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RESEARCH ARTICLE

PRELIMINARY CHARACTERIZATION, ANTIOXIDANT ACTIVITIES AND ULCER CURATIVE EFFECT OF *OPUNTIA FICUS INDICA F. INERMIS* ROOTS POLYSACCHARIDES IN RATS

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ABSTRACT

The present study was undertaken to investigate the *in vitro* antioxidant activity and the *in vivo* curative effect of *Opuntia ficus indica f. inermis* polysaccharides (OFIP) against ethanol- induced ulcer in rats. According to gas chromatography-mass spectrometry and Fourier transform infrared spectroscopy studies OFIP was an hetero-polysaccharide composed of rhamnose, arabinose, fucose, mannose, glucose and galactose at the molar ratio of 4.91; 4.94; 3.87; 32.51; 7.8; 7.1. *In vitro* tested for their potential antioxidant activity OFIP exhibited high radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) when compared with butylated hydroxyanisole (BHA) activity. Whereas OFIP hydroxyl radical scavenging activity, reducing power and the lipid peroxidation inhibitory effect appeared low but not weak when compared with the BHA and vitamin C activities. *In vivo* the treatment with OFIP at oral doses 100, 200, and 400 mg/kg b.w., was found to provide a dose-dependent protection against ethanol-induced gastric ulcer by reducing the gastric juice output, enhancing the healing rate and increasing the mucus production. The antiulcerogenic activity of OMFE might be due to a possible synergistic antioxidant, anti-excretory and healing mechanisms.

KEYWORDS: *Opuntia*; Polysaccharides; Arabinose; Antioxidant; Ethanol; Ulcer.

INTRODUCTION:

Opuntia ficus indica f. inermis belongs to the *Opuntiodeae* subfamily among the *Cactaceae*. Several cultivars are found in the Mediterranean area [1]. *Opuntia ficus indica f. inermis* species grows throughout Tunisia and is mainly cultivated for its sweet and juicy fruit (prickly pear), which was shown to be rich in antioxidant compounds such as polyphenols, flavonoids, betalains, and ascorbic acid [2]. *Opuntia* fruits were found to display interesting properties such as antiulcerogenic [1], antioxidant [3], and neuroprotective [4]. Moreover, prickly pear is used for the treatment of gastritis, hyperglycemia, arteriosclerosis, diabetes, and prostate hypertrophy [5]. *Opuntia* cladodes are modified stems which replace the photosynthetic function of leaves. This part of cactus plant are mainly used for livestock forage and are consumed mainly as staple food, but according to Mexican popular medicine, some diseases like mellitus diabetes, hyperlipidemy, obesity and gastrointestinal disorders can be alleviated by eating *Opuntia* stems [6]. The flowers of *Opuntia ficus indica f. inermis* were used in traditional Tunisian medicine for their diuretic activity, their capacity to expulse renal calculus and to cure ulcer. The various parts of cactus plant flowers, fruit and cladodes showed an antioxidant and a pharmacological benefit. Literature reports few data about cactus roots as well their bioactive compounds. Our previous study reported the preventive effect of *Opuntia ficus indica f. inermis* roots extract against ulcer induced by ethanol and we suggest that phenolic and flavonoids are the main bioactive constituents responsible for antiulcer property [7]. In the present study, our goal is the preliminary characterization of *Opuntia ficus indica f. inermis* roots polysaccharides and to test their *in vitro* antioxidant and their potential curative effects against ethanol- induced ulcer in rats. Therefore, the extracted *Opuntia ficus indica f. inermis* roots polysaccharides (OFIP) were preliminary characterized by gas chromatography (GC-MS) and Fourier transform-infrared spectroscopy (FT-IR). In vitro tested for their antioxidative activities using the 1,1-diphenyl-2-picrylhydrazyl radical, the hydroxyl radical, the reducing power and the lipid peroxidation assays. Finally, the OFIP curative effects against ethanol- induced ulcer in rats were investigated.

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MATERIALS AND METHODS:

CHEMICALS AND EQUIPMENTS:

The fresh roots of *Opuntia ficus indica f. inermis* was collected from municipal areas of Gafsa, state of Tunisia. *Opuntia* root was washed with distilled water, cuted into slices, oven-dried at 40 °C and grounded with moulinex blinder. The material that passed through a 60-mesh sieve was then kept in sealed polyethylene bags until use.

DEAE-cellulose, Sephadex G-100, Sephadex G-25,1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid (TCA), pyridine, trifluoroacetic acid (TFA), acetic anhydride, 2-Propanol, Sodium borohydride, Ammonium hydroxide, were purchased from Sigma–Aldrich (St. Louis, MO, USA). The solvents for GC–MS were of chromatographic purity. All other reagents used were of analytical grade.

EXTRACTION AND PURIFICATION OF OFIP:

The *Opuntia ficus indica f. inermis* powdered roots (200 g) were extracted with 95% ethanol (200 ml,×3) at 75 °C for 2h under reflux to remove lipids. The residue was then extracted with distilled water (150 ml) at 90 °C for 3 times at 1 h for each time. After centrifugation (5000 ×g for 15 min), the supernatant was concentrated to one tenth of the volume, and precipitated with 4 vol of 95% ethanol at 4°C for 24 h. The precipitate was dissolved in 50 ml of distilled water and deproteinized by Sevag reagent (chloroform/butanol 4:1, v/v) as described by Navarini et al. (1999) [8], followed by exhaustive dialysis with water for 48 h. The concentrated dialyzate was then precipitated with 4 vol of 95% EtOH at 4°C for 24 h. The precipitate was washed with absolute ethanol, acetone, and ether. The washed precipitate was the crude polysaccharide (6.4 g).

