

Protective role of polyphenolic compounds extracted from *Cyperus rotundus* rhizomes and taurine on troponin-I and some oxidant/antioxidants parameters of female rats treated with isoproterenol – induced myocardial infraction.

Mohammed A . Auda

Email: aja_sci.dean@sci.utq.edu.iq,

Alyaa Majid

Email: aliaa.s_mschem@sci.utq.edu.iq,

College of Science, Department of Chemistry,

University of Thi-Qar, Iraq.

Khalid G . Al-Fartosi

Email: khalid.f_bio@sci.utq.edu.iq,

College of Science, Department of Biology,

University of Thi-Qar, Iraq.

Abstract

The present study was designed to investigate the effects of polyphenolic compounds extracted from *Cyperus rotundus* rhizomes and taurine on isoproterenol induced myocardial infraction in female rats. 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, serum MDA, tissue MDA, Cp, Alb, uric acid, serum GSH and tissue GSH. The effect of polyphenolic compounds from *Cyperus rotundus* rhizomes 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. The results showed a significant increase in the level of serum troponin-I, serum MDA, tissue MDA, Cp and uric acid in ISO-treated animals as compared with the control animals. While, the results showed asignificant decrease in the level of serum Alb, serum GSH and tissue GSH in ISO- treated animals as compared with the control animals. Polyphenolic compounds from *Cyperus rotundus* rhizomes and taurine

showed a significant cardioprotective activity by lowering the levels of serum troponin-I, serum MDA, tissue MDA, Cp and uric acid and elevate the levels of Alb, serum GSH and tissue GSH. This study concluded that the polyphenolic compounds from *Cyperus rotundus* rhizomes and taurine possess cardioprotective activity against Isoproterenol induced myocardial infraction in rats.

Keywords: Cardioprotective; Antioxidants; *Cyperus rotundus*; Isoproterenol; Taurine, Myocardial infraction .

الدور الوقائي لمركبات متعددة الفينول المستخلصة من درنات السعد المستدير وبعض معايير الاكسدة/ مضادات الاكسدة في اناث I والتايورين على تروبونين – الجرذان المصابة بأحتشاء عضلة القلب المستحدث بواسطة الايزوبروتيرينول. أ.د. محمد عجة عودة. أ.د. خالد كاطع الفطوسي. م. علياء ماجد.

الخلاصة:

هدفت الدراسة الحالية لمعرفة تاثير مركبات متعددة الفينول المستخلصة من درنات السعد المستدير والتايورين على الايزوبروتيرينول المستحدث احتشاء عضلة القلب في اناث الجرذان. استخدمت في هذه الدراسة 60 من اناث الجرذان المختبرية وقسمت الحيوانات الى 10 مجاميع كل مجموعة تحتوي على (6 جرذ) ز استحدث احتشاء عضلة القلب بواسطة حقن الايزوبروتيرينول وبتركيز (85 ملغم/ كغم) في الغشاء البريتوني لأناث الجرذان المختبرية مرتين كل 24 ساعة . اظهرت النتائج ان حقن الايزوبروتيرينول في الغشاء البريتوني لاناث الجرذان المختبرية قد ادى الى ارتفاع معنوي في مستويات troponin-I, serum MDA, tissue MDA, Cp and uric acid في مجموعة الثانية مقارنة مع المجموعة الاولى. في حين اظهرت النتائج ان حقن الايزوبروتيرينول قد ادى الى انخفاض معنوي في مستويات Alb, serum GSH and tissue GSH في المجموعة الثانية مقارنة مع المجموعة الاولى. وتبين من خلال الدراسة الحالية ان معالجة اناث الجرذان المصابة باحتشاء عضلة القلب بمركبات متعددة الفينول مستخلصة من درنات السعد المستدير والتايورين قد سبب انخفاضا معنويا في مستويات troponin-I, serum MDA, tissue MDA, Cp and uric acid وارتفاعا معنويا في مستويات Alb, serum GSH and tissue GSH. ومن ذلك نستنتج ان مركبات متعددة الفينول المستخلصة من درنات السعد المستدير والتايورين لها دور وقائي ضد احتشاء عضلة القلب.

