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Using polyphenolic extract for tubers *Cyperus rotundus* to prevent myocardial infarction

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Abstract:
The present study aimed at determining the effect polyphenolic extract for tubers cyperus *rotundus* on myocardial infarction in female rats. In this study, 36 female rats were used and the animals were divided into 6 groups of each group containing (6 rats). Myocardial infarction was induced by injection of isoproterenol at (85 mg/kg) in the intraperitoneally of female rats at an interval of 24 h for two days. The results showed that the injection of isoproterenol in the intraperitoneally of female rats has caused a significant increase in levels of Troponin-I, lactate dehydrogenase(LDH), creatine phosphokinase (CPK), alanine transferase (ALT), aspartic transferase (AST) and necrosis in the second group compared to the first group. It was found in the current study that treatment of female rats with myocardial infarction with phenolic extract of tubers of *cyperus rotundus* has caused a significant decrease in levels of Troponin-I, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), alanine transferase (ALT), aspartic transferase (AST). Necrosis decreased in the group treated with phenolic extract of tubers of *cyperus rotund*us. From this we conclude that the phenolic extract of *cyperus rotundus* of tubers has protective role against myocardial infarction.
Keywords: Cyperus rotundus, Myocardial infarction, cardiac markers, polyphenol.

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). MI is a condition of heart muscles damge and refers to tissue death when one or more coronary arteries which supply oxygen-rich blood to the heart muscle become suddenly blocked (Bishop et *al*., 2005). Usually this is because one of the coronary arteries that supplies blood to the heart develops a blockage due to an unstable buildup of white blood cells, cholesterol and fat. MI can be defined from a number of different perspectives related to clinical, (ECG), biochemical and pathologic characteristics. One of the proposed mechanisms of infraction refer to the free radicals that produced by multiple mechanisms and play an important role in cell membrane damage including mitochondrial membrane damage, thereby produce the necrosis which lead to infraction (Opie, 2004).

Studies on the use of plant extracts for controlling disease have shown the importance of natural chemicals (phytochemicals) as possible sources of non-phytotoxic and easily biodegradable, alternative fungicides and antibiotics (Okigbo *et al*., 2009) . 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 , Majid *et al*., 2018, Auda *et al*., 2018).

2- Materials and Methods:

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).

Drugs and chemicals:

ISO was purchased from (Cayman, British), taurine was purchased from (BDH, England), Troponin-I was purchased from (CDH, India), Alanine transaminase (ALT), Aspartate transaminase kits were purchased from Randox (UK), creatine phosphokinase, Lactate dehydrogenase kits were purchased from biolabo (france).

Polyphenol extraxted:

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 was placed in conical flask volume (2L) 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.

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: pretreated orally with (15mg/kg) of phenolic extract 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-6: pretreated orally with (30mg/kg) of phenolic extract 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).

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), Troponin-I was purchased from (CDH, India), Alanine transaminase (ALT), Aspartate transaminase were measured with commercial kits. the used reagents were supplied by (Randox, UK) creatine phosphokinase, Lactate dehydrogenase were measured with commercial kits. the used reagents were supplied by (Biolabo, france).

Statistical Analysis:
Statistical analysis was done using the software SPSS version 15.0; The results were expressed as mean ± standard deviations (mean ± 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:

Table (1) and figure (2) showed a significant increase ($p \leq 0.05$) in the concentration of serum Troponin-I in group (2) in comparison with group (1). There was non significant differences in the concentration of serum Troponin-I in group (3,4,5 and 6) in comparison with group (1) and between them. In table (1) and figure (2) there was a significant increase ($p \leq 0.05$) in the concentration of serum lactate dehydrogenase (LDH) in group (2) in comparison with group (1). There was non significant difference in the concentration of serum lactate dehydrogenase (LDH) in groups (3,4,5 and 6) in comparison with group (1) and between them. Also the same table (1) and figure (3) showed a significant increase ($p \leq 0.05$) in the concentration of serum creatine phosphokinase (CPK) in group (2) in comparison with group (1). The results showed non significant difference in the concentration of serum creatine phosphokinase (CPK) in groups (3,4,5 and 6) in comparison with group (1) and between them. The present study showed a significant increase ($p \leq 0.05$) in the concentration of serum alanine transaminase (ALT) in group (2) in comparison with group (1). Also, there was non significant difference in the concentration of serum alanine transaminase (ALT) in groups (3 and 5) in comparison with group (1) and between them. In the same table, the results indicated a significant increase ($p \leq 0.05$) in the concentration of serum alanine transaminase (ALT) in groups (4) in comparison with group (1). There was a significant increase ($p \leq 0.05$) in concentration of serum alanine transaminase (ALT) in group (6) in comparison with group (1, 3 and 5). The results indicated non significant difference in concentration of serum alanine transaminase (ALT) between groups (3, 4 and 5). Also table (1) and figure (5) showed a significant increase ($p \leq 0.05$) in the concentration of serum aspartate transaminase (AST) in group (2) in comparison with group (1). There was non significant difference in the concentration of serum aspartate transaminase (AST) in groups (4 and 6) in comparison with group (1). Also, there was a significant increase ($p \leq 0.05$) in the concentration of serum aspartate transaminase (AST) in groups (3 and 5) in comparison with group (1). There was non significant difference in concentration of serum aspartate transaminase(AST) between groups (3, 4, 5 and 6).

Table (1): Effect of phenolic extract and taurine on cardiac markers

Group	Troponin-I (ng/mL)	LDH (U/L)	CPK (U/L)	ALT (U/L)	AST (U/L)
Group (1)	0.38 ± 0.09 ^{e}	238.20 ± 28.73 ^{e}	125.50 ± 8.26 ^{e}	32.40 ± 3.45 ^{g}	55.70 ± 1.41 ^{f}
Group (2)	3.55 ± 0.50 ^{a}	521.60 ± 34.28 ^{a}	281.26 ± 36.76 ^{a}	63.56 ± 5.21 ^{a}	99.33 ± 12.34 ^{a}
Group (3)	0.50 ± 0.04 ^{e}	250.86 ± 33.88 ^{e}	126.70 ± 2.53 ^{e}	33.80 ± 3.79 ^{fg}	62.51 ± 2.42 ^{e}
Group (4)	0.51 ± 0.03 ^{e}	242.36 ± 31.76 ^{e}	129.36 ± 5.26 ^{e}	36.72 ± 2.42 ^{ef}	59.24 ± 3.24 ^{ef}
Group (5)	0.49 ± 0.02 ^{e}	249.92 ± 28.51 ^{e}	133.36 ± 5.37 ^{e}	34.80 ± 3.14 ^{fg}	61.88 ± 5.25 ^{e}
Group (6)	0.53 ± 0.03 ^{e}	246.76 ± 44.09 ^{e}	131.14 ± 2.86 ^{e}	39.60 ± 1.81 ^{e}	57.60 ± 3.32 ^{ef}
LSD	0.31	40.82	17.78	3.26	6.03

❖ Values refer to mean ± SD.

- Different letters refer to a significant difference at ($p \leq 0.05$).
- Same letters refer to non significant difference at ($p \leq 0.05$).

