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Original Article

THE POSSIBLE CARDIOPROTECTIVE EFFECTS OF DIFFERENT FRACTIONS OF ARTICHOKE EXTRACTS AGAINST 5-FU INDUCED CARDIOTOXICITY IN ALBINO RATS

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ABSTRACT

Objective: The present study aimed to evaluate the cardioprotective effects of ethyl acetate and methanolartichoke extracts(*Cynara scolymus* L.) against 5-Flurouracil (5-FU) induced cardiotoxicity in rats.

Methods: Thirty-six albino rats were divided randomly and equallyin to six groups (each group with 6 rats): I, negative control, received (2 ml/kg/d)of dimethyl sulfoxide (DMSO) orally for 30 successive d; II, positive control, received (2 ml/kg/d) of (DMSO) orally for 30 successive d, and subsequently administered a single dose of 5-FU (150 mg/kg) by intraperitoneal injection on 27thd in assossiation with DSMO; III and IV, received (200 mg/kg/d) of oral methanol and ethyl acetate artichoke extracts respectivelyfor 30 successive d; V and VI, received(200 mg/kg/d) of oral methanol and ethyl acetate artichoke extracts respectively for 30 successive d,with a subsequently received single dose of 5-FU (150 mg/kg) by intraperitoneal injection on 27th d of the experiment.

Results: Prophylactic treatment of ethyl acetate and methanol artichoke extracts significantly attenuates the increased level of serum cardiac troponin T (CTn-T) and tumor necrosis factor-α(TNF-α)caused by 5-FU-induced cardiotoxicity in experimental albino rats while it increases the serum level of total antioxidant capacity (T-AOC).

Conclusion: Results of the present study suggest that methanol and ethyl acetate artichoke extracts may be an effective modulator in mitigating 5-FU induced cardiotoxicity.

Keywords: Artichoke extracts, 5-fluorouracil, Cardioprotective, Cardiac troponin T, TNF-α and T-AOC.

INTRODUCTION

Antimetabolite, 5-fluorouracil (5-FU) has been effectively used for the treatment cancers of head, neck, breast and alimentary system [1], it is still a widely used anticancer drug since 1957 [2]. 5-FU is a heterocyclic aromatic organic compound with a structure similar to that of a pyrimidine molecules of DNA and RNA with an analogue of uracil with a fluorine atom at the C-5 position in place of hydrogen [3]. Due to its structure, 5-FU interferes with nucleoside metabolism and can be incorporated into RNA and DNA, leading to cytotoxicity and cell death [4, 5]. The alternative activation pathway of 5-fluorouracil requires initial activation of the drug by the enzyme thymidine phosphorylase, which catalyses conversion of 5-FU to fluorodeoxyuridine (FUDR), the later is then phosphorylated by thymidine kinase (TK) to 5-fluoro-2'-deoxy uridine 5'-monophosphate (5-FdUMP),an inhibitor of thymidylate synthase (TS)which is involved in the synthesis of thymidylate, thus preventing DNA synthesis; that leads to imbalanced cell growth and ultimately cell death [6]. In addition to bone marrow depression, gastrointestinal tract reaction, or even leucopenia and thrombocytopenia [7], 5-FU has several side effects such as cardio toxicity, nephro toxicity and hepatotoxicity which restrict its wide and extensive clinical use. Most of th 5-FU related organ toxicity is coupled with increased oxidative stress and apoptosis [8].