1- Introduction:

Myocardial infarction (MI) is a clinical problem defined as acute necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (Kesarwani and Azmi,

2014). Ischemia caused due to reduced blood supply to heart causes several biochemical alterations which may lead to cardiac dysfunction and ultimately cell death (Kumar and Kumar, 2013). MI is a condition of heart muscles death when one or more coronary arteries which supply oxygen-rich blood to the heart muscle become suddenly blocked (Bishop, 2005). Important risk factors are earlier cardiovascular disease, old age, tobacco smoking, high blood levels of certain lipids (LDL and TG) and small levels of (HDL) cholesterol, diabetes, high blood pressure, lack of physical activity, obesity, chronic kidney disease, excessive alcohol consumption and the use of cocaine and amphetamines (Graham *et al.* , 2007).

Scientists have reported that rhizomes and tubers of *C. rotundus* possess antidiarrheal, antioxidant, anti-inflammatory, antimutagenic, antiperiodic, anticonvulsant, anti-saturative, antipyretic, antifungal, antidiabetic, antimalarial, antilipidemic, antibacterial, antiviral, anti-tumoral, cardioprotective, and wound-healing properties (Shivakumar *et al.*, 2009 ; Dang *et al.*, 2011).

Taurine, 2-aminoethanosulphonic acid, is one of the most abundant amino acids in the human body (Lourenço and Camilo, 2002). 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 (Pop-Busui *et al.*, 2001; Li *et al.*, 2005; Schaffer *et al.*, 2009). According to animal studies, taurine may reduce blood lipid levels (Niittynen *et al.*, 1999). The cholesterol-lowering effect of taurine can be explained by its effects on bile acid metabolism. Therefore, the aim of the present study to evaluate the effect of polyphenolic compounds extracted from *cyperus rotundus* rhizomes and taurine on some biochemical parameters of ISO induced Myocardial infraction in female rats .

2- Materials and Methods:

2-1-Plant Collection:

Samples of *Cyperus rotundus* rhizomes were collected from local market of the Nasiriya city, Thi-Qar, Iraq. It cleaned after that broke and grinded it by using electric grinder. (Denver,Germany).

2-2- Drugs and chemicals:

ISO was purchased from (Cayman, British), taurine, trichloro acetic acid (TCA), thiobarbituric acid (TBA), para phenylene diamine (PPD), azide sodium, Tris- HCL and sodium carbonate anhydrate was purchased from (BDH, England), Sodium phosphate acidic NaH_2PO_4 , Sodium phosphate basic Na_2HPO_4 were purchased from Fluka Garnatie (Switzerland), Reduced Glutathione was purchased from (CDH, India).

2-3-Extraction of Polyphenols:

Polyphenols compounds were extracted according to the method of (Gayon, 1972). (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 Whatman 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

2-4- 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 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 22th and 23th 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 22th and 23th day).

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

Group-10: injected with taurine (200mg/kg). once daily for 21 days, then injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h , i.e ., on 22th and 23th day).

2-5- a- 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 (My biosource, USA), MDA was measured according to the method of (Muslih et al.,2002), Cp was measured according to the method of (Menden et al., 1977), Albumin and uric acid were measured with commercial kits , the used reagents were supplied by (Biolabo, france) and GSH was measured according to the method of Ellman s method (Ellman, 1959).

2-5-b- Biochemical Estimation in Heart Tissue Homogenate:

The heart tissues were dissected out and washed in ice-cold saline. The tissue (100mg) was weighted and homogenized in 5mL of 0.1 M Tris-HCL buffer (pH 7.4) in ice-cold condition. The homogenate was centrifuged at 2500 and the clear supernatant solution was taken for assay of malondialdehyde (MDA) according to the method (Utely, 1967) and GSH according to the method Ellman s (Ellman, 1959)..

2-6- Statistical Analysis:

Statistical analysis was done using the software **SPSS** version 15.0; The results were expressed as mean \pm standard deviations (mean \pm SD) and LSD. Two way ANOVA-test was used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant.

