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Name Authors ⇐Time New Roman, Size 11

*Department of Genetic Engineering, Institution of Genetic Engineering and Biotechnology for postgraduate Studies , University of Baghdad, Baghdad, Iraq.

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[1]Corresponding author E-mail: ⇐Time New Roman, Size 12

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Introduction

Is escalating in recent year's and remains as a leading cause of mortality in developing countries (1). It is can occur during the therapy with several cytotoxic drugs, including 5-fluorouracil and may be the dose limiting factor in cancer treatment and hence tumor response (2). Cardiac toxicity includes a wide range of cardiac effects from small changes in blood pressure and arrhythmias to cardiomyopathy. In literature, different mechanisms of chemotherapy induced cardiac toxicity are postulated including cellular damage due to the formation of free oxygen radicals and the induction of immunogenic reactions with the presence of antigen presenting cells in the heart. Moreover, the influence of the cytotoxic agents on certain phospholipids, especially cardiolipin, may also explain the development of cardiac toxicity (2).

5- Fluorouracil (5-FU) is an antimetabolite that acts during the S phase of the cell cycle and is activated by thymidine phosphorylase into fluorodeoxyuridylate (5 fluoro 2'deoxyuridine 5'monophosphate, 5-FdUMP) that inhibits thymidylate synthase, thus preventing DNA synthesis that leads to imbalanced cell growth and ultimately cell death (3-5). It is a potent antineoplastic agent commonly used for treatment of various malignancies including gastrointestinal, breast and head and neck cancer. In addition to bone marrow depression, gastrointestinal tract reaction, or even leucopenia and thrombocytopenia [(6)], it has diverse adverse effects such as cardiac toxicity, hepatotoxicity and nephrotoxicity which restrict its wide and extensive clinical usage, also it

Cardiovascular disease causes marked organ toxicity coupled with increased oxidative stress and apoptosis (7).

Medicinal plants and their derivatives are widely used all over the world as medicinal, salutistic or functional food. Some medicinal plants are promising natural source (8). Artichoke (*Cynara scolymus L.*) is one of the world's oldest medicinal plants. It is an important crop of ancient Greece, grows in Egypt, Mediterranean area and other countries .It is belonging to the family (Asteraceae) (9). It has medical properties and used in traditional folk medicine mainly because of their choleretic (increasing bile secreation), diuretic and hypocholesterolemic activities (10). It is a good source of natural antioxidants such as vitamin C, hydroxycinnamic acids and caffeoylquinic acid derivatives (cynarin and chlorogenic acid) (11). Artichoke plant is rich with flavonoids (luteolin, apigenin), which its potential protective effect as antioxidant have been demonstrated for the extracts of this vegetable in reducing reactive oxygen species (ROS) from stimulated human neutrophil and in protection of hepatocyte from t-butyl hydrogen peroxide induced cytotoxicity (12). The artichoke extracts were assessed for their protective role in the control of oxidative damage to biological molecules (proteins, lipids and DNA), caused by free radicals such as RCOO and/or OH, using the b- potential antioxidant effect. Several *in vitro* studies have shown that the antioxidant potential effect of ALE is dependent on radical scavenging and metal ion chelating effect of its constituents such as cynarin,

chlorogenic acid and flavonoids. Pure constituents of ALE have also been shown to produce less inhibitory activity on free radical production than the extract itself (13).

Materials and Methods

Chemicals and drugs

5-Fluorouracil (5-FU) obtained from EBEWE pharma, AUSTRIA. Dimethyl sulfoxide (DMSO) and ethanol solvent obtained from warehouse chemicals of College of Pharmacy/ University of Baghdad.

Reagents

Standard assay rat's kits for AST/GOT and ALT/GPT were obtained from Egyption Company For Biotechnology (S.A.E) and for CK obtained from Biolabo SA, 02160 ,Maizy, France.

Plant materials

The plant was collected from the Garden of Medicinal Plants at the Department of Pharmacognosy and Medicinal plants / College of Pharmacy / University of Baghdad. The leaves of the plant were dried in shade at room temperature, then rendered into a fine powder by using electrical mill and weighed.