Artichoke (*Cynara scolymus* L.) is one of the world's oldest medicinal plants with 2000 y history. It belongs to the family (Asteraceae) [9]. Originally it comes from the Mediterranean region and North Africa and then cultivated around the world. Artichoke was used as a food (as a digestive aid) and medicine by the ancient Egyptians, Greeks, and Romans [9]. It grows to a height of about 2 m and produces a large, violet-green flower head. The flower petals and fleshy flower bottoms are eaten as a vegetable throughout the world, which has led to its commercial cultivation in many parts of South and North America (chiefly California) as well as in Europe [9]. This plant is widely distributed in Iraq and normally located and found in the outer lines of the fields, water lines and humid watery soil. The plant flourishes in winter and it is usually harvested in February and March. Because of the artichokes durability, it is hardy to temperature below freezing [10]. *Cynara scolymus* L. is not only a good food, known for its pleasant bitter taste, but also an interesting and widespread herbal drug [11]. Artichoke leaves contain up to 2% phenolic acids, mainly 3-caffeoylquinic acid (chlorogenic acid), plus 1.3-di-O-caffeoylquinic acid (cynarin), and caffeic acid; 0.4% bitter sesquiterpene lactones of which 47-83% is cynaropicrin; 0.11.0% flavonoids including the glycosides luteolin-7-β-rutinoside (scolymoside), luteolin-7-β-Dglucoside and luteolin-4-β-D-glucoside; phytosterols (taraxasterol); sugars; inulin; enzymes; and a volatile oil consisting mainly of the sesquiterpenes β-selinene and caryophyllene [12]. Artichoke leaf extract has been used as hepatoprotective [13], antimicrobial [14] and cholesterol reducing purposes [15]. Also it has been found to decrease the production of reactive oxygen species, the oxidation of low-density lipoproteins [16], lipid peroxidation [13], protein oxidation and increase the activity of glutathione peroxidase [17].

Several clinical studies have suggested that serum level of tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), and C-reactive protein (CRP) are elevated in patients with congestive heart failure (CHF), regardless of the etiology of the condition [18].

The hepatoprotective,anti-inflammatory and anti-oxidant properties of artichoke extracts motivated us for designing this novel study to investigate the protective effects of different fractions of artichoke extracts (ethyl acetate and methanol)against 5-FU induced cardiotoxicity in albino rats.

MATERIALS AND METHODS

Reagents: Standard assays rat's kits for Cardiac Troponin T(cTn-T) and TNF-α were abtained from CUSABIO BIOTECH CO., LTD., China. And for Total Antioxidant Capacity (TAOC) obtained from RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY.

Chemicals and drugs: 5-Fluorouracil (5-FU) obtained from EBEWE Pharma Ges.b.H.KG, Austria and (DMSO) from Sigma ,USA.

Plant material: 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 the shade at room temperature, then rendered into a fine powder by using electrical mill.

Extraction of the plant: 750 g of powdered leaves were extracted by soxhlet, using 3000 ml from each of petroleum ether, ethyl acetate and methanol successively as solvents, each extract was filtered and evaporated to dryness under reduced pressure using rotary evaporator,after that the collected amount was weighted.

Preparation of extracts for injection: A known weights from the dried ethyl acetate and methanol artichoke extracts was dissolved in (DSMO) to get a concentration of 40 mg/ml.

Animals and treatment

Thirty-six female albino rats of 1-2 mo old (average body weight 150-220 g) were obtained from animal house of the College of Pharmacy/University of Baghdad. The animals were acclimatized under standard laboratory conditions for 2w prior to treatment. They had free access to standard diet and water. They were maintained under standard condition of temperature(30°C), humidity and light/dark cycles. All the experimental studies were conducted by inconformity with the guidance for care and standard experimental animals of our college ethical protocol. The animals used in this study were divided equally into six groups each group with 6 rats as follow: Group I, negative control, received (2 ml/kg/d) of (DMSO) orally for 30 successive d; Group II, positive control,received (2 ml/kg/d)of (DMSO) orally for 30 successive d, and subsequently administered a single dose of 5-FU (150 mg/kg) by intraperitoneal injection on 27thd in association with DMSO; Group III, received(200 mg/kg/d)of methanol artichoke extract orally for 30 successive d; Group IV, received (200 mg/kg/d)of ethyl acetate artichoke extract orally for 30 successive d; Group V, received (200 mg/kg/d) of methanol artichoke extract orally for 30 successive d with a subsequently single intraperitoneal dose of 5-FU (150 mg/kg) on 27th d in association with methanol extract; Group VI,received(200 mg/kg/d)of ethyl acetate artichoke extracts orally 30 successive d with a subsequently single intra peritoneal dose of 5-FU (150 mg/kg) on 27th d in association with ethyl acetate extract. After 24 h of the end of the experimental period (30 d), all the animals were sacrificed under light diethyl ether anesthesia and blood samples were collected in clean test tubes by intracardiac puncturing of rats 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 and assessment of biochemical parameters: Rat Cardiac Troponin T (cTn-T), Tumor Necrosis Factor-alpha (TNF-α) and Total Antioxidant (TAO).