3- Results :

The results showed a significant increase ($p \leq 0.05$) in the concentration of serum troponin-I, serum malondialdehyde (MDA) and tissue malondialdehyde in group (2) in comparison with group (1). There was non significant difference in the concentration of serum troponin-I, serum malondialdehyde (MDA) and tissue malondialdehyde (MDA) in groups (3, 4, 5 and 6) in comparison with group (1) and between them. Also, there was a significant decrease ($p \leq 0.05$) in the concentration of serum troponin-I, serum MDA and tissue MDA in groups (7, 8, 9 and 10) in comparison with group (2). The results indicated non significant difference in the concentration of serum MDA and tissue MDA between groups (9 and 10) .(table, 1).

The results showed a significant increase ($p \leq 0.05$) in the concentration of serum ceruloplasmin (Cp) and uric acid in group (2) in comparison with group (1). There was non significant difference in the concentration of serum ceruloplasmin and uric acid in groups (3 and 4) in comparison with group (1) and between them. Also, there was a significant decrease ($p \leq 0.05$) in concentration of serum uric acid in groups (5 and 6) in comparison with group (1). Results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum Cp and uric acid in groups (7, 8, 9 and

10) in comparison with group (2). While there was non significant difference in the concentration of serum Cp and uric acid between groups (7 and 8) , while showed non significant difference in the concentration of serum Cp and uric acid between groups (9 and 10). In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum albumin, serum GSH and tissue GSH in group (2) in comparison with group (1). There was non significant difference in the concentration of serum Alb, serum GSH and tissue GSH in groups (3, 4, 5, 6) in comparison with group(1) and between them. Also, there was a significant increase ($p \leq 0.05$) in the concentration of serum Alb, serum GSH and tissue GSH in groups (7, 8, 9 and 10) in comparison with group (2), while non significant difference in the concentration of serum Alb, serum GSH and tissue GSH between groups (7and 8). Results indicated non significant difference in concentration of serum GSH and tissue GSH between groups (9 and 10). Also, there was a significant decrease ($p \leq 0.05$) in concentration of serum Alb in group (10) in comparison with group (9).(table, 2).

Table (1): Effect of polyphenol extract and taurine on troponin-I and malondialdehyde concentration .

Group	Troponin (ng/mL)	MDA Serum ($\mu\text{mol/L}$)	MDA Tissue (nmol/mg protein)
Group (1)	0.09^e ±0.38	1.51 ± 0.28^f	1.41 ± 0.11 ^e
Group (2)	0.50^a ±3.55	4.65 ± 0.37^a	3.91 ± 0.27 ^a
Group (3)	0.04^e ±0.50	1.56 ± 0.31^f	1.42 ± 0.41 ^e
Group (4)	0.03^e ±0.51	1.65 ± 0.33^f	1.46 ± 0.29 ^e
Group (5)	0.49 ±0.02^e	1.60 ± 0.22^f	1.44 ± 0.39 ^e
Group (6)	0.53 ± 0.03^e	1.62 ± 0.35^f	1.43 ± 0.14 ^e
Group (7)	2.45 ± 0.31^b	3.99 ± 0.37^b	3.21 ± 0.32 ^b
Group (8)	2.07 ± 0.32^c	3.42 ± 0.79^d	2.75 ± 0.48 ^c

Group (9)	1.85 ± 0.58^c	2.20 ± 0.17^e	2.23 ± 0.35 ^d
Group (10)	0.98 ± 0.23^d	2.20 ± 0.17^e	1.88 ± 0.50 ^d
LSD	0.31	0.40	0.38

-Value refer to mean ± SD.

-Different letters refer to a significant difference at (p≤0.05).

- Same letters refer to non significant difference at (p≤0.05).

Table(2):Effect of polyphenol extract and taurine on antioxidants parameters.