The crude polysaccharide was then purified with DEAE-cellulose (2.5cm×25 cm) equilibrated with distilled water. The column was firstly eluted with distilled water and then with stepwise gradient of NaCl aqueous solution (0.1-0.5 M) at a flow rate of 1ml/min. Different fraction (2ml) was collected and total sugar content of each tube was measured at 490 nm using the phenol-sulfuric acid assay [9]. The fraction which contains the high content of sugar was then purified further on a Sephadex G-100 column (3 cm×50 cm) with 0.15 M NaCl at a flow rate of 1 ml/min and then was applied to a Sephadex G-25 column (3 cm×50 cm) to remove salts. The extract-purification steps were repeated five times to recuperate purified polysaccharides OFIP yielded 2.3g.

MONOSACCHARIDE COMPOSITION ANALYSIS:

The monosaccharide composition of *OFIP* was analyzed according to the reported method [10]. Briefly, OFIP (2 mg) was hydrolyzed with 2.0 ml 2 M trifluoroacetic acid at 120 °C for 2 h. The resulting hydrolyzate was repeatedly co-concentrated with methanol to dryness and converted into aldonitrile acetates by the addition of a mixture of methanol, pyridine and acetic anhydride. The derivatives were then analyzed by a GC-MS (Varian 3800) equipped with flame-ionization detector (FID) and a HP-5 fused silica capillary column (30 m × 0.32 mm × 0.25 mm) used with a 4min solvent delay and a flow rate of 1.5ml/min. Injected samples are subjected to the following temperature program: Initial hold at 160°C for 2 min; a 20°C/min ramp to 200°C and hold for 5 min; a 20°C/min ramp to 245°C and hold 12 min; spike to 270°C and hold for 5 min before cooling to the initial temperature 160°C. Peaks are identified by mass profiles.

FT-IR SPECTROMETRIC ANALYSIS:

Fourier transform infrared (FT-IR) spectra of the OFIP were recorded on FT-IR Shimadzu, FTIR-8400S spectrophotometer equipped with IRsolution 1.10 Shimadzu software in the range of 4000–500 cm^{-1}. FT-IR scans were collected on completely dried thin films of the OFIP polysaccharide cast on KBr discs. The spectra covered the infrared region 4000–500 cm^{-1}, the number of scans per experiment was 10 and resolution was 6 cm^{-1}.

IN VITRO ANTIOXIDANT ACTIVITY OF OFIP:

DPPH RADICAL SCAVENGING ASSAY:

DPPH radical scavenging assay was performed according to the previous method reported by Grzegorczyk et al. (2007) [11]. Fresh prepared DPPH solution (0.1 mM, in 95% ethanol) was used on the day of each test. OFIP solution (1 ml) was mixed with 1ml DPPH solution. The mixture was vigorously shacked, kept in dark for 30 min and then the absorbance was recorded at 517 nm using an Analytik jena 40, spectro-photometer. The ability to scavenge DPPH was calculated as a percentage according to the following equation:

$$\text{Scavenging activity \%} = (1-A_{sample}\ 517/A_{control}\ 517)\times 100\%.$$

REDUCING POWER ASSAY:

The reducing power of OFIP was determined according to the method reported by Qi et al. (2005) [12]. OFIP solution (1 ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%, w/v). The mixture was incubated at 50 °C for 20 min, followed by addition of trichloroacetic acid (2 ml, 10%,

w/v) and centrifugation at 1000 × *g* for 15 min. A 2.5 ml aliquot of the supernatant was mixed with 2.5 ml of water and 0.5 ml ferric chloride (0.1%, w/v), and the absorbance was measured at 700 nm.

HYDROXYL RADICAL SCAVENGING ASSAY:

Hydroxyl radical scavenging assay was conducted according to the previous method with a modification [13]. Deoxyribose (2.67 mM) and EDTA (0.13 mM) were dissolved in phosphate buffered saline (PBS, 0.2 M, pH 7.4). The PBS solution (0.6 ml) was mixed with 0.1 ml OFIP solution, 0.2 ml ferrous ammonium sulfate (0.4 mM), 0.05 ml ascorbic acid (2.0 mM) and 0.05 ml H_2O_2 (20 mM). The solution was incubated at 37 °C for 15 min, and then 1 ml thiobarbituaric acid (1%, w/v) and 1 ml trichloroacetic acid (2%, w/v) were added. The mixture was boiled for 15 min and cooled in ice, and its absorbance was measured at 532 nm. The scavenging activity was calculated according to the following equation:

Scavenging activity % = $(1-A_{sample}\ 532/A_{control}\ 532)\times 100\%$

LIPID PEROXIDATION ASSAY:

The lipid peroxidation assay used in this study was based on the method of Dasgupta and De (2004) [14]. Egg yolk homogenate (10%, v/v) was prepared as a lipid-rich media. OFIP solution (0.1 ml) was mixed with 0.5 ml egg yolk homogenate and 0.4 ml pure water. Ferrous sulfate (50 µl, 70 mM) was then added to induce lipid peroxidation and the mixture was incubated at 37.5 °C for 30 min. Subsequently, 1.5 ml acetic acid (20%, v/v, pH 3.5) and 1.5 ml thiobarbituaric acid (0.8%, w/v, in 1.1% sodium dodecyl sulfate) were added and the mixture was shaken and heated at 95 °C for 60 min. After the reaction solution was cooled, 5 ml of 1-butanol was added and the mixture was centrifuged at 5000 ×g for 15 min. The upper layer was collected and its absorbance at 532 nm was measured. The inhibition of lipid peroxidation was calculated according to the equation:

Inhibition% = $(1-A_{sample}\ 532/A_{control}\ 532)\times 100\%$

ULCER HEALING ACTIVITY OF OFIP:

EXPERIMENTAL ANIMALS:

Adult male Wistar rats weighing 240–260 g purchased from SIPHAT (Tunis, Tunisia) were used for the acute toxicity and antiulcerogenic studies. Before any experience, all animals were kept for 2 weeks adaptation period under the same laboratory conditions of temperature (22 ± 2 °C), relative humidity (70 ± 4%) and a 12 h light/dark cycle, and received a nutritionally standard diet (SICO, Tunisia) and tap water. All animals were fasted prior all assays and kept in cages with raised floors of wide mesh to prevent coprophagia. Standard drugs and RTE were administered orally by gastric intubation. Animals were cared for under the Tunisian Code of Practice for the Care and Use of Animals for Scientific Purposes.

EFFECT OF OFIP ON ETHANOL-INDUCED ULCERS:

After 24 h of fasting, with only water provided, 96% ethanol at an oral dose of 1ml/rat/day was given in three doses at an interval of 72 h to induce ulcer in the experimental animals (n= 90). During and post-induction ulcer periods, control animals (n = 18) was scheme treated with distilled water. One hour after the administration of the final dose of ethanol, experimental animals were randomly divided in to five groups (n = 18 each) and treated with distilled water (dw), OFIP or sucralfate (a standard drug known by its healing effect of the gastric ulcer) as showed in the figure 1. Fore hours after the last treatment the designated groups of animals were sacrificed, their stomachs ligatured in esophageal and pyloric canals and excised. The stomachs were then opened along the greater curvature; the gastric juice and the mucus covering each stomach were then carefully collected into clean tubes. This were centrifuged at 12,000 × *g*, 4 °C for 10 min and analyzed for gastric juice volume, and mucus weight. Each stomach were then rinsed with saline solution (0.9%), photographed and the extent of the lesions were measured (mm^2) and taken as ulcer index using the ImageJ software according to the method of Khan (2004) [15]. The curative ratio (%) was then measured as described by Takagi et al. (1969) [16] using the following formula:

Curative ratio = 100 × [Control (ulcer index) – Test (ulcer index)/ Control (ulcer index)]

STATISTICAL ANALYSIS:

All *in vitro* tests are performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical significance between groups was assessed by Student's test, $p < 0.05$ being considered statistically significant.

RESULTS:

PURIFICATION AND CHARACTERIZATION OF OFIP:

Figure 2A represents the chromatogram of crude polysaccharides subjected to DEAE-cellulose. Two distinct peaks were observed. The first peak (peak-1) showed prominent biological activity and was further purified with

two successive Sphadex columns, G-100 and G-25 to obtain one prominent peak of OFIP with yellow color (Fig. 2B).
According to the analysis of monosaccharide using GC–MS, OFIP was a hetero-polysaccharide composed of rhamnose, arabinose, fucose, mannose, glucose and galactose at the molar ratio of 4.91; 4.94; 3.87; 32.51; 7.8; 7.1.

FTIR SPECTRAL ANALYSIS OF OFIP:

Fig. 2C showed the FTIR spectrum of OFIP. One characteristic absorptions of polysaccharides is at about 3000-2800 cm^{-1}, were due to the stretching vibration of –OH and C–H. The absorption at 1700–1750 cm^{-1} was the special absorption of uronic acid. No absorbance was recorded at this region which correlates with the limited content of uronic acid determinate or be concealed by the strong absorption at 1623 cm^{-1}. The absorption at 897 cm-1 indicated that β–glycosidic linkages were present between the sugar units of OFIP. Each particular polysaccharide has a specific band in the 1200–1000 cm^{-1} regions. This region is dominated by ring vibrations overlapped with stretching vibrations of (C–OH) side groups and the (C–O–C) glycosidic band vibration. The absorptions at 1247 cm^{-1} and 1043cm^{-1} indicated a pyranose form of sugar.

ANTIOXYDANT ACTIVITY OF OFIP:

The radical-scavenging activity of OFIP was tested using an ethanolic solution of DPPH radical and compared with the activity of the Butylated hydroxyanisole (BHA) used as standard. Fig. 3A, shows that the radical-scavenging activities of OFIP and BHA on DPPH radicals increased in dose-dependant manner and ranged respectively from 38.6% to 75.8% and 34.2% to 72.9%. The EC_{50} values calculated from the graph (Fig. 3. A.) shows that the radical-scavenging activity of OFIP (EC_{50} = 3.2 ± 0.4 mg/ml) appeared higher then that of BHA (EC_{50} = 3.46 ± 0.6 mg/ml).