Statistical analysis

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

RESULTS

5-FU in (Group II) significantly (∗P<0.05) increases serum level of cardiac troponin T (table 1) and TNF-α (table 2) with respect to Group I,and significantly (∗P<0.05) decreases serum level of TAO (table 3) with respect to Group I. Administration of methanol and ethyl acetate artichoke extracts in association with 5-FU(Group V& VI respectively) significantly (∗P<0.05) decreases the serum level of cardiac troponin T (table 1) andTNF-α(table 2), while it significantly (∗P<0.05) increases the TAO serum level with respect to Group II(table 3).

Group IV shows no significant difference (∗P<0.05) in serum levels of cardiac troponin T, TNF-α, and TAO with respect to Group I, also group III shows no significant difference (∗P<0.05) in serum level of cardiac troponin T with respect to Group I, but it revealsa significant difference (∗P<0.05) in the serum level of TNF-α and TAO with respect to Group I. Groups V and VI revealed a significant difference (∗P<0.05) in serum level of cardiac troponin T, TNF-α, and TAO with respect to group I, they(Group V and VI) also revealed significant difference (∗P<0.05) in serum level of cardiac troponin T, TNF-α, and TAO with respect to Group III and I V respectively as shownin table 1, 2 and 3.

Group V shows no significant difference (∗P<0.05) in serum level of cardiac troponin T and TAO with respect to group VI, while it shows a significant difference (∗P<0.05) in serum level of TNF-αwith respect to group VI, as shown in table.1, 2 and 3.

Table 1: The effects of ethyl acetate and methanol artichoke extracts (200 mg/kg) on serum level of cardiac troponin T in albino rats with 5-FU induced cardiotoxicity, data are expressed as mean±SD,n =6,∗p<0.05. SD is standard deviation,5-FU is5-Fluorouracil and DSMO is dimethyl sulfoxide

Groups	Type of treatment	Serum cardiac troponin T ng/l (mean±SD)
I	DMSO (2 ml/kg/d) orally for 30successive d	24.8±8.262
II	DMSO (2 ml/kg/d) orally for 30 successive d+5-FU (150 mg/kg) single intraperitoneal dose on 27th d	60.8±8.72 *
III	Methanol extract (200 mg/kg/d) for 30 successive d	32.83±8.6 ^{sa}
IV	Ethyl acetate extract (200 mg/kg/d) for 30 successive d	31.11±6.66 ^{sA}
V	Methanol extract (200 mg/kg/d) for 30 successive d+5-FU(150 mg/kg) single intraperitoneal dose on 27th d	43.8±5.65 ^{bs*}
VI	Ethyl acetate extract (200 mg/kg/d) for 30 successive d+5-FU(150 mg/kg) single intraperitoneal dose on 27th d	47.8±7.13 ^{Bs*}

-(*): Significantly different with respect to group I (∗p<0.05), -Values of groups III & V with non-identical subscripts (a, b) are significantly different (∗p<0.05), -Values of groups IV & VI with non-identical subscripts (A, B) are significantly different (∗p<0.05), -s subscript: significant difference with respect to the 5-FU treated group, -n: Number of animals**.**

Table 2: The effects of ethyl acetate and methanol artichoke extracts (200 mg/kg) on serum level of TNF-α (tumor necrosis factor - alpha) in albino rats with 5-FU induced cardiotoxicity,data are expressed as mean±**SD, n =6,∗ p<0.05. SD is standard deviation, 5-FU is 5-Fluorouracil and DSMO is dimethyl sulfoxide**

Groups	Type of treatment	Serum TNF-α ng/l (mean±SD)
I	DMSO (2 ml/kg/d)orally for 30 successive d	23.33±5.6
II	DMSO (2 ml/kg/d) orally for 30 successive d+5-FU (150 mg/kg) single intraperitoneal doseon 27th d	107.5±45 *
III	Methanol extract (200 mg/kg/d) for 30 successive d	13.93±4.24 ^{as*}
IV	Ethyl acetate extract (200 mg/kg/d)for 30 successive d	32±25.86 ^{As}
V	Methanol extract (200 mg/kg/d)for 30 successive d+5-FU(150 mg/kg) single intraperitoneal dose on 27th d	49.3±22 ^{bcs*}
VI	Ethyl acetate extract (200 mg/kg/d)for 30 successive d+5-FU(150 mg/kg) single intraperitoneal dose on 27th d	68.5±14.8 ^{BCs*}