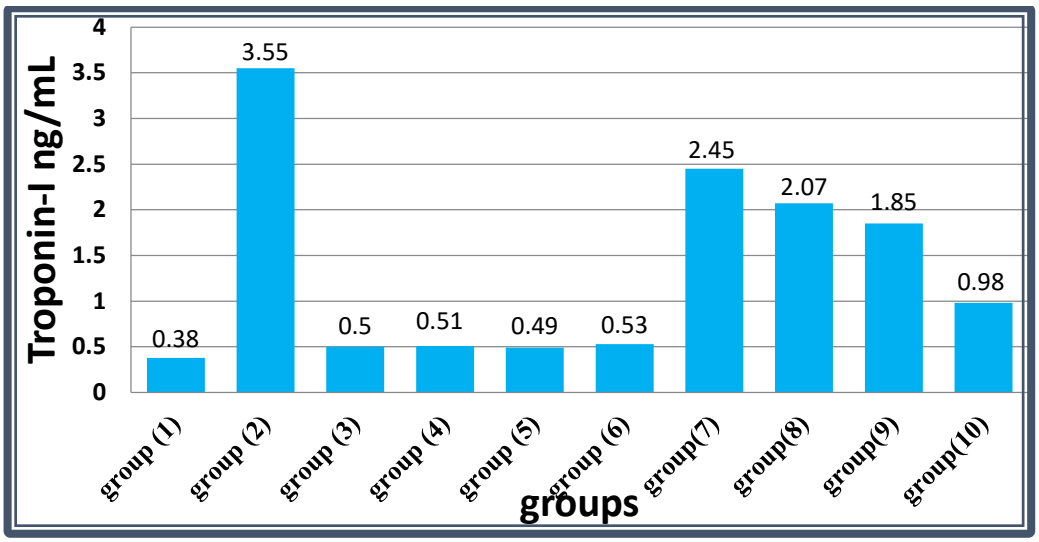

Figure (1): Effect of phenolic extract and taurine on Troponin-I levels.

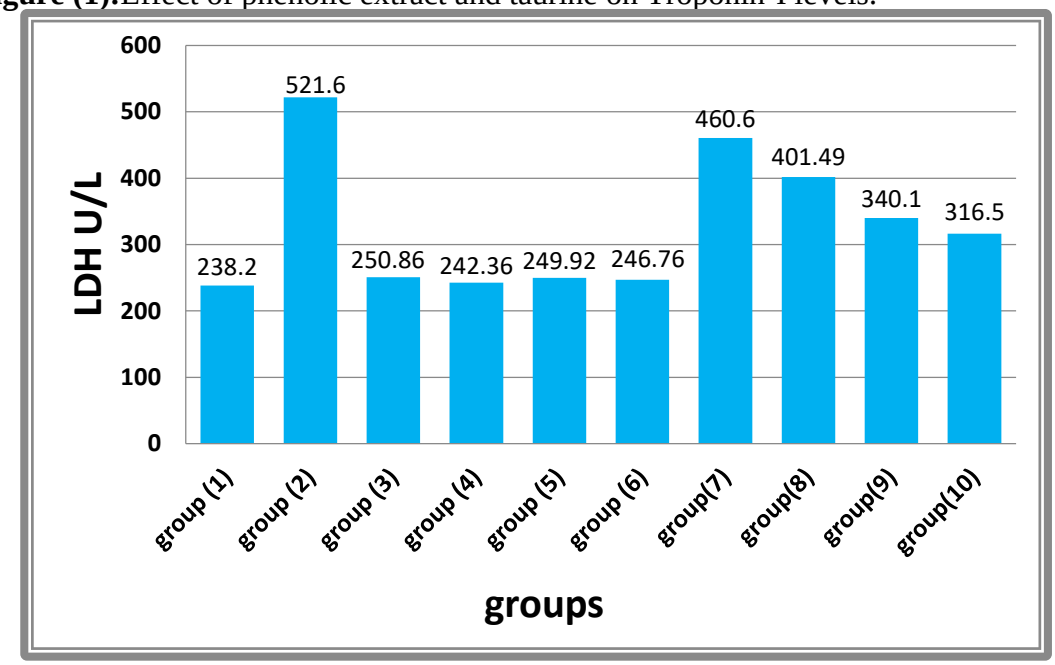

Figure (2): Effect of phenolic extract and taurine on LDH levels.