Group	Cp (g/L)	Alb (g/dL)	Uric acid (mg/dL)	GSH serum μmol/L	GSH tissue μg/mg protein
Group (1)	3.03 ± 0.59^c	4.91 ± 0.62^a	3.23 ± 0.35^e	586.11 ± 74.41^a	0.63 ± 0.04 ^a
Group (2)	5.59 ± 0.58^a	3.03 ± 0.47^d	6.51 ± 0.47^a	365.60 ± 51.02^d	0.26 ± 0.04 ^d
Group (3)	3.08 ± 0.26^c	4.80 ± 0.16^a	3.51 ± 0.50^e	595.59 ± 63.70^a	0.68 ± 0.08 ^a
Group (4)	3.05 ± 0.06^c	4.83 ± 0.12^a	3.92 ± 0.57^{de}	591.99 ± 44.97^a	0.73 ± 0.05 ^a
Group (5)	3.06 ± 0.56^c	4.85 ± 0.38^a	4.01 ± 0.35^d	597.17 ± 38.53^a	0.71 ± 0.07 ^a
Group (6)	3.09 ± 0.61^c	4.89 ± 0.14^a	4.11 ± 0.32^d	589.17 ± 58.12^a	0.74 ± 0.06 ^a
Group (7)	3.96 ± 0.35^b	3.65 ± 0.16^c	5.90 ± 0.50^b	459.15 ± 23.76^c	0.41 ± 0.05 ^c
Group (8)	3.89 ± 0.52^b	3.70 ± 0.61^c	5.75 ± 0.43^b	481.36 ± 67.54^{bc}	0.44 ± 0.06 ^{bc}
Group (9)	3.78 ± 0.62^b	4.25 ± 0.58^c	4.93 ± 0.44^c	496.47 ± 67.54^{bc}	0.48 ± 0.06 ^b
Group (10)	3.68 ± 0.35^b	4.30 ± 0.62^b	4.69 ± 0.54^c	519.93 ± 56.62^b	0.50 ± 0.11 ^b
LSD	0.51	0.46	0.48	60.70	0.07

Legend as in the table (1).

4- Discussion :

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 (Sunmonu and Afolayan, 2010, Afroz *et al.*, 2016, Jagadeesh *et al.*, 2016). 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 (Takeda *et al.*, 2003). Treatment with polyphenolic compounds extracted from *Cyperus rotundus* rhizomes 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 (Jahan *et al.*, 2012; Parikh *et al.*, 2015; Jagadeesh *et al.*, 2016). The decrease of troponin level in rats challenged with ISO after pretreatment with taurine, it probably did so by maintaining the delicate balance of tonicity in cells of the myocardium. Taurine concentration is a major factor involved in the processes of cell volume regulation (Trachtman *et al.*, 1990). 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.

In the present experiment, the generation of free radicals in the myocardium might have exceeded the ability of the free radical scavenging enzymes to dismutate isoproterenol-generated free radical formation resulting in myocyte lesions and reduction of scavengers. Pathogenesis of myocardial injury is associated with the generation of reactive oxygen species. (Roig-Perez *et al.*, 2004; Sivakumar *et al.*, 2007). The decrease of MDA levels as reported in this study was

compatible with the findings of Djeridane *et al.* (2006), who reported that phenolic compounds especially the flavonoids act as scavengers of free radicals as well as inhibitors of lipid peroxidation. *Cyperus rotundus* is rich in flavonoids such as rutin (Al-sanfi, 2016), its valuable compounds and their antioxidant properties (Nagulendran *et al.*, 2007).

In the present study, the groups (9 and 10) rats treated with taurine showed a significant ($p \leq 0.05$) decrease in the level of lipid peroxidation in serum and heart tissue compared to group (2) isoproterenol-injected rats. This was probably achieved by means of its antioxidant nature (Rodriguez-Martinez *et al.*, 2004) against lipid peroxidation induced by isoproterenol. The unpaired electron present in the hydroxyl radicals generated by isoproterenol might have been trapped and subsequently dismutated by taurine.

