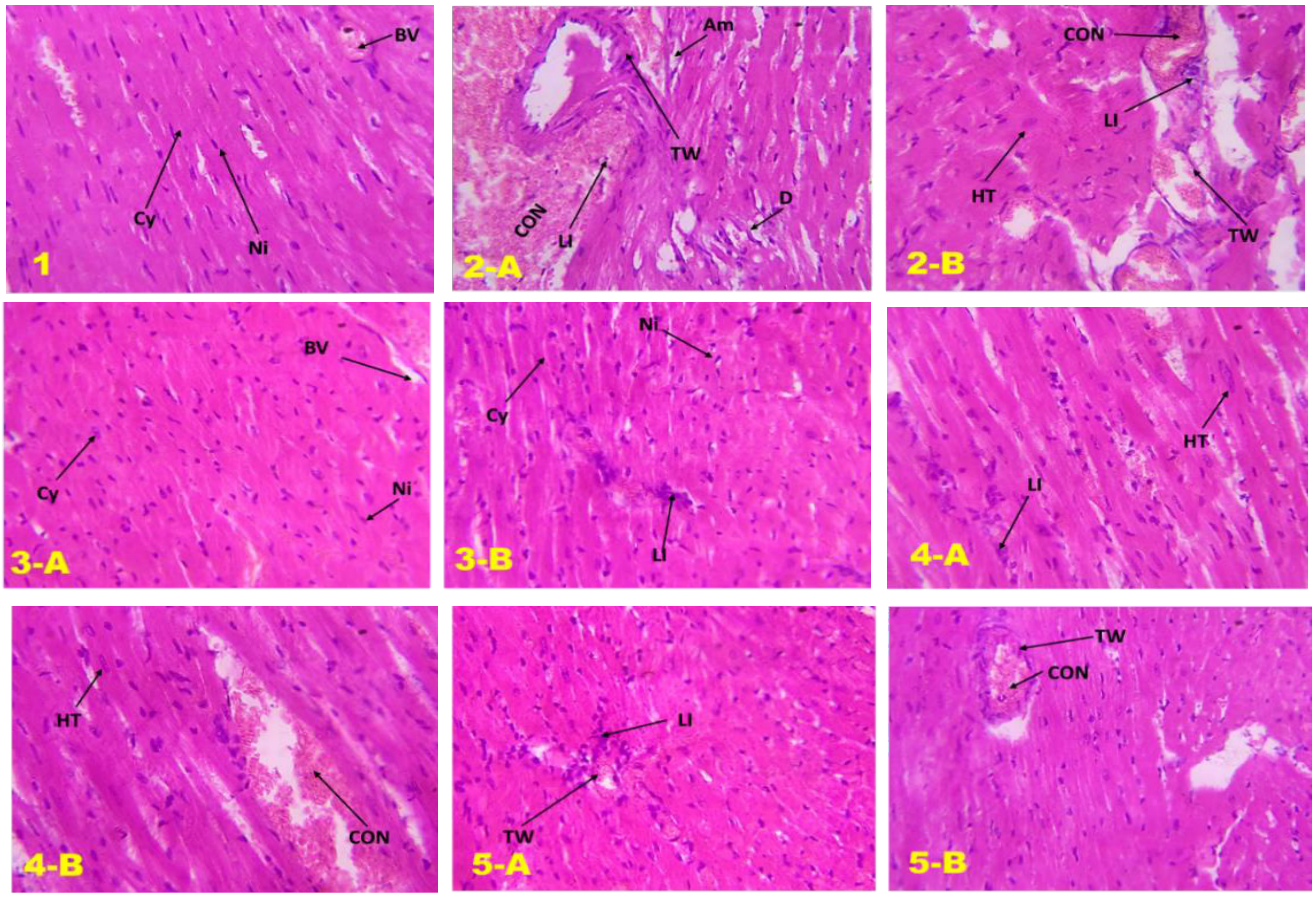

Figure 1. The effect of PTU (0.05% orally), alcoholic extract of Costus root (300 mg/kg), alcoholic extract + PTU, and LT4 after PTU treatment on female rats' heart tissue. Section (1) of the GI shows the normal cardiac cells (Cy) and their nuclei (Ni). Section (2 A,B) of the GII (Hypothyroid) shows a severe thickening of the coronary artery wall (TW) with severe congestion(CON), mild degeneration (D) and moderate hypertrophy (HT) of cardiac cells, significant lymphatic infiltration (LI) is also present. The coronary artery wall (TW), together with signs of cardiac cell hypertrophy (HT), is somewhat thickened in sections (3A,B) and (4A,B) of the GIII and IV, respectively. Section (5) of the GV indicates the presence of traces of degeneration (D) in the cardiac cells accompanied by mild lymphatic infiltration (LI) and mild thickening in the coronary artery wall (TW)

future. Significant changes in the level of cardiac markers (LDH, CPK) were seen in the group after oral administration of ECR (300 mg/kg) (III, IV). Our results are consistent with what was reported by Saleem and his team (2013). This may be due to the high content of flavonoids in the Costus root Compared to other plant parts (Naseer *et al.* 2022), which protect against inflammation and increase blood flow (Ullah *et al.* 2020).While the results of the histological analysis of the cardiac tissue of the G III-IV groups showed a remarkable improvement. These results are in agreement with what was reported by Elgharabawy (2021) and his group. Compared to the healthy group, the GII of rats had an increase in TSH, MDA, cholesterol, and triglycerides, a decrease in T4, T3 hormones, GSH, and SOD antioxidant, and a decrease in HDL level, while the (III, IV) groups demonstrated the opposite result. The present study's findings are consistent with those of Bolkiny *et al.* (2019), as well as Alnahdi *et al.* (2017), and Hegazy *et al.* (2020) and his team, this may be because the root of the Costus plant includes a number of physiologically active compounds, such as phenols and flavonoids, in addition to Dehydro Costus lactone, alkaloids, costunolide, and other medicinally significant chemicals. (Choi *et al.* 2009; Chang *et al.* 2012; Zhang *et al.* 2022). Researchers hypothesize that the ability of Indian Costus roots to reduce serum fat levels is due to the presence of the chemical component costunolide, which has the ability to increase HDL-cholesterol in the blood and decrease total cholesterol, triglyceride, and LDL-

cholesterol levels in the serum (Eliza *et al.* 2009). Finally, the groups administered with LT4 showed significant improvement in all variables studied possibly may be because LT4 is the homolog of T4 in terms of action (Kahaly 2021).

In conclusion, the current investigation revealed that hypothyroidism in female rats was related with abnormalities in the LDH and CPK indices, and that therapy with Costus ameliorated these variations in blood, suggesting a beneficial therapeutic impact of Costus in thyroid diseases. In the future, maybe molecular studies will reveal how the roots of this plant might reduce the amounts of LDH and CPK. Consequently, our findings suggest that As an adjuvant cotherapy for hypothyroidism disorders treated with levothyroxine, costus root extract may be used.

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