Cardioprotective Activity of Polyphenolic Extract of Tubers of Cyperus Rotundus and Taurine against Isoproterenol - induced Myocardial Infraction in Rats: Troponin-I and Histological Findings

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Abstract

Objective: The polyphenolic extract of tubers of cyperus rotundus and taurine was studied for the cardio protective activity using isoproterenol induced myocardial infraction in female rats. Materials and Methods: Sixty of female rats (Rattus norvegicus) were used in the present study. Animals were divided into 10 groups (6 for each group). MI was induced in rats with ISO (85 mg/kg) twice at an interval of 24 hrs. ISO produced significant alterations in the troponin-I and necrosis in the heart. The effect of polyphenolic extract of tubers of cyperus rotundus oral treatment for 21 days at two doses (15 mg and 30 mg/kg, body weight) and taurine at two doses (100 mg and 200 mg/kg, body weight) was evaluated against ISO-induced cardiac necrosis. Results: Significant myocardial necrosis and increase in serum levels of troponin-I were observed in ISO-treated animals as compared with the normal control animals. Polyphenolic extract of tubers of cyperus rotundus and taurine showed a significant cardio protective activity by lowering the levels of serum troponin-I and less myocardial necrosis. Conclusion: These findings the synergistic cardio protective effects of polyphenolic extract of tubers of cyperus rotundus and taurine during ISO-induced myocardial infraction in rats.

Keywords: Isoproterenol, Cardiac troponin I, Myocardial necrosis, Histology, Cyperus rotundus, Taurine.

Introduction

Myocardial infarction (MI) is an irreversible necrosis of tissue of a region of myocardium caused by ischemia, which is a perfusion imbalance between demand and supply of blood to the heart via the coronary circulation [1]. There is substantial evidence that ischemic tissue generates oxygen – derived free radical (oxygen radicals). Oxygen molecules containing odd number of electron, making them chemically reactive and often leading to chain reaction [2]. Recently, attention has been focused on non-nutrient phytochemicals and polyphenols such as the flavonoids, alkaloids, and xanthones derived from different plant species as potential therapeutic agents in the prevention and management of cardiovascular diseases due to their antioxidant nature [3]. Cyperus rotundus L. is one such plant, well-known in both Unani and Ayurveda for various potential including cardio protective activity. Locally called Motha, C. rotundus is known for its analgesic, antiarthritic, antidiabetic, anti diarrhoeal, anti-inflammatory, antimicrobial, antimutagenic, antioxidant, antipyretic, apoptotic as well as cytoprotective potential in traditional system of medicine [4]. The tubers of the plant have reported to possess antioxidant, free radical scavenging, hypolipidemic and hypotensive properties [4, 5]. Taurine, 2-aminoethanolsulfonic acid is one of the most abundant amino acids in the human body [6]. Taurine has since been found to act as an organic osmolyte, an antioxidant, a scavenger of carbonyl compounds, a modulator of cytosolic calcium, an analgesic, and to have neurotrophic properties [7, 8, 9]. According to animal studies, taurine may reduce blood lipid levels [10]. The cholesterol-lowering
effect of taurine can be explained by its effects on bile acid metabolism. Therefore, the aim to present study evaluates the effect of polyphenolic extract of tubers of *Cyperus rotundus* and taurine on ISO induced myocardial infraction in female rats.

**Materials and Methods**

**Experimental Animals**

Sixty healthy adult female rats (*Rattus norvegicus*) weighing (190-200 g) of 9-10 weeks old were used in the present study. Animals were housed in the animal house of Biology Department, Science College, Thi-Qar University, Iraq. Experimental animals were divided into ten groups (6 rats in each group). Upon the following designed.

Group-1: Control group; treated orally with distill water for 21 days.

Group-2: ISO (Isoproterenol hydrochloride) group; were injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h, i.e., on 22 th and 23 th day).

Group-3: treated orally with 15 mg /kg polyphenols of tubers of *C. rotundus* once daily for 21 days.

Group-4: treated orally with 30 mg /Kg polyphenols of tubers of *C. rotundus* once daily for 21 days.

Group-5: injected I.P. with (100mg/kg) of taurine for 21 days.

Group-6: injected I.P with (200mg/kg) of taurine for 21 days.

Group-7: pretreated orally with (15mg/kg) of polyphenols of tubers of *C. rotundus*. Once daily for 21 days, then injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h, i.e., on 22 th and 23 th day).

Group-8: pretreated orally with (30mg/kg) of polyphenols of tubers of *C. rotundus*. Once daily for 21 days, then injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h, i.e., on 22 th and 23 th day).

Group-9: injected with taurine (100mg/kg). Once daily for 21 days, then injected I.P. with (85 mg/ kg) of ISO, twice an interval of 24 h, i.e ., on 22 th and 23 th day).

Group-10: injected with taurine (200mg/kg). Once daily for 21 days, then injected I.P. with

**Plant Collection**

Samples of tubers of *Cyperus rotundus* were from local market of the Nasiriya city, Thi-Qar, Iraq. It cleaned after that broke and grinded it by using Electric grinder.