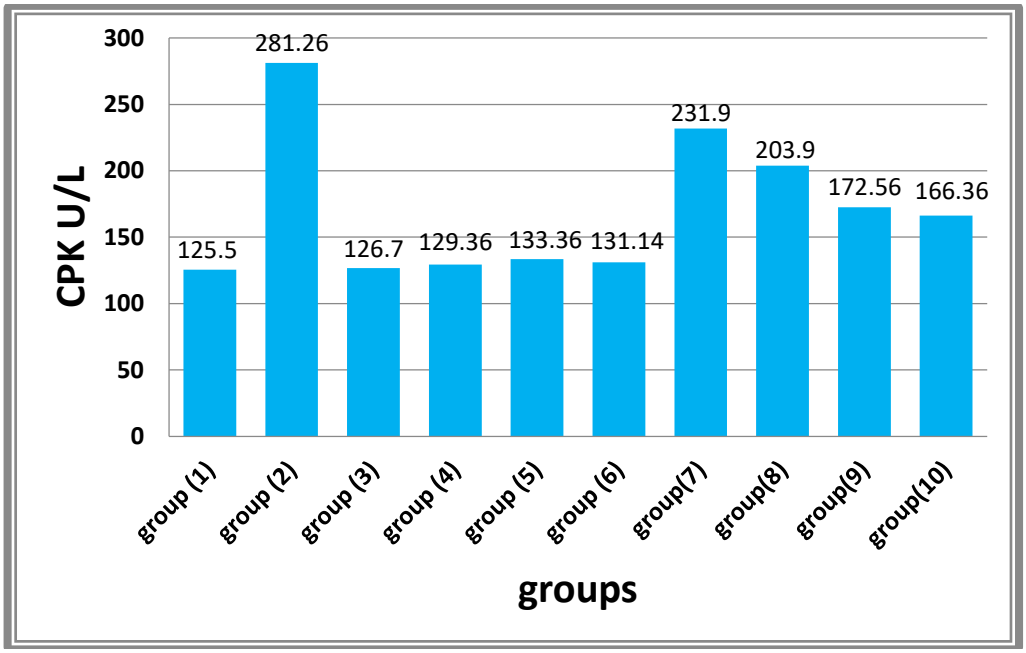

Figure (3): Effect of phenolic extract and taurine on CPK levels.

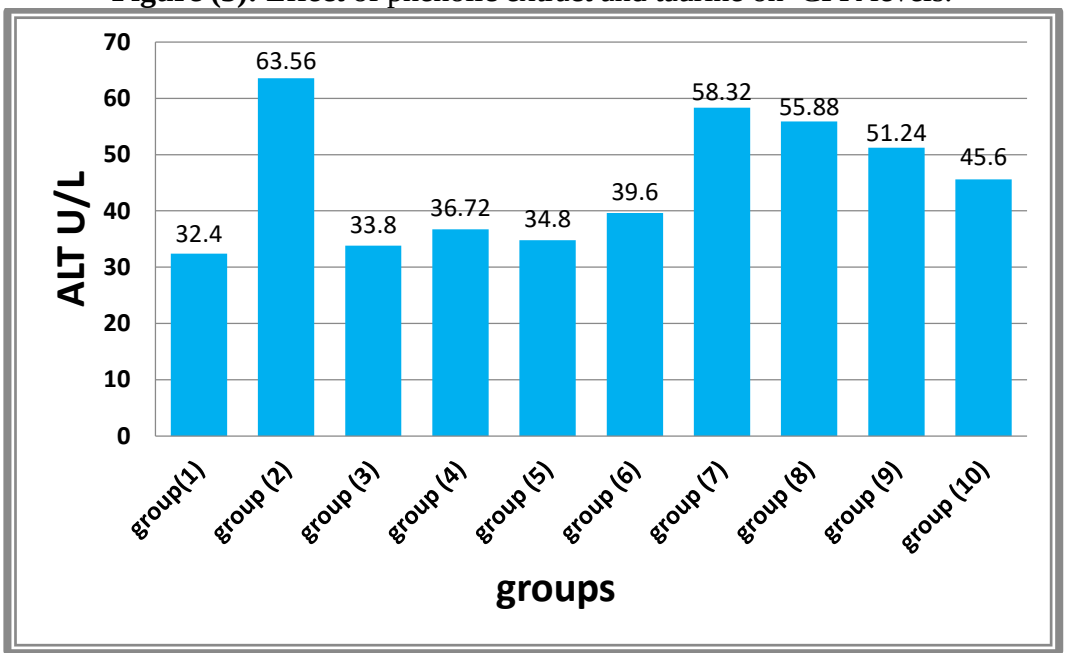

Figure (4): Effect of phenolic extract and taurine on ALT levels.