Fig. 3B, shows that the hydroxyl radical scavenging activity of OFIP and vitamin C (Vitam C) increased with increase of concentrations and reaches respectively 59.8% and 90.9% for the same concentration 8 mg/ml. The effective concentration (EC_{50}) of OFIP (EC_{50} = 6.68±0.49 mg/ml) providing a 50% hydroxyl radical scavenging effect, appeared lower than that of vitamin C (EC_{50} = 0.27±0.07 mg/ml) used as standard.

Fig. 3C, shows that the lipid peroxidation inhibition effect of OFIP and BHA increased with the increase of sample concentration. At a concentration of 0.5 mg/ml, the lipid peroxidation inhibitory rate of OFIP (48.9%) appeared lower than that of BHA (78.6%) used as standard.
This observation was confirmed by the determination of the effective concentration providing 50% inhibition of the lipid peroxidation rate, which appeared low down in the case of OFIP (EC_{50} = 0.73±0.02 mg/ml) when compared with those of BHA (EC_{50} = 0.31±0.08 mg/ml).

Fig. 3d, shows the reducing ability of OFIP and BHA increased with the increase of concentration and respectively reaches their maximums of absorbance 0.91 and 1.01 for the same concentration 8 mg/ml. Whereas The effective concentration (EC_{50}) of OFIP (EC_{50} = 1.75±0.43 mg/ml) providing a 0.5 of absorbance, appeared lower than that of BHA (EC_{50} = 0.36±0.02 mg/ml) used as standard.

EFFECT OF OFIP ON ETHANOL-INDUCED ULCERS:

Table 1 shows that chronic ethanol-induced ulcer was evidenced with a significant increase ($p < 0.01$) of the gastric juice output and a significant decrease of gastric mucus weight ($p < 0.01$) when compared with control group. Whereas treatment of ethanol ulcerated rats with OFIP normalized as dose-dependant manner the above cited parameters to near values registered in control and sucralfate-treated groups.

Table 2 also shows the effects of OFIP and sucralfate, as function of time and doses, on the lesion area of ethanol-ulcerated rats. The three consecutive acute-ethanol intoxications significantly increased ($p < 0.01$) the ulcer index (UI) in ethanol group when compared with control group. The ulcer index of ethanol-ulcerated rats decreased spontaneously as function of time in groups sacrificed after 10 and 15 days of ulcer induction, but still significantly ($p < 0.01$) higher than those recorded in treated rats. The treatment of ulcerated rats with the low dose of OFIP (OFIP1 = 100 mg/kg b.w), for 5 days, significantly ($p < 0.01$) reduced ulcer index giving a curative ratio of 21.29% in comparison with ethanol group. The curative ratio of OFIP1 group increased with time-treatment to achieve 60.36% after 15 days of treatment. Whereas the treatment of ulcerated rats with the high dose of OFIP (OFIP3 = 400 mg/kg b.w) rapidly reduced the ulcer index giving an 82.69% curative ratio in only 5 days, this ratio appeared near those registered in sucralfate treated group and both raised to reach 93%. In fact the treatment with OFIP3 for 15 days appeared the most choice of doses and time period treatment when compared sucralfate treatment.