-(*): Significantly different with respect to group I (∗p<0.05), -Values of groups III & V with non-identical subscripts (a, b) are significantly different (∗p<0.05), -Values of groups IV & VI with non-identical subscripts (A, B) are significantly different (∗p<0.05), -Values of groups V & VI with non-identical subscripts (C,c) are significantly different (∗p<0.05), -s subscript: significant difference with respect to the 5-FU treated group, -n: Number of animals

Table 3: The effects of ethyl acetate and methanol artichoke extracts (200 mg/kg) on serum level of TAO(total antioxidant) in albino rats with 5-FU induced cardiotoxicity, data are expressed as mean±**SD, n =6,∗ p<0.05. SD is standard deviation, 5-FU is 5-Fluorouracil and DSMO is dimethyl sulfoxide**

Groups	Type of treatment	Serum TAO ng/l (mean±SD)
I	DMSO (2 ml/kg/d) orally for 30 successive d	1.40±0.044
II	DMSO (2 ml/kg/d) orally for 30 successive d+5-FU (150 mg/kg) single intraperitoneal dose on 27th d	1.30±0.075 *
III	Methanol extract (200 mg/kg/d) for 30 successive d	1.70±0.112 sa*
IV	Ethyl acetate extract (200 mg/kg/d) for 30 successive d	1.42±0.021 As
V	Methanol extract (200 mg/kg/d)for 30 successive d+5-FU(150 mg/kg) single intraperitoneal dose on 27th d	1.59±0.034 sb*
VI	Ethyl acetate extract (200 mg/kg/d)for 30 successive d+5-FU(150 mg/kg) single intraperitoneal dose on 27th d	1.48±0.057 B*

-(*): Significantly different with respect to group I (∗p<0.05), -Values of groups III & V with non-identical subscripts (a, b) are significantly different (∗p<0.05), -Values of groups IV & VI with non-identical subscripts (A, B) are significantly different (∗p<0.05), -s subscript: significant difference with respect to the 5-FU treated group, -n: Number of animals

DISSCUSION

Cardiotoxicity 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 [19-24]. The incidence of 5-FU induced cardiotoxicity varies between 0-35% and this may depend on dose, cardiac comorbidity and schedule of chemotherapy [19,20,22]. The clinical handling of 5-FU-induced cardiotoxicity is difficult as the pathophysiological mechanisms underlying this cardiotoxicity remain undefined [19,25]. 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 thrombo genicity due to altered rheological factors [25].

The pathogenesis of 5-FU induced cardiotoxicity may involve oxidative stress with increased levels of superoxide anion after 5-FU treatment [26]. The activities of Super Oxide Dismutase (SOD) and Glutathion Peroxidase(GSH-Px)were lowered in 5-FU treated guinea pigs [27] 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 [28,29]. Increased reactive oxygen species (ROS) levels inside cells lead to oxidation of macromolecules, including lipids, nucleic acids, and proteins, thereby disturbing cellular functions [29]. The present study confirms the cardiotoxicity of 5-FU, as evidenced by the significantly (∗P<0.05) elevation in serum level of cardiac troponin T and TNF-α with increased oxidative stress manifested as significantly (∗P<0.05) decrease in TAO serum level.

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 haemo dynamic stress [30]. Creatinine kinase (CK), serum AST, lactate dehydrogenase, myoglobin, and troponins are some of these markers [31].

In the last decade, many studies focused on the possibility that inflammation may complicate the clinical course of heart failure (HF) via impairing cardiac contractility, promoting apoptosis and fibrosis and ultimately leading to myocardial remodeling [32, 33]. Furthermore, the chemotherapy initiates the release of (ROS) and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukins (IL)-1 and 6, which play an indirect role in the amplification of intestinal damage [34].

