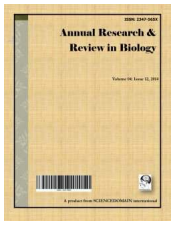

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Stimulation of Hepatocytes Repair by Fruit Juice of *Opuntia ficus indica* in Anti Cancer Drug Cyclophosphamide (CP)-Induced Liver Toxicity in Mice

Khalid N. Al-Kubaisy[1], Luay Y. Al- Essa[2] and Maissa' T. Shawagfeh[1*]

[1]Department of Biomedical Science, Zarqa University College, Applied Balqa University, Zarqa, Jordan.
[2]Department of Pharmacy, Faculty of Pharmacy, Al-Zaytoona University, Amman, Jordan.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cyclophosphamide (CP) is a cytotoxic alkylating agent that has been used extensively in medicine as anti neoplastic agent for the treatment of different cancers worldwide. Chemotherapy with CP is associated with significant toxicity due to overproduction of reactive oxygen species (ROS) and free radicals resulting in increased levels of oxidative stress (OS) aiming to identify natural substances that would be effective in reducing the severity of CP.

Cactus (*Opuntia ficus indica*) is an indigenous medicinal plant possesses wide range of medicinal properties. It is used in traditional folk medicine and nutrition in many countries. In this study, we examined the effects of prickly pear fruit's juice on liver damage in order to evaluate its hepatoprotective effects against hepatotoxicity, oxidative stress, and cytotoxicity induced in mice by CP at a dosage of (75 mg/kg) body weight.

The degree of liver injury was analyzed using serum biochemical markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH), the total protein and albumin contents. Further, the liver analyzed for the degree lipid peroxide (LPO)

**Corresponding author: E-mail: mtash48@bau.edu.jo;*

as (MDA) and enzymatic and non-enzymatic antioxidants. Moreover, the cytotoxicity induced by CP treatment was substantiated by the reduction of hepatic cells nuclieic acids, protein, glutathione and increased lipid peroxide levels due its prooxidant nature.
Treatment of mice with cactus fruit juice after CP- dosing statistically restored the serum hepatic markers (AST, ALT, LDH), total protein and albumin, hepatic cells nucleic acids and antioxidant levels.

Keywords: Cactus fruit juice; cyclophosphamide; oxidative stress; cytotoxicity.

1. INTRODUCTION

Cyclophosphamide (CP) is a cytotoxic alkylating agent that has been used extensively in medicine as antineoplastic agent for the treatment of different cancers [1]. This drug has significant immunosuppressive activity and is used clinically in the treatment of autoimmune diseases and for renal and bone marrow transplantation [2]. CP first is converted by the liver cells into two active chemicals; acrolein and phophoramide mustard by oxidase enzymes via cytochrome P-450 systems [3-5]. CP application can be associated with serious side effects including vomiting, nausea, skin rashes, chest pain, cystitis jaundice, hemopoetic suppression [6,7]. It can also cause hepatotoxicity, nephrotoxicity, cardiotoxicity [8], genotoxicity (14) and infertility [9].

A large number of studies are aiming to identify natural substances that would be effective in reducing the severity of toxic chemicals and drug abuse. Although, there are a number of drugs on market their long term use can have numerous side effects, such as CP. For this reason many researches are carried out to find antioxidant drugs of natural compounds that help in protection against many toxic chemicals including CP with minimize or no side effects [10-14].

Cactus (*Opuntia ficus indica*) commonly known as prickly pear belongs to the family cactaceae, is an important forage crop for livestock in many arid and semi-arid regions of the world. Although Opuntia ficus-indica is found in Jordan, it comes originally from Central America. Cactus fruit may be useful as functional foods are drug against reactive species mediated diseases. Different parts of cactus are used in folk medicine, based on source of plant and country origin. Several studies demonstrating both cactus fruit and cladode juice possess several health beneficial properties.

Chemical analysis of the extract revealed the presence of polyphenol compounds, flavanoids, flavonols, quercetin protein, sugars, vitamins A, B1, B6, E, and ascorbic acid, carotenoids and betalains pigments. These compounds are found to exhibit diverse biological properties [15-19]. Besides, several studies are known to share a spectrum of pharmacological activities including anticancer [20,21], neuroprotective [22], anti ulcer activity [23], antihepatotoxic action against CCl4, nickel, mycotoxins and insecticide the chlorpyrifos [23-25] cytotoxicity and antigenotoxic [26,27], antibacterial and antifungal [28], as natural macromolecular coagulant and wound healing [29], anti-inflammatory activity [30], antidiabetic property, hypolipidemic [31,32], and antiviral activities has been recorded [33]. Pretreatment test in animal model revealed a protective action against ethanol-induced kidney injury [34].