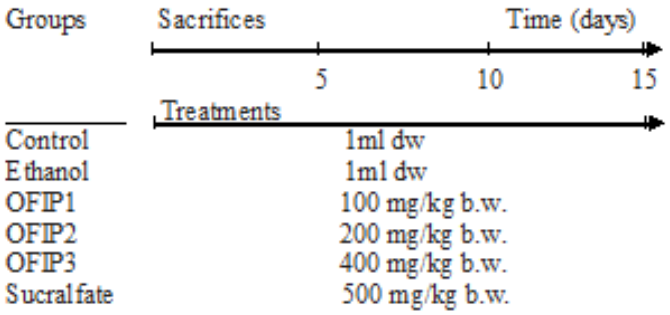
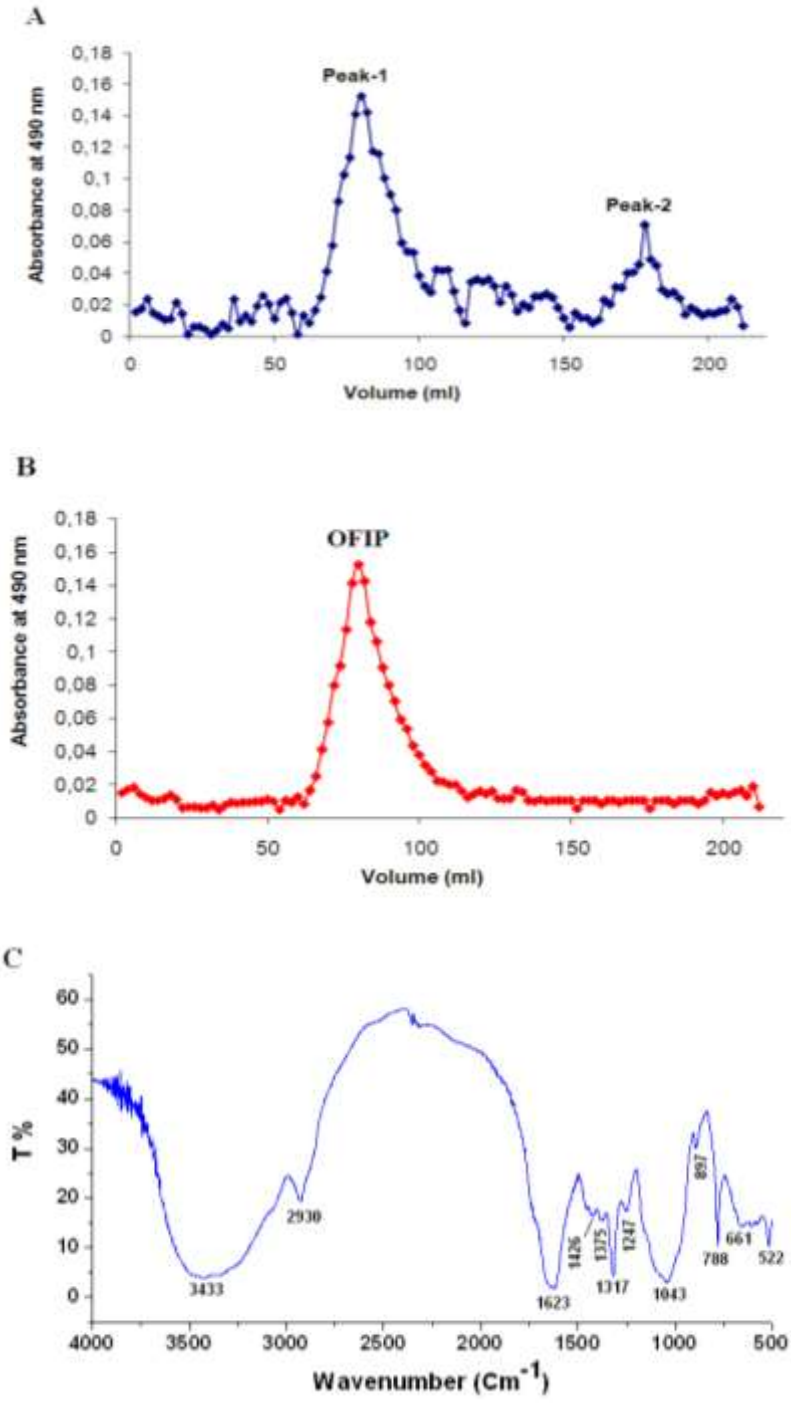


Figure1: Scheme of drugs treatments. Control and ethanol-ulcerated (Ethanol) groups are both treated along the treatments period with (dw) distilled water (1ml/rat). OFIP1, OFIP2 and OFIP3 are the groups respectively treated with 100 mg/kg, 200 mg/kg and 400 mg/kg b.w of OFIP after ethanol ulcer induction. Sucralfate used as positive drugs was administered at 500 mg/kg b.w. Six animals are sacrificed form each group with in interval of 5 days.