Extraction of the plant

Four grams of powdered leaves were extracted by maceration with 2500 ml of absolute ethanol for one week, then the extract was filtered and evaporated to dryness under reduced pressure by using rotary evaporator, after that the collected amount was weighted (14).

Preparation of the extract for injection

A specific weight (4.5 gm.) from the dried ethanolic artichoke extract was dissolved in dimethyl sulfoxide (112.5 ml) to get a concentration of 40 mg/ml (as a stock solution) [13].

Experimental animals

Twenty -four female albino rats of 1-2 month old (average body weight 150-200gm), were obtained from animal house of the college of pharmacy/ university of Baghdad. The animals were acclimatized under standard laboratory conditions for 2 weeks prior to treatment .They had free access to standard diet and water. They were maintained under standard condition of temperature (30ºC), humidity and light / dark cycles. All the experimental studies were conducted inconformity with the guidance for care and standard experimental animals of our College ethical protocol. The animals were used in this study divided equally into four groups, each group with 6 rats they were treated as following: Group I: (negative control) received oral daily dose of DMSO (2 ml/kg /day) for 10 successive days. Group II: (positive control), received oral daily dose of DMSO (2 ml/kg /day) for 10 successive days with administered single dose of 5-FU (150 mg/kg) intraperitoneally on 8th day [7] in association with DMSO. Group III: received 200 mg/kg/day [13] of ethanolic artichoke extract orally for 10 successive days. Groups IV: received 200 mg/kg/day [13] of ethanolic artichoke extract orally 10 successive days with subsequently single intraperitoneal dose of 5-FU (150 mg/kg) on 8th in association with the ethanolic artichoke extract (13).After 24 h of the end of the experimental period (10 days), all the animals were anesthetized under light diethyl ether anesthesia and blood samples were collected in clean test tubes by intracardiac puncturing and allowed to clot at room temperature.

Biochemical assessment

The serum was separated by centrifugation for 20 min at 3600 round per minute (r.p.m.) and stored into eppendorff tubes at – 20 °C to be used for determination of creatine Kinase (CK), aspartate

aminotransferase (AST) and alanine aminotransferase (ALT) [13].

Results and Discussion

Data were subjected to statistical analysis, data were expressed as the mean values mean± standard deviation (SD) of samples. The Statistical significance of the differences between various groups was determined by student unpaired t- test. Differences were considered statically significant for p-value < 0.05.

The effects of ethanolic artichoke extract on 5-fluorouracil (5-FU) induced cardiac-toxicity in rats:

5-FU (Group II) significantly (P<0.05) increased serum levels of AST, ALT, and CK with respect to Group I. Administration of ethanolic artichoke extract in association with 5-FU (Group IV) significantly (P<0.05) decreases the serum levels of AST, ALT and CK with respect to Group II. Groups III show no significant differences (P<0.05) in ALT and CK with respect to Group I, but there was significant difference in AST with respect to group I. While group IV showed significant elevation (P<0.05) in AST, ALT and CK with respect to Group I, as shown in Table (1).

Cardiac toxicity is one of the dangerous side effect of 5-FU , which often presents as myocardial ischemia, but to a lesser extent cardiac arrhythmia, hyper and hypotension, left ventricular dysfunction, cardiac arrest and sudden death (15-20). The incidence of 5-FU induced cardiac toxicity varies between 0-35 % and this may depend on dose, cardiac comorbidity and schedule of chemotherapy (15, 16, 18). The clinical handling of 5-FU-induced cardiac toxicity is difficult as the pathophysiological mechanisms underlying this cardiac toxicity remain undefined (15, 21). However several mechanisms have been proposed, including vascular endothelial damage followed by coagulation, ischemia secondary to coronary artery spasm, direct toxicity on the myocardium and thrombogenicity due to altered rheological factors (21).

