CHEMOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF OPUNTIA FICUS – INDICA FRUITS EXTRACT

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ABSTRACT

The activity elicited by the extract might be due to its ability to activate antioxidant enzymes. The findings suggest the potential use of the ethanolic extract of opuntia Ficus indica as a novel therapeutically useful nephroprotective agent. Our goal was to investigate the effect of Opuntia Ficus indica fruit extract on CYP induced toxicity in albino Wister rats. To this end, we evaluated the effect of Opuntia Ficus indica 400 mg/kg b.w. tested in albino Wister rats by monitoring its effects on oxidative stress, by CYP; we chose this dose because our studies have shown that it can do a good prevention against toxicity induced by CYP. Our result suggests that extract of Opuntia Ficus indica has the potential to prevent CP induced cellular toxicity. The extract of Opuntia Ficus indica shown to act as a potent antioxidant activity.

Keywords: - *Opuntia Ficus – Indica; CYP induced toxicity; Chemoprotective; Antioxidant; Albino Wister Rat.*

1. INTRODUCTION

The prickly pear cactus [Opuntia ficus-indica] has a global distribution and is an important nutrient and food source [1, 2]. The prickly pear variety Opuntia ficus-indica var. saboten (OFS) is widely cultivated on Cheju Island, in southwestern Korea, and is used as a functional food in Korea [3, 4]. Previous study on crude compositions of the OFS showed that the major component of OFS is nitrogen freeextract. However, very little information is currently available on the active constituents present in OFS that are responsible for its antioxidant activity. Furthermore, the mechanism(s) of the antioxidant actions of OFS has yet to be determined. Hence, the purposes of this work were to determine the antioxidant property of OFS and to preliminarilydefine the active components and mechanism of its antioxidant action. The term cactus (Cactaceae) refers to a group of approximately 1,600 species in 130 genera subdivided in the three subfamilies Pereskioideae, Opuntioideae and Cactoideae [5]. The cactus pear fruit also known as prickly pear, tuna or fico d'india. The plant is used mainly for fruit production, although in some countries it is used as a vegetable for human consumption and also as fodder. The high season for harvesting Opuntia cactus fruits is from April to August in Africa and America, and November to December in the Mediterranean regions [5]. Unfortunately, cactus fruits have a short shelf life from 3–4 weeks, thus limiting long-term storage and worldwide distribution. Typically, a high pH value which varies from 5.3 to 7.1 is found, and the very low acidity (0.05- 0.18% citric acid equivalents) compromises extended fruit storage. Phenolics comprise a wide variety of compounds,divided into several classes such as hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, proanthocyanidins, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes and lignans, that occur in a

great number of fruits (grapefruits, oranges, berries, dark grapes, apples, etc.) andvegetables (onions, broccoli, cauliflower, Brussels sprouts,tomatoes, peppers, etc.), wine, tea, chocolate and other cocoa products in varying quantitative and qualitative amounts [5]. The presence of phenolics has been detected in cactus pulp fruit. Kuti [5] has reported anantioxidative effect due to the major flavonoids encountered in cactus fruits. The presence of several antioxidants (ascorbic acid, carotenoids, reduced glutathione, cysteine, taurine and flavonoids suchas quercetin, kaempferol and isorhamnetin) has been detected in the fruits and vegetables of different varieties of cactus prickly pear. Cancer is the uncontrolled growth of cells coupled with malignant behavior: invasion and metastasis. Cancer is thought to be caused by the interaction between genetic susceptibility and environmental toxins. In the broad sense, most chemotherapeutic drugs work by impairing mitosis (cell division), effectively targeting fast-dividing cells. As these drugs cause damage to cells, they are termed cytotoxic. Some drugs cause cells to undergo apoptosis (so-called "self-programmed cell death"}.

Figure 1. Opuntia ficus-indica flower

Figure 2. Opuntia ficus-indica (Indian fig)

2. MATERIALS AND METHODS

2.1. Plant extract collection

Plant Extract was provided by Prof. Mauro Ballero, Department of Botanical Science, Cagliari University, Italy.
Dipartimento di Scienze Botaniche,
Universita degli Studi di Cagliari,
Viale Sant'Ignazio da Laconi, 13,
09123 University, Italy.