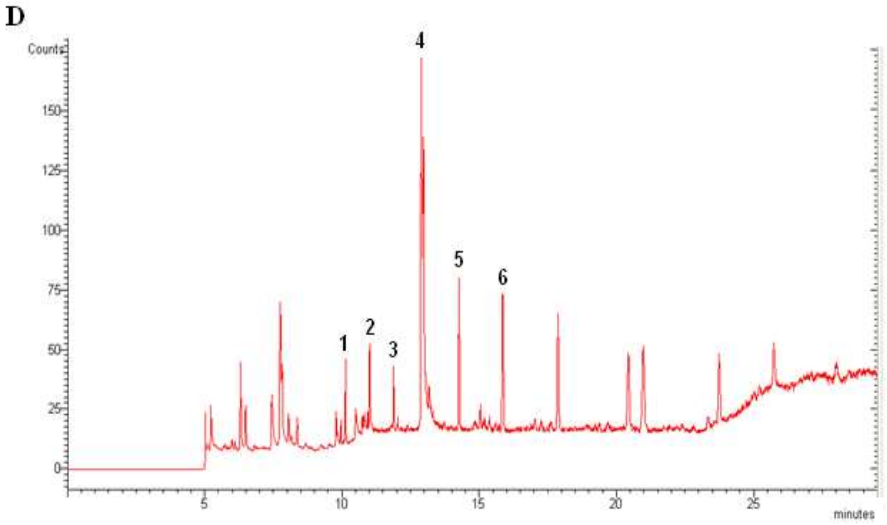
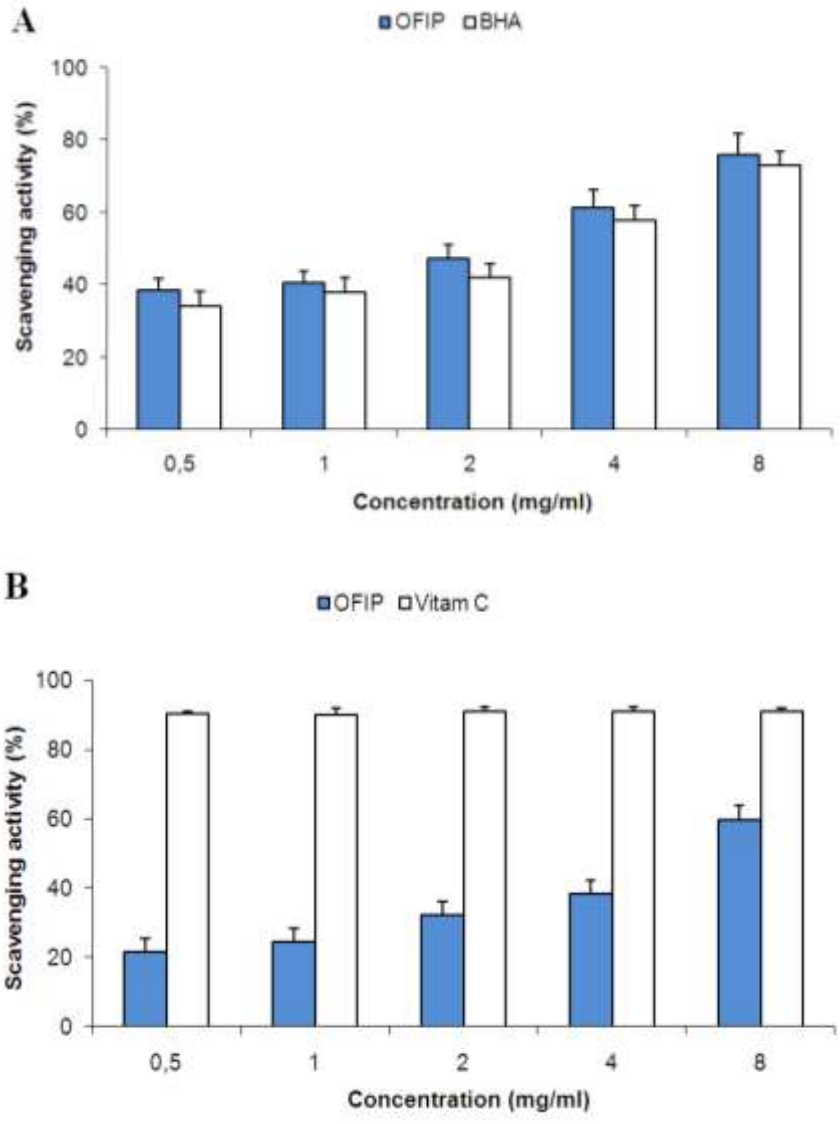


Figure2: Purification and characterization of OFIP. (A) Chromatogram of crude polysaccharides purified by DEAE-cellulose; (B) chromatogram of the second peak (peak-2) purified by Sephadex G-100 and Sephadex G-25; (C) FTIR spectra of OFIP. (D) GC-MS chromatogram of OFIP polysaccharides showing (1) rhamnose, (2) arabinose, (3) fucose, (4) mannose, (5) glucose and (6) galactose. The other peaks are not identified.