In the present study, we have evaluated the beneficial effects of fruit juice of *Opuntia ficus indica* against anti cancer drug CP- induced hepatotoxicity, oxidative stress and cytotoxicity in mice.

2. MATERIALS AND METHODS

2.1 Preparation of Cactus Fruit Juice

The orange-yellow cactus pear fruits were purchased from a local market in Amman-Jordan, during summer season (2014). The sample of the plant was identifies by a professor from plant production department- University of Jordan. Whole fresh fruits were stored, washed with tap water, peeled then juice extracted from the whole edible pulp using food processor (moulinx-masterchef 8000) with no addition of water then centrifuged at 3000/10 min. to remove hard fibers and seeds. Supernatants were stored frozen at (-20℃) until use.

2.2 Animal Model

All animals were handled according to the guidelines of the animal ethical committee. CP was purchased from Sigma-Aldrich Co. (USA).

A total of 18 male albino mice weighing between (25-30 g) obtained from the animal house/ University Applied Sciences-Amman were used. The animals were maintained under standard conditions of temperature 25±1℃ with a 12 h/12 h light/dark cycle. All animals were allowed free access to food and water ad libitum. The animals are divided randomly into three groups as follows; six mice each:

- Group (1) treated with normal saline only, for 10 days (control)
- Group (2) was administered with (75 mg/kg) cyclophosphamide (CP)/ intoxicated control group were used for comparison with group (1).
- Group (3) was treated once daily with 2 ml/kg body weight of the fruit juice for 10 days (the dose was selected by performing an effective dose fixation study) and subsequently exposed to a single dose of CP, (6 h) after the last fruit juice treatment.

2.3 Blood Serum and Liver Tissue Preparations

At the end of experimental period, blood was collected from the heart puncture and serum was separated by centrifugation at 3000/10 min. Serum samples were subjected to estimation of serum hepatic markers.

Then the mice scarified under ether anesthesia and their livers were excised washed with ice-cold (1.15 potassium chloride) and blotted to dryness, samples of liver tissue were homogenized in Elvehjem Teflon glass homogenizer for 2 min. to make 10% w/v liver homogenate.

2.4 Biochemical Analysis

2.4.1 Blood serum parameters estimation

The activity of serum enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) beside total protein and albumin levels, assays were carried out according to the procedure described by Randox Lab. Ltd-UK diagnostic kits.

2.4.2 Oxidative stress estimation

The level of tissue lipid peroxidation (as MDA) served as an index of the intensity of oxidative stress (OS). Amalondialdehyde (MDA) was determined in the liver homogenate as described by Ohkawa et al. [35] with KCl for 30 min at 37℃. After incubation with thiobarbituric acid to generate pink color produced, which has max absorption at 532 nm and the values were expressed as nmoles MDA/g wet tissue samples.

Estimation of total reduced glutathione (GSH) was determined in the liver homogenate by method of Ellman [36] when 5,5 dithio-bis-2-nitrobonzoic acid (DTNB) is added to the supernatant and was measured at 412 nm against reagent blank with no homogenate. The values were expressed as µg/g wet tissue.

2.4.3 Antioxidant enzymes (CAT and SOD) estimation

Catalase activity was determined by method Hohasson et al. [37] and Superoxide dismutase (SOD) activity was done by the method Kakkar et al. [38] using available kits obtained from Calbiochem (USA) according to the manufacturer instructions.

2.4.4 Protein and nucleic acids in hepatic cells estimation

Total hepatocytes protein was determined by method of Lowery et al. [39] using folin-ciocalteu reagent and modified in a few details. The method of Herbert et al. [40] was used to determine the concentrations of nucleic acids. Liver tissues were homogenized in Elvehjem Teflon glass homogenizer for 2 min. to make 10% w/v liver homogenate and the homogenate was extracted in different concentration of cold and hot perchloric acid ($HClO_4$) after the final extraction in $HClO_4$, incubation and centrifugation the supernatant was used to determine the levels of DNA and RNA.