The pathogenesis of 5-FU induced cardiac toxicity may involve oxidative stress with increased levels of superoxide anion after 5-FU treatment [22].The activities of superoxide dismutase (SOD) and glutathion peroxidase (GSH-Px) were lowered in 5-FU treated guinea pigs (23) demonstrating a reduced antioxidant capacity. If not eliminated by cellular antioxidant systems, superoxide anions can generate the highly reactive and toxic hydroxyl radicals through the Haber–Weiss reaction, which is catalyzed by iron (24,25). Increased reactive oxygen species (ROS) levels inside cells lead to oxidation of macromolecules, including lipids, nucleic acids, and proteins, thereby disturbing cellular functions (25). In addition to ROS, pro-inflammatory cytokines, such as tumour necrosis factor- α (TNF-α) and interleukins (IL)-1 and 6, also play an indirect role in production toxicity and organs damage that generate by chemotherapy agents, as 5-FU [26].

The present study confirms the cardiac toxicity of 5-FU, as evidenced by the significantly (P<0.05) elevation in serum level of AST, ALT and CK. Detection of elevated concentrations of cardiac biomarkers in blood is a sign of cardiac injury which could be due to supply–demand imbalance, toxic effects, or haemodynamic stress [27]. Creatine kinase (CK), AST, lactate dehydrogenase, myoglobin, and troponins are some of these markers (28).

The present study has shown a successful reduction in cardiac toxicity induced by 5-FU in albino rats after treatment with ethanolic artichoke

extract, this was reflected by reduction in serum level of AST, ALT and CK. This is give a strongly refers to the possible cardiac protective effects of artichoke extract against 5-FU induced cardiac toxicity in the rats. This protective effect of artichoke extract related to the power antioxidant effect of phenolic acids especially chlorogenic acid and cyanine (29). When the biological activity of artichoke extracts is considered, the presence of luteolin-7- glucoside and hydrolysable tannins, besides caffeoylquinic derivatives, in the phenolic fraction of these extracts must be taken into account: all these phenolics possess a good antioxidant activity against peroxyl and hydroxyl radicals by decreasing the release of ROS, which is produced by effect of cytotoxic drugs, when assessed using the beta-carotene/linoleate assay and the metmyoglobin assay (30).

Table 1. Effects of ethanolic artichoke extract (200 mg/kg) on the serum levels of AST, ALT and CK in albino female rats with 5-FU induced cardiac toxicity, data are expressed as Mean ± SD, n =6,∗ p <0.05. Standard deviation (SD), 5-Fluorouracil (5-FU) and dimethyl sulfoxide (DSMO).

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Treatment group n=6	Type of treatment	Serum Aspartate aminotransferase (S.AST) Up to 40 IU/L (Mean ± SD)	Serum Alanine aminotransferase (S.ALT) Up to 40 IU/L (Mean ± SD)	Serum Creatine kinase (S.CK) Up to 190 IU/L (Mean ± SD)
I	Dimethyl sulfoxide (DMSO) only	27.33 ± 0.42	7.33 ± 0.81	51.33 ± 4.35
II	5-Fluorouracil (5-FU)	74.06 ± 11.52*	32.53 ± 1.43*	555.66 ± 67.77 *
III	200 mg/kg of Ethanolic artichoke extract	22.33 ± 2.60 *	7.2 ± 4.73	48.42 ± 9.40
IV	200 mg/kg of Ethanolic artichoke extract + 5-FU	31 ± 1.93 s	16 ± 0.44 ∗s	63.6 ± 5.05 ∗s

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(∗): Significant difference with respect to negative control group (P< 0.05),

(s): Significant difference with respect to 5-FU treated group.

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Conclusion

The results of this study suggested that the ethanolic artichoke extract has protective effects against 5-FU-induced cardiac toxicity in albino female rats. However, before a conclusive statement can be made on the potential antioxidant activity of artichoke extract as an adjunct to 5-FU therapy, there is a need for further.

References

1. Reddy, K.S and Ansari, N. (2010). New Delhi. Cardiovascular diseases in the developing countries: dimensions, determinants, dynamics and directions for public health action. Coronaries, American Heart Hospital Journal, 8(2):111-12
2. Costi, M. P.; Ferrari, S. and Venturelli, A. et al. (2005). Thymidylate synthase structure, function and implication in drug discovery. Current. Medical Chemistry, 12:2241–58.