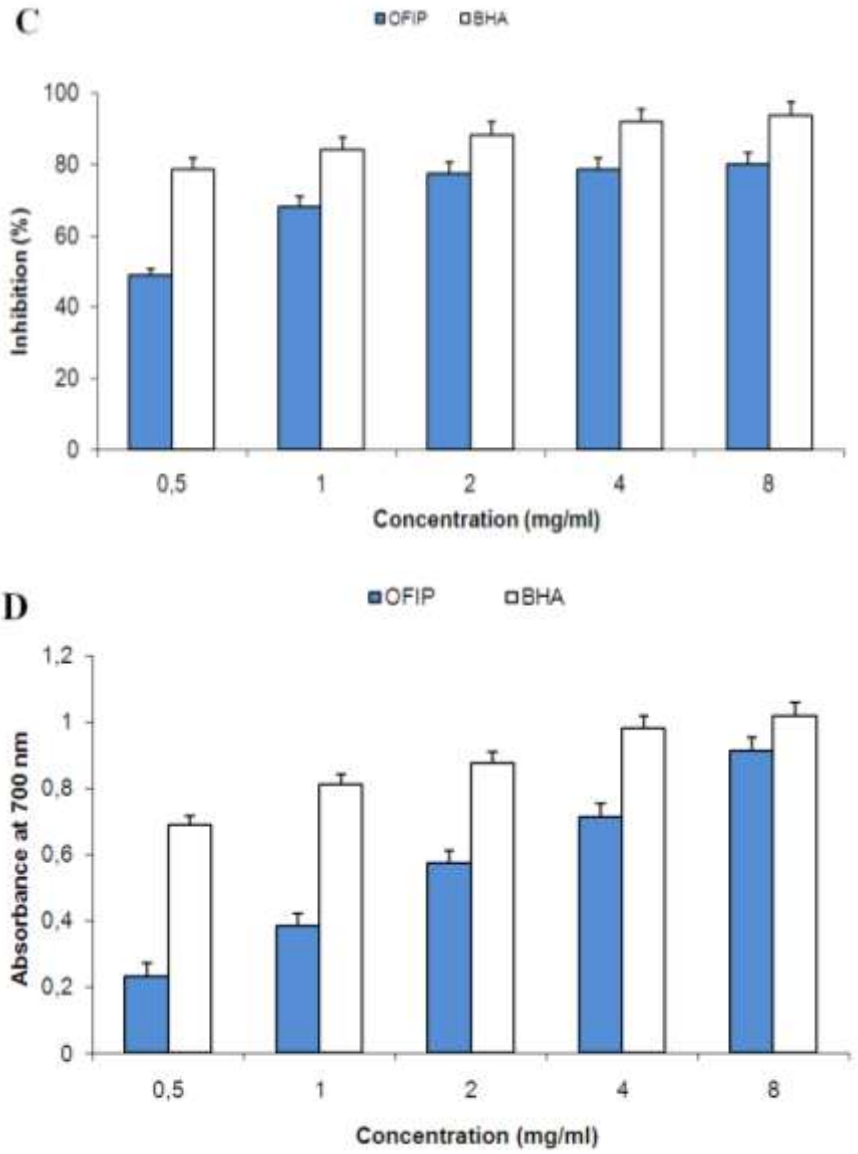


Figure 3: Radical-scavenging activity (A, DPPH; B, hydroxyl), (C) lipid peroxidation inhibition and (D) reducing power of OFIP compared to the butyl hydroxyanisole (BHA) and vitamin C (Vitam C) activities.

Table 1: Effect of ethanol and OFIP treatments on gastric juice volumes (Gv) and mucus weight (M).

Groups	Sacrifices (days)						
	5		10		15		
	Gv	M	Gv	M	Gv	M	*P*
Control	1.12 ± 0.3	129.1 ± 0.8	1.3 ± 0.1	127.3 ± 0.4	1.09 ± 0.6^{bb}	125.7 ± 0.9	**
Ethanol	3.6 ± 0.5	71.6 ± 0. 7	3.1 ± 0.2	87.4 ± 0.6	2.7 ± 0.1^{bb}	102.5 ± 1.1	♦♦
OFIP1	2.1 ± 0.4	101.7 ± 0.4	1.8 ± 0.3	126.2 ± 1.1	1.7 ± 0.6^{b}	138.2 ± 0.8	*
OFIP2	1.4 ± 0.2	150.3 ± 0.6	1.2 ± 0.4	173.6 ± 0.5	1.08 ± 0.4^{bb}	186.3 ± 0.6	**
OFIP3	1.03± 0.4	183.3 ± 0.9	1.04 ± 0.2	211.2 ± 0.1	1.01 ± 0.2^{bb}	234.2 ± 0.2	**
Sucralfate	0.9 ± 0.3	186.4 ± 0.8	1.01± 0.3	216.3 ± 0.4	1.03 ±0.4^{bb}	235.1 ±0.3	**

Values are expressed as means ± SD, for six rats in each group. * $p < 0.05$, ** $p < 0.01$ when compared with ethanol group. $^{♦♦}p < 0.05$; when compared with control group. OFIP1, OFIP2 and OFIP3 are the groups respectively treated with 100 mg/kg, 200 mg/kg and 400 mg/kg b.w of OFIP after ethanol ulcer induction. Sucralfate was used as positive drugs.

Table 2: Healing effect of OFIP and sucralfate as function of time and doses in experimental gastric ulcer induced by ethanol into the rats

Groups	Sacrifices (days)					
	5		10		15	
	UI (mm^2)	CR (%)	UI (mm^2)	CR (%)	UI (mm^2)	CR (%)
Control	0	–	0	–	0	–
Ethanol	52.6 ± 4.9 ^{aa}	–	39.1±7.2 ^{aa}	–	38.1±3.6^{aa}	–
OFIP1	41.4 ± 6.7 ^{b}	(21.29%)	30.1±2.2 ^{b}	(23.01%)	15.1±1.5^{b}	(60.36%)
OFIP2	28.6 ± 5.1 ^{bb}	(45.62%)	17.8±1.9 ^{bb}	(54.4%)	7.5±6.9^{bb}	(80.31%)
OFIP3	9.1 ± 4.8 ^{bb}	(82.69%)	4.9 ±1.6 ^{bb}	(87.4%)	2.6±5.7^{bb}	(93.1%)
Sucralfate	8.7 ± 6.3 ^{bb}	(83.46%)	4.6±0.8 ^{bb}	(88.2%)	2.3±0.8^{bb}	(93.9%)

Values are expressed as means ± SD, for six rats in each group. UI: ulcer index expressed as mm2, CR: curative ratio expressed as %. OFIP1, OFIP2 and OFIP3 are the groups respectively treated with 100 mg/kg, 200 mg/kg and 400 mg/kg b.w of OFIP after ethanol ulcer induction. Sucralfate was used as positive drugs. ^{b} $p < 0.05$, ^{bb} $p < 0.01$ when compared with ethanol group. ^{a} $p < 0.05$; when compared with control group.

DISCUSSION:

Since 20 years ago, interest on the cactus plants has been increased and there are numerous researches on their nutritional and therapeutic compounds. In fact, *Opuntia* species are known for their ability to treat gastritis, hyperglycemia, arteriosclerosis, diabetes, and prostate hypertrophy [5]. Our previous study has also shown that methanolic root extract from *Opuntia ficus indica f. inermis*, has an antioxidant and a gastro-protective effect on ethanol-induced gastric ulcer [7]. Whereas the bioactive(s) compound(s) of *Opuntia ficus indica f. inermis* root extract and their real antiulcerogenic mechanism are still unknown. In this study, the *Opuntia ficus indica f. inermis* root polysaccharides are isolated, preliminary characterized and studied for their antioxidant and antiulcerogenic effects.