The DNA levels were determined by treating with diphenylamine reagent (give blue color) and reading the O.D. at 600 nm. To determine the levels of RNA, the extract was treated with orcinol reagent and the green color was measured at 660 nm at full computerized spectrophotometer to determine the amount of nucleic acids present.

2.5 Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) and student t-test. Results were presented as means. P- value < 0.05 was considered to be significant.

3. RESULTS

Hepatoprotective activity of cactus fruit juice against cyclophosphamide (CP) studied by estimating serum enzyme levels AST, ALT, and LDH showed significant ($p< 0.05$) reduction of CP-induced elevated serum levels of enzyme activities, with parallel significant ($p < 0.05$) increase in total protein and albumin (Table 1) indicating the cactus fruit juice of *O. ficus indica* could preserve the normal functional status of the liver.

In Table 2 Cactus fruit juice of *O. ficus indica* was screened for the protective effect against CP-induced lipid peroxidation (LPO) in mice. A single dose of CP significantly elevated the level of (LPO) with decrease levels of free radical enzymes (CAT, SOD) and non-enzymatic reduced glutathione (GSH) in liver homogenate. The pretreatment of mice with fruit juice of cactus significantly ($p< 0.05$) decreased the CP-induced oxidative stress in mice.

Table 3 indicates toxic effects on DNA, RNA and proteins of hepatic cells upon exposure and pretreatment. The DNA, RNA levels in hepatic cells were significantly reduced after treatment mice with CP as compared with control. Pretreatment with cactus fruit juice was found to significantly elevate the levels of DNA, RNA and protein in hepatic cells.

4. DISCUSSION

Major function of liver is to detoxify xenobiotics and toxins. Among therapeutics for liver diseases, protective drugs such as antioxidants have attracted more and more attention and radical- scavenging action is well known as an important mechanism of antioxidation [41]. Chakrabobrty et al. [22], suggested that antioxidant supplementation can influence the response to chemotherapy as well as the development of adverse side effects that result from treatment with antineoplastic agents.

Table 1. Effect and pretreatment of cactus fruit juice on serum biochemical responses of mice to CP

Parameter	Control	CP	CP+ Cactus fruit juice
AST 1U/L	43.51±3.20	81.73±5.92*	57.11±6.53*
ALT 1U/L	31.63±3.71	54.00±5.88*	27.32±5.13*
LDH 1U/L	141.52±5.41	164.71±9.22*	153.13±4.33*
Total Protein mg/dl	5.81±0.41	3.93±0.338*	4.86±1.27*
Albumin mg/dl	4.78±0.71	2.11±0.478*	3.92±0.83*

**Results are expressed as means ± SE for six mice, statistical significance at p<0.05 in comparison between the normal group and tested groups (CP) and CP + fruit juice of cactus*

Table 2. Effect of pretreatment with cactus fruit juice on lipid peroxidation and antioxidant status levels of hepatic cells in mice response to CP

Groups	MDA nmole/g wet liver tissue	GSH µg/g wet liver tissue	CAT (U/ml)	SOD (U/ml)
Control	277.3±5.66	7.24±2.83	123.46±3.10	31.11±2.10
CP	305.5±7.18*	4.66±3.11*	81.55±1.68*	18.64±1.62*
CP + cactus fruit juice	286.8±4.31*	6.14±2.67*	98.87±1. 37*	26.38±1.13*

**Values are given as means ± SE for groups of six mice each values are statistically significant at (p<0.05) treated mice compared with control*

Table 3. Effect of pretreatment of cactus fruit juice on nucleic acids and protein levels in hepatic cells of mice response to CP

Groups	DNA µg/g	RNA µg/g	Protein mg/g
Control	1850.9±22.13	645.16±13.2	130.80±0.41
CP	1442.8±11.43*	571.28±14.72*	110.71±0.46*
CP + cactus fruit juice	1673.2±12.33*	608.62±15.10*	118.83±0.51*

**Values are given as means ± SE for groups of six mice each. Values are statistically significant at (p< 0.05) treated mice with control group*

CP is an alkylating agent and the most commonly used anticancer and chemotherapy drug, its cytotoxic effects are the result of chemically reactive metabolites that alkylate DNA and proteins [42,43]. The harmful effects of chemotherapeutic agents have been documented. The toxic effects of these agents are due to increased overproduction of free radicals and reactive oxygen species (ROS). The increased generation of ROS by CP can produce oxidative damage, which play a major role in the pathogenesis of several oxidative related diseases. ROS cause tissue damage via multiple mechanisms. According to Luper [44], most plants contain polyphenols as antioxidative compounds, as free radicals considered to play a major role in liver disorders characterized by degenerative, necrosis and functional impairment and provide hepatoprotectiion against poisoning by toxic xenobiotics.