Table 2.1 Detail of Plant Extract

Authentication Codex	Family	Species	Drug
OF13022011SMN	Cactaceae	*Opuntia Ficus Indica*	Fruits

2.3. Preliminary phytochemical screening

- Detection of carbohydrates, flavonoids, glycosides, saponin glycoside and tannins.
- Detection of phenol, steroids, alkaloids, amino acid and protein.

General Test	Reagent	Procedure	Observation
Polyphenol test	Potassium permanganate	Extract the diluted with water and add	Color of potassium

		small portion of potassium permanganate	permanganate disappear

Table 2.3.1 Test for Polyphenol

Table 2.3.2 Test for Inulin

General Test	Reagent	Procedure	Observation
Inulin	a-napthol sulphuric acid	To the test solution add a solution of a –napthol and sulphuric acid	Brownish red color produce

Table 2.3.3 Test for Amino Acid

General Test	Reagent	Procedure	Observation
Ninhydrine test	Ninhydrine reagent	2 ml test solution and add 2 ml ninhydrine reagent	Blue or violet color appears.

3. ANIMAL USED AND HOUSING DETAIL

Table 3.1 Detail of Animal Use

Species	Balb-C mice
Sex	Male
Number of animals	24

4. PRINCIPLE OF THE TEST

It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing is needed, − dosing of three additional animals, with the same dose − dosing of three additional animals at the next higher or the next lower dose level.

5. DESCRIPTION OF THE METHOD

The preferred rodent species is the rat, although other rodent species may be used. Normally females are used [6]. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within $\pm$ 20 % of the mean weight of any previously dosed animals. The temperature in the experimental animal room should be 22ºC ($\pm$ 3ºC). The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not normally exceed 1mL/100g of body weight: however, in the case of aqueous solutions 2 mL/100g body weight can be considered. The test substance is administered in a single dose by gavages using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose, should be delayed until one is confident of survival of the previously dosed animals.

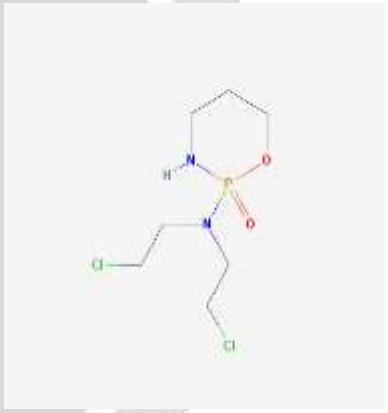
Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days,

except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead.

- **Group A:** This group was administered only vehicle according to body weight orally. Once a day for 15 days. And served as control.
- **Group B:** This group was administered with the plant extract of 400mg/kg orally according to body weight. Once a day for 15 days.
- **Group C:** This group was administered with 200mg/kg body weight CYP i.p. Once a day for 15 days. And served as the negative control.
- **Group D:** This group was administered with CYP 200mg/kg i.p and then the extract 400mg/kg orally 48 hour later for 13 days.
- **Group E:** This group was administered with the plant extract of 400mg/kg orally according to body weight. Once a day for 15 days. On 15th day one hour after extract administration 200mg/kg CYP i.p administered.
- **Group F:** This group was administered with CYP 200mg/kg i.p and then the silymarin 100mg/kg orally 48 hour later for 13 days.

6. INDUCTION OF TOXICITY (CYCLOPHOSPHAMIDE)

The present study was undertaken to evaluate the chemo protective efficacy of *Opuntia ficus indica* against CP-induced toxicity albino wistar rats. Cyclophosphamide (CP) is one of the most widely used alkylating antineoplastic agents that damage normal cells while killing cancerous cells in vivo. The use of CP in treating cancer patients is limited due to its severe toxicities induced mainly by oxidative stress in brain, hepatotoxic and nephrotoxic. *Opuntia ficus indica* extract is a natural product shown to act as a potent antioxidant and chemoprotective in chemically induced murine toxicity and carcinogenesis models in vivo. In the present study, this compound has been evaluated for its protective potential against CP-induced toxicity in albino wistar rats [7].



6.1. Introduction of cyclophosphamide

Cyclophosphamide is an alkylating agent widely used in cancer chemotherapy. Its cytotoxic effects are the result of chemically reactive metabolites that alkylate DNA and protein, producing cross-links. An alkylating agent adds an alkyl group (CnH2n+1) to DNA. It attaches the alkyl group to the guanine base of DNA, at the number 7 nitrogen atom of the imidazole ring [7].