**Drugs and Chemicals**

ISO was purchased from (Cayman, British), taurine was obtained from (BDH, England). The cTnI ELISA kit, the used reagents were supplied by (Cayman, British).

**Extraction of Polyphenols**

Polyphenols compounds were extracted according to the method of Gayon, 1972[12]. (500 g) of plant powdered material was defatted by washing five times with n-hexane(1L) at (60°C), then it was mixed with (800mL) of acetic acid (2% v/v), the mixture were placed in conical flask volume (2000mL) and put in water bath (60°C) for 8 hrs, then the extraction process done by reflex condenser.

The mixture was heated at 50°C (water bath) for 15 min and left to cool. The suspension was filtered by Buchner funnel by what man No.1 filter paper and by the use of vacuum pump. The precipitate was canceled and the filtrate volume was measured. N-propanol was added in to filtrate with the same volume of filtrate. Then (NaCl) was added until to become solution super saturated. Then, it was evaporator by using rotary evaporator until drying.

**Biochemical Estimation in Serum**

5mL of blood were drawn from each animal of experimental groups, the sample was transferred into clean tube, left at room temperature for 15 minutes for clotting, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in a clean tube in the refrigerator at (-20 °C) until the time of assay. The serum was used for the estimation of troponin-I. It was measured according to the method of the cTnI ELISA kit; the used reagents were supplied by (Cayman, British).

**Histopathology of Heart Tissue**

Animals were sacrificed on the day of withdrawal of blood; hearts were removed, washed immediately with saline and then
fixed in 10% buffered formalin. The hearts stored in 10% buffered formalin were embedded in paraffin, sections cut at 5 mm and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histoarchitectural changes.

Statistical Analysis
Statistical analysis was done using the software SPSS version 15.0; the results were expressed as mean ± standard deviations (mean ± SD) with LSD. Two ways ANOVA-test was used to compare parameters in different studied groups. P-values (P ≤ 0.05) were considered statistically significant.

Results
Effect of Polyphenol Extract and Taurine on Troponin-I
Table 1 showed a significant increase (p≤0.05) in the concentration of serum troponin-I in group (2) in comparison with group (1). There were no significant differences in the concentration of serum troponin-I in groups (3, 4, 5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant decrease (p≤0.05) in the concentration of serum troponin-I in groups (7, 8, 9 and 10) in comparison with group (2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Troponin (ng/mL)</th>
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</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>0.38 ± 0.09</td>
</tr>
<tr>
<td>Group (2)</td>
<td>3.55 ±0.50</td>
</tr>
<tr>
<td>Group (3)</td>
<td>0.50 ±0.04</td>
</tr>
<tr>
<td>Group (4)</td>
<td>0.51 ±0.03</td>
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<tr>
<td>Group (5)</td>
<td>0.49 ±0.02</td>
</tr>
<tr>
<td>Group (6)</td>
<td>0.53 ±0.03</td>
</tr>
<tr>
<td>Group (7)</td>
<td>2.45 ±0.31</td>
</tr>
<tr>
<td>Group (8)</td>
<td>2.07 ±0.32</td>
</tr>
<tr>
<td>Group (9)</td>
<td>1.85 ±0.58</td>
</tr>
<tr>
<td>Group (10)</td>
<td>0.98 ±0.23</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D values with non-identical superscript (a, b or c …etc.) were considered significantly differences (P ≤ 0.05). SD: Standard deviation, LSD: Least Significant Difference.

Histopathological Studies of Heart Tissue of Control and Experimental Rats
No morphological damage was observed in heart of the rats in group (1) (Fig.1). Histopathological studies showed that animal’s treatment with ISO caused heart damage including severe necrosis, which was also accompanied by congestion and accumulation of inflammatory cells.

In contrast, cardiomyocytes necrosis and congestion appeared less marked in polyphenol extract and taurine treated rat. Degenerative changes in various degrees were observed in microscopic examination of heart in animal group treated with ISO to induced MI and heart damage in this group, showed a large ventricle area of degenerated, vacuolated myocardial cell associated with infiltration of mononuclear cell, and myocardial necrosis (Fig. 2). Light microscopically findings in groups (3, 4, 5 and 6) the animals treated with (15 mg/kg polyphenol extract,30 mg/kg polyphenol extract, animals treated with 100 and animale treated with 200mg/ kg taurine) respectively. It were noticed these groups have the same histological appearance as the control group, cardiomyocytes were normal and fibrosis, necrosis and proliferation or inflammatory infiltration was not observed.