In this investigation, the levels of Cp in serum of group (2) were significantly higher ($p \leq 0.05$) compared with group (1), the increase of CP levels were independently associated with increased risk of cardiovascular and all causes mortality in CAD represented by angiography results (Grammer *et al.*, 2014). In the present study, the groups (7 and 8) rats treated with polyphenolic compounds extracted from *Cyperus rotundus* rhizomes showed a significant decrease ($p \leq 0.05$) in the level of ceruloplasmin in compared to group (2). may be due to the polyphenols decrease an activity of numerous proteins associated with oxidative stress. (Park and Dong, 2003). In the present study. prior administration of taurine resulted in a significant reduction ($p \leq 0.05$) in the levels of ceruloplasmin in serum of groups (9 and 10) rats compared with group (2), indicating the prevention of oxidative stress (Franconi *et al.*, 2004). which may associate with the beneficial role against the complications. Administration of taurine protected the tissue damage produced by the acute sublethal dose of γ - irradiation in rats by decreasing oxidative stress (Monira *et al.*, 2015). There was a significant decrease ($p \leq 0.05$) noticed in the level of Albumin in serum of group (2) compared to group (1), could be due to the increase in synthesis of lipid peroxide and elevation in formation of free radicals which result in the increase of membranes permeability and leaking the proteins outside the vascular system (Vlassara *et al.*, 2001).

In the present study, the groups (7 and 8) rats treated with polyphenolic compounds extracted from *cyperus rotundus* rhizomes showed a significant increase ($p \leq 0.05$) in the level of Albumin in compared to group (2), could be due to the that polyphenols could induce decrease in lipid peroxidation processes as well as increase in the activities of plasma protein thiols as albumin and other serum proteins in both animal and human (Vita, 2005). In the present study. prior administration of taurine resulted in a significant increase ($p \leq 0.05$) in the levels of Albumin in serum of groups (9 and 10) rats compared with group (2), this protective effect of taurine may therefore be related to its direct antioxidative effect. (Timbrell *et al.*, 1995; Balkan *et al.*, 2005). Although the exact mechanism for its antioxidative effect is unclear, this effect has been attributed to its scavenging activity (Cetiner *et al.*, 2005). In the present study, the groups (7 and 8) rats treated with polyphenolic compounds extracted from *cyperus rotundus* rhizomes showed a significant decrease ($p \leq 0.05$) in the level of uric acid in compared to group (2), the *C. rotundus* significantly inhibited the activity of xanthine oxidase (Swaminathan *et al.*, 2010). In the present study. prior administration of taurine resulted in a significant decrease ($p \leq 0.05$) in the levels of uric acid in serum of groups (9 and 10) rats compared with group (2), the effect of taurine on MI showed the results of improvements in oxidative stress (Madsen-Bouterse and Kowlura, 2008).

In other study, reported that this decrease GSH level in ISO induced rats might be due to its increased utilization in protecting – SH group containing proteins from free radicals (Patel *et al.*, 2010). treatment with polyphenolic compounds extracted from *cyperus rotundus* rhizomes prevented the ISO induced LPO and increase the level of GSH in serum and tissue, which is due to antioxidant of *cyperus rotundus* (Safriani *et al.*, 2016). Active phyto constituents may protect against myocardial ischemia reperfusion injury by enhancing antioxidant mechanism mediated by glutathione and alpha tocopherol in rats (Upaganlawar *et al.*, 2009). A significant rise ($p \leq 0.05$) in the level of GSH was noticed in serum and heart tissue in groups (9 and 10) compared to group (2), indicating that antioxidant status was operating at a higher rate in taurine-treated rats for the counteraction of lipid peroxides.

5- Conclusion:

The present study demonstrated that polyphenol compounds of *Cyperus rotundus* rhizomes and taurine have a cardioprotective effect against ISO-induced MI in rats. The mechanism of the cardioprotective effect may involve prevention of lipid peroxidation and preservation of antioxidant (ceruloplasmin, Albumin, uric acid and GSH) as well as scavenging of free radicals. Polyphenol compounds of *Cyperus rotundus* rhizomes and taurine reduced the deleterious effects in the setting of myocardial infarction through potent antioxidant actions. These findings indicate that *Cyperus rotundus* and taurine could be a useful intervention in the management of cardiovascular disease.

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