FT-IR and GC-MS, carbohydrate composition, analysis indicated that OFIP was a heteropolysaccharide with pyran group, composed of rhamnose, arabinose, fucose, mannose, glucose and galactose with the molar ratio 4.91; 4.94; 3.87; 32.51; 7.8; 7.1. Further more our results demonstrated that OFIP had a significant antioxidant activity. As shown in Fig.2, OFIP was observed to have obvious scavenging activities against DPPH and hydroxyl radicals. The radicals scavenging activities of OFIP could arise from their activity as hydrogen or electron donors [17]. The FT-IR analyses of OFIP demonstrated an intense peak at 3433 cm^{-1} which is the characteristic absorption of hydroxyl groups, the main responsible of OFIP hydrogen or electron release, hence their radical scavenging activity. Our observations are consistent with those demonstrated by Li et al., 2007 [18]. In addition, OFIP effectively inhibited the lipid oxidation of egg yolk homogenate and exhibited strong reducing power. The reducing power is associated with antioxidant activity and may serve as a significant reflection of the lipid peroxidation prevention [19]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so they can act as primary and secondary antioxidants. It is well known that *O. ficus indica* polysaccharide constitute a polymer of monosaccharide with repetitive hydrogen, hydroxide, and acids groups [20]. These bounded contents could contribute to the OFIP antioxidant activity as good hydrogen donors and therefore should be able to reduce Fe^{3+} to Fe^{2+}form. The OFIP scavenging ability tested *in vitro* could foresee a possible *in vivo* antiulcerogenic effect.

Acute alcohol consumption has been considered as one of several factors causing the gastro-duodenal disorders such as gastric ulcer [21]. For the treatment of gastric ulcer, many pharmaceutical products including histamine (H2) receptor antagonists, protons pump inhibitors, antacids and anti-cholinergic have been used [22]. While most of prescribed synthetic drugs produce several adverse reactions when used at long term [23]. Hence, the search is still on to find a natural drug possessing antioxidant and antiulcerogenic properties. The major research study demonstrated the ulcer preventive effect of natural compounds but few data reports the curative effect [24]. A key question when looking for potential antiulcer compounds is if whether the product displays curative effect in animal models. The present study demonstrated for the first time the ulcer curative effect of *Opuntia ficus indica f. inermis* root polysaccharides (OFIP).

The present study showed that copious acute ethanol intoxication induced gastric ulcer evidenced by a significant increase of ulcer index, gastric juice output and a significant decrease of gastric mucus. Our results are in agreement with previous reports which demonstrated that,

after consumption, ethanol rapidly penetrates the gastric mucosa, induced mucus and protein degradation, and increased intracellular membrane permeability to acid, sodium, calcium and water [25]. The massive intracellular infiltration of calcium leads to cell death and exfoliation in the surface epithelium. The gastric juice and acid outputs extend the inter-glandular spaces, promote the neutrophiles infiltration to the ulcerated zones and causes inflammation [26].

Our result also showed the diminution of the gastric juice output and a slight increase of the gastric mucus as function of time after ethanol abstinence. This fact indicate that the spontaneous ulcer healing require a long time. Whereas the treatment of the ulcerated rats with *Opuntia* roots polysachrides (OFIP) significantly reduced the ulcer index, the gastric juice output and enhanced the mucus production as dose dependant manner. When compared with sucralfate effect our results demonstrated that the treatment within 15 days with 400 mg/kg b.w of OFIP appeared to be the most choice of doses and time period treatment.

Sucralfate is an aluminum salt of sucrose octasulphate used as a cytoprotective barrier in cases of gastric ulceration [27]. It has been demonstrated that sucralfate polymerized in acid medium and became a viscous substance charged negatively. The sucralfate preferentially shown a great affinity to bind to the ulcerous craters proteins charged positively, therefore enhanced ulcer curative effect [28]. Galati et al., (2001) [29] reported that the cactus contain a high molecular weight polysaccharides mainly formed by uronic acid, negatively charged, strongly viscous and exhibited an antiulcer effect. Our results also showed that the OFIP are mainly formed by uronic acid, showed a strongly viscous propriety and exhibited a strong ulcer curative effect when compared with sucralfate. Hence the possible mechanism for the ulcer curative activity of the OFIP may be due to their ability to (1) bind to the surface mucosa and function as a protective coating, (2) to act as an anti-excretory compound, (3) to protect the mucosa by increasing mucus synthesis, and (4) to accelerate lesions healing [30].

In conclusion this is the first evidence that *Opuntia ficus indica f. inermis* root polysaccharides (OFIP) has a curative effect on ethanol-induced gastric ulcer. Due to the radical-scavenging activity, the reducing powers, the ability to prevent lipid oxidation, the anti-excretory and the healing effects. We show that OFIP is a potential therapeutic option in the effective management of ulcer and we suggest that OFIP exhibited a powerful ulcer curative effect through a possible synergistic antioxidant, anti-excretory and healing mechanisms.

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