In addition, in these groups, ventricle areas of degenerated and vacuolated myocardial cell were not seen. In the animal of group (7) which treated with ISO and 15 polyphenol extract revealed area of myocardial cell degeneration, moderate of degree of mononuclear cell infiltration and vacuolated myocardial cell (Fig.7). But, in animals treated with ISO and 30 polyphenol extract...
in the group (8) showed minimal of inflammatory cells and vacuolated myocardial cells (Fig.8). Also in present study we found the animals treated with ISO and 100 taurine, there was decreasing of heart damage, ventricular area of fibrosis, edema and inflammatory infiltration compared to ISO group (Fig.9). A decreasing in degenerative changes was noticed in animals treated with ISO and 200 taurine in (Fig. 10), we showed the section of heart of rat a few of mononuclear cells infiltration at the peripheral of ventricle.
Figure 4: Group 4: normal muscle without any pathologic change, (H&E) 10x

Figure 5: Group 5: normal muscle without any pathological change, (H&E) 40x

Figure 6: Group 6: normal muscle without any pathological change, (H&E) 40x

Figure 7: Group 7: showing interstitial moderate degree of infiltration of inflammatory cells (IC) and vaculated myocardial cell, (H&E) 10x
Discussion

Effect of Polyphenol Extract and Taurine on Troponin-I

The results indicated a significant increase of troponin level in group (2). In the present study agrees with the results of Afroz et al. 2016 and Jagadeesh et al. 2016 [12,13]. Myocardial infarction occurs when blood flows to an area of the cardiac muscle, is suddenly blocked leading to ischemia and to death of myocardial tissue. The heart becomes inflamed and necrotic at the point of obstruction [14]. When myocytes become necrotic, the integrity of the sarcolemmal membrane is compromised and intracellular macromolecules such as cTnI begin to diffuse into the cardiac interstitium and ultimately into the microvasculature and lymphatics in the region of the infarct; and eventually, they
are detected in the peripheral circulation [15]. The released kinetics of the various cardiac biologic markers depends in part on their location in the myocyte, their molecular weights, and the route by which they are cleared from the circulation [16]. Pretreatment with cyperus rotundus extract showed considerable decreased of cardiac troponin-I level in the serum of ISO induced myocardial infarcted rats.

The cardio protective property of cyperus rotundus extract, maintains myocardial membrane integrity and it could be due to the reduction of the degree of damage in the myocardium against free radicals produced by ISO autoxidation thereby restricting the leakage of cardiac troponin-I into the circulation [13,17,18]. The decrease of troponin level in rats challenged with ISO after pretreatment with taurine, it probably did so by maintaining the delicate balance of toxicity in cells of the myocardium.

Taurine concentration is a major factor involved in the processes of cell volume regulation [19]. Cell volume affects the most basic processes of cell function and as such it exerts an important role in the onset, severity, and outcome of myocardial infarction.

Histopathological Studies of Heart Tissue of Control and Experimental Rats

Microscopical examination of heart tissue sections of group (1) and groups (3, 4, 5 and 6) revealed normal architecture of the heart. But histopathological alterations were noticed in heart tissue sections of group (2) isoproterenol – injected rats as compared to normal cardiac architecture of group (1). Administration of isoproterenol –induced pathological alterations in the myocardial tissue such as a large area of the ventricle degenerated, vacuolated of myocardial cell, mononuclear cell infiltration, area of myocardial cell degeneration, myocardial necrosis.

This pathological aberration is probably related a decline in oxygen supply with paramount rise in wall – stress [20, 21, 22]. This indicates involvement of oxidative stress and inflammatory processes in ISO-induced myocardial injury [23]. Isoproterenol administration in large dose induces morphological and functional changes in heart leading to myocardial necrosis [24]. The toxic effects of catecholamines could be accounted due to the oxidation of hydroxyl groups in catecholamines leading to the conversion into quinones and the subsequent formation of adrenochromes which cause cell necrosis and contractile failure in the rats heart. Highly toxic oxygen-derived free radicals are produced during this phase which is detrimental to extra- and intracellular enzymes and proteins [25].

In contrast, degenerative changes appeared less marked in rat treated with polyphenol extract. This is line with the works of Muruganandam et al. 2002 and Prabhu et al. 2006 [26, 27] who reported that treatment with the polyphenol protect the cardiac tissue of diabetic and isoproterenol myocardial infarction in rats. Other study also that the active constituents of cyperus rotundus mainly flavonoids and other polyphenolic compound could be beneficial due to their antioxidant properties [28].

A decreasing in degenerative changes was noticed in animals pretreatment with taurine. Previous report by Tadros et al. 2005 [29] reported that taurine significantly ameliorated the morphometrical and histopathological aberrations in an experimental animal model of Huntington’s disease due to its antioxidant effect and γ-aminobutyric acid agonistic action. Histopathological study by Cetiner et al. 2005[30] showed that taurine protected against methotrexate-induced oxidant organ injury and inhibited leukocyte apoptosis in experimental rats. In the present study, the histopathological observations carried out in the heart tissue of control and experimental groups of rats confirmed the cytoprotective action of taurine in experimentally induced myocardial infarction condition.

Conclusion

The present study demonstrates that polyphenolic compounds of tubers of cyperus rotundus and taurine have potential to protect against MI by improving cardiac function and preserving histopathology. These findings indicate that polyphenolic compounds of tubers of cyperus rotundus and taurine could be a useful intervention in the management of cardiovascular disease.
References