Cyclophosphamide Systematic (IUPAC) name (RS)-N, N-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide

Cyclophosphamide (INN, trade names Endoxan, Cytoxan, Neosar, Procytox, Revimmune), also known as cytophosphane, is a nitrogen mustard alkylating agent, from the oxazophorines group [8]

7. SUB ACUTE ORAL TOXICITY

Extract	*Opuntia ficus indica* fruits extract
Vehicle	Water

Table 7 Test substance for acute oral toxicity

Table 7.1 Test animals (species, sex, number)

Species	Balb-C mice
Sex	Male
Number of animals	27

Table 7.2 Test conditions at different concentration, route, and time

Test sample	The route	The Dose	Time of injection
Solution	Oral	5,50,300&2000 mg/kg	12:00 PM

8. RESULTS AND DISCUSSIONS

8.1. Dose – Response

S.No.	No. Of Animas	Dose	Behavioral Alteration	Mortality
1.	03	5mg/kg	No major alteration	0/3
2.	03	5mg/kg	No major alteration	0/3
3.	03	50mg/kg	No major alteration	0/3
4.	03	50mg/kg	No major alteration	0/3
5.	03	300mg/kg	No major alteration	0/3
6.	03	300mg/kg	No major alteration	0/3
7.	03	2000mg/kg	No major alteration	1/3
8.	03	2000mg/kg	No major alteration	0/3

Table 8.1 Dose for mice

9. ANTIOXIDANT ASSAY

9.1. DPPH assay

9.1.1. Absorbance of test solution at 517nm

S. NO.	CONCENTRATION µg/ml	ABSORBANCE	
		Without DPPH	With DPPH
1)	10	0.002	0.039
2)	20	0.004	0.034

3)	40	0.005	0.030
4)	60	0.007	0.023
5)	80	0.010	0.020
6)	100	0.12	0.015

Table 9.1.1 Absorbance of test solution at 517nm.The results are the means of three separate experiments

S. NO.	CONCENTRATION nm	ABSORBANCE AT 517nm	% INHIBITION
1)	10	0.042	49.39
2)	20	0.036	56.62
3)	40	0.032	61.44
4)	60	0.028	66.26
5)	80	0.023	72.2
6)	100	0.018	78.31

Table 9.1.1.1 Concentration vs. absorbance & Calculation of % inhibition in DPPH

10. CONCLUSION

In present study it was observed that fruits extract of *opuntia ficus indica* family Cactaceae was rich in carbohydrates, flavonoids, polyphenols and steroids. Extract was not having saponins and tannins. It is widely accepted and established that flavonoids and phenols are good source of antioxidant agents. To summarize, the result of present study indicates that CYP exposure result in the pronounced tissue damaged and cellular oxidative stress. Administration of *Opuntia ficus indica fruit extract* protect against Hepatotoxicity, nephroprotective and Oxidative stress. These observations supported that Opuntia ficus indica has potential for its evolution as Chemo protective agent against CYP induced oxidative injury in various vital organs. Therefore, it can be concluded from this investigation that *opuntia ficus indica fruit* extract exhibited hepatoprotective activity and this may be due to its rich contents of flavonoids. In conclusion, that the ethanol extract of *opuntia ficus indica fruit* can prevent renal damage from CYP induced nephrotoxicity in rats and it is likely to be mediated through its antioxidant activities. The activity elicited by the extract might be due to its ability to activate antioxidant enzymes. The findings suggest the potential use of the ethanolic extract of *opuntia ficus indica* as a novel therapeutically useful nephroprotective agent. Our goal was to investigate the effect of *Opuntia ficus indica* fruit extract on CYP induced toxicity in albino wister rats. To this end, we evaluated the effect of *Opuntia ficus indica* 400 mg/kg b.w. tested in albino wister rats by monitoring its effects on oxidative stress, by CYP; we chose this dose because our studies have shown that it can do a good prevention against toxicity induced by CYP. Our result suggests that extract of *Opuntia ficus indica* has the potential to prevent CP induced cellular toxicity. The extract of *Opuntia ficus indica* shown to act as a potent antioxidant activity.

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