Tumor Necrosis Factor-alpha (TNF-α) is a major mediator of inflammation [35]. ROS generation, after TNF-α binding, has been reported to be involved in both cell survival and cell death, and the main source of ROS generation that contributes to TNF-α-induced cell death is the mitochondrion [35, 36]. ROS modulator 1 (Romo1) is localized in the mitochondria and induces mitochondrial ROS production through complex III of the mitochondrial electron transport chain [37-39]. Romo1 expression is induced by an external stimulus such as 5-FU, and it is up regulated in senescent cells and in most cancer cells [37,38,40]. Romo1 is a molecular bridge between TNF-α signaling and the mitochondria for ROS production that triggers TNF-α-mediated apoptosis [35]. Another study reported that NADPH oxidase is the source of ROS generation after TNF-α treatment [41]. ROS are known to contribute to cell death by inducing mitochondrial membrane permeabilization and sustaining c-Jun N-terminal kinase (JNK) activation [42, 43]. There are several reports regarding the mechanism of JNK-induced apoptosis in response to TNF-α [35].

The present study has shown a successful reduction in cardiotoxicity and inflammation induced by 5-FU in albino rats after treatment with ethyl acetate and methanol artichoke extracts, this was reflected by attenuation in serum level of cardiac troponin T and TNF-α with significant decrease in the oxidative stress manifested by elevation of the serum levels of total antioxidant capacity (T-AOC) of treated rats. This antioxidant and anti-inflammatory effects of artichoke extracts are attributed to phenolic compounds [44,45]. Different studies about artichoke extracts have demonstrated their health-protective potential, especially their hepatoprotective [46,47] and health promoting properties in preventing cardiovascular disease (CVD) by its hypo lipidemic action [48] or by upregulation of endothelial nitric-oxide synthase (eNOS)gene expression and eNOS protein expression [49]. Also, artichoke extracts have anticancer effects by increasing apoptosis either in hepatocellular carcinoma in rats [50] or human hepatoma cells [51]. In fact, artichoke is potentially good source of antioxidant activity because it contains large amounts of caffeic acids [52, 53]. Caffeic acid derivatives are the main phenolic compounds in artichoke heads, with a wide range of caffeoylquinic acid derivatives as (cynarin) [54] with chlorogenic acid (5-Ocaffeoylquinicacid) as the most important of these derivatives [55]. Other phenolics such as the flavonoids apigenin and luteolin (both glucosides and rutinosides) [56] as well as different cyanidin caffeoylglucoside derivatives [57] have been identified.

The effects of artichoke extracts and its constituents have also been investigated for activity against oxidative stress in studies using human leucocytes [58]. The extracts demonstrated a concentration-dependent inhibition of oxidative stress induced by several agents, such as hydrogen peroxide, that generates reactive oxygen species. The constituents; cynarin, caffeic acid, chlorogenic acid and luteolin also showed concentration-dependent oxidative stress inhibitory activity [58]. In addition,artichoke extracts has marked protective properties against oxidative stress induced by inflammatory mediators and oxidize-LDL in cultured endothelial cells and monocytes [16]. *In vivo*, the administration of an edible artichoke in rats has shown that artichoke extract increased the level of glutathione peroxidase activity in erythrocyte and decreased the level of 2-Aminoadipic semialdehyde (a protein oxidation biomarker) [59].

Flavonoids protective effects against many diseases, in particular cardiovascular diseases and cancer, are attributed to two properties: (1) antioxidant activity and (2) inhibition of certain enzymes such as xanthine oxidase (enzyme catalyzes the conversion of both hypoxanthine to xanthine and xanthine to uric acid while reducing O2 to O2-• and H2O2 [60]) [61, 62]. Many studies have suggested that flavonoids exhibit biological activities, including anti allergenic, antiviral, anti-inflammatory, vasodilationactions. These pharma-cological effects are linked to the antioxidant properties of

flavonoids. Flavonoids can express these properties by: (1) suppressing ROS formation through inhibiting some enzymes or chelating trace elements involved in free radical production, (2) scavenging radical species and more specially the ROS, and/or (3) up-regulating or protecting antioxidant defense [61].

CONCLUSION

Our results suggest that ethyl acetate and methanol artichoke extracts have protective effects against 5-FU-induced cardiotoxicity in albino rats. However, before a conclusive statement can be made on the potential antioxidant activity of artichoke extracts as an adjunct to 5-FU therapy, there is a need for further long-term chronic studies for different fractions of artichoke extracts.

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CONFLICT OF INTERESTS

Declared None

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