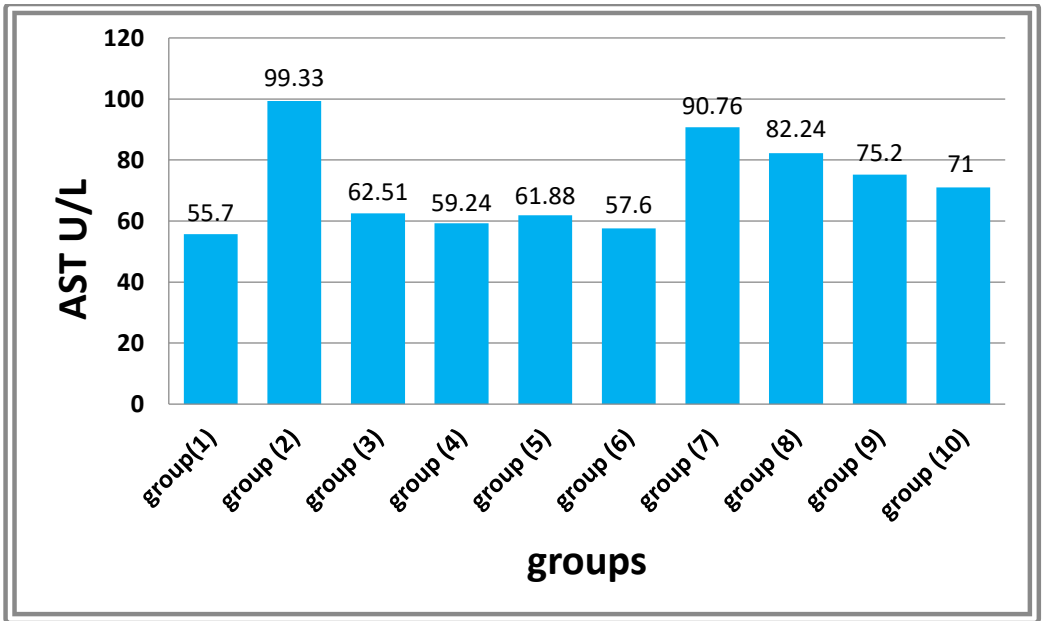

Figure (5): Effect of phenolic extract and taurine on AST levels.

Cardiac Troponin-I is the troponin regulatory complex protein involved in contractile process of the myocardium which is considered as a gold standard marker with high sensitivity and specificity for the diagnosis of MI (O'Brien *et al*., 1997). This contractile protein released during myocardial necrosis and its elevation in the serum predicts the risk of MI. Metabolically damaged myocardium will release the diagnostic markers of MI into the extra cellular fluid (Upaganlawar *et al*., 2009).

Treatment with tubers of *cyperus rotundus* extract showed a considerable decreased level of cardiac Troponin-I in the serum of groups (7 and 8). The cardio protective property of *cyperus rotondus* 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 e*t al*., 2012; Parikh *et al*., 2015; Jagadeesh *et al*., 2016).

Also, the increased of (LDH, CPK, ALT and AST) levels in the isoproterenol – administrated rats compared with control group, which is indicative of the severity of isoproterenol-induced necrotic damage to the myocardial membrane. This observation is in accordance with earlier studies (Kulkarni and Swamy, 2015; Abdel-Reheim, 2016), which indicated these cardio specific marker enzymes are released from the heart into the blood during myocardial damage due to deficiency of oxygen supply, the cell membrane become permeable or may rupture and results in the leakage of enzymes in the serum (Sabeena Farvin *et al*., 2004; Shi *et al*., 2013).

Oral treatment with tubers of *cyperus rotundus* extract restored the activities of myocardial marker enzymes. This could be due to the protective effect of *cyperus rotundus extract* on the myocardium, reducing the myocardial damage by restricting the leakage of (LDH, CK, ALT and AST). Our present results are in agreement with that of Sadiya *et al*. (2016), who also reported the protective role of *cyperus rotundus* extract on a gainst isoprotrenol – induced myocardial necrosis in rats by measuring marker enzymes. *Cyperus rotundus* extract decreased the activities of marker enzymes in the serum of ISO administered rats which could be due to free radical scavenging property of the extract due to the presence of phytochemicals such as Flavonoids and Tannins (Raza *et al*., 2012; Abi *et al*., 2014). These phytochemicals have been proved for their antioxidant activity which may be responsible for the cardioprotective effect of extract against ISO induced MI.

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