Recently, they have been isolated and identified flavinoiuds and flavonols from pulp fruit juice of *O. ficus indica* with antioxidant and free radicals scavengers [45-48], betalain pigments [49], multivitamins and organic acids [50]. However, Lee et al. [48] reported that *O. ficus indica* juice possess antioxidant, free radical scavengering activity due to its high amount of phenolics (180-3 mg/g) which might be the active compounds responsible for the antioxidant properties, and so may provide a possible therapeutic alternative in hepatic disorders. Although, many investigations were carried out by several authors to study the protective effects of polyphenols and flavinoids from different plant extracts on experimental animals against various types of chemical toxins and suggested that phenolics and flavinoids have contributed directly to the antioxidant activities by neutralizing the free radicals [51-55].

The present study investigates the possible ameliorative effects of cactus fruit juice against anticancer drug CP-induced hepatotoxicity and oxidative stress (OS) in mice. The three experimental groups were evaluated (Groups 1,2,3). CP (75 mg/kg) caused severe hepatotoxicity as evidenced by elevation of serum hepatic markers (ALT, AST, LDH) significantly ($P< 0.05$) increased (Table 1). These biomarkers are more sensitive used in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into blood circulation after cellular damage. Since albumin is a key component of serum proteins, because is synthesized in the hepatocytes, one element used to monitor the liver function.

In the present study there were a decrease in serum albumin and total protein levels in CP-treated mice. The results obtained in the present study regarding the effect of CP-intoxication on the serum hepatic enzyme activities, total proteins and albumin are in agreement with these reported [56-58] who are documented that acute hepatic injury induced by various hepatotoxins continuous to be one of the major causes of progressive liver disorder causing oxidant/antioxidant imbalance. The present study revealed the significant decrease in reduced glutathione (GSH) content, catalase (CAT) and superoxide dismutase (SOD) activities and significant increase in malondialdehyde (MDA) level indicated that CP- induced hepatotoxiciy was mediated through oxidative stress. In contrast, cactus fruit juice treatment significantly improved CP-induced biochemicals alterations in mice (Table 2). These results were consistent with recent studies [51].

It is worth to mention that the lipid peroxidation is belives to play an important role in the liver injury. On other hand, glutathione (GSH) is thought to be important factor in cellular function and defense against oxidative stress. In current study significant increase of (MDA) and decrease of (GSH) were shown in liver tissue in CP-treated animals as compared to control groups (Table 2). It has been reported that acroline of CP could interact with tissues antioxidant defense system, produces highly reactive oxygen, free radicals and mutagenic to mammalian cells. In present study revealed the increase of MDA and reduction of GSH levels and antioxidant enzyme activity (CAT, SOD) in CP treated animals, this might due to more flux of hydrogen radicals and hydrogen peroxide (H_2o_2) [57,58]. Moreover, CP- treatment was found to be cytotoxic and it inhibited the levels of nucleic acids and proteins in hepatic cells (Table 3). These biochemical changes induced by CP are possibly attributed to its preoxidant property. The reduction of nucleic acids, proteins, glutathione and antioxidant enzymes levels and increased in malondialdehyde proberly indicates their preoxidant nature. The pretreatment of mice with cactus fruit juice significantly restore these biochemical readings to near normally.

Study by Romero et al. [58] found that ant oxidation with xenobiotics induces changes in the process of protein synthesis and the levels of DNA are decreased and this result is accompanied with increased concentrations of hepatic enzyme levels (AST and ALT). This

finding was in agreement with our results. The pretreatment of mice with cactus fruit juice significantly ($p< 0.05$) restore these biochemicals to near normally.

5. CONCLUSION

It can be concluded from the results that *O. ficus indica* fruit juice has a protective action against CP- hepatotoxicity, oxidative stress and cytotoxicity and suggest that cactus fruit juice may find clinical application against a variety of toxins and drugs where cellular damage is consequence of reactive oxygen species (ROS).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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