ANTI-INFLAMMATORY PROPERTIES OF BIOACTIVE COMPOUNDS FROM PRICKLY PEAR FRUIT (OPUNTIA SPP.) IN VITRO

A Senior Scholars Thesis

by

MATTHEW FELTMANN, JULIA NELSON, AND BENJAMIN WALKER

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLARS

May 2012

Major: Nutrition Science, Food Science and Technology

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Approved by:

Research Advisor: Susanne Talcott
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ABSTRACT

Anti-Inflammatory Properties
of Bioactive Compounds from Prickly Pear Fruit
(Opuntia SPP.) in Vitro. (May 2012)

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Prickly pear is a long-domesticated cactus crop that is important in agricultural economies throughout arid and semiarid parts of the world. Secondary plant compounds found in similar consumable fruits and vegetables have been found to possess anti-inflammatory and anti-oxidant properties. Prickly pear fruit has been shown to contain potentially anti-inflammatory compounds that also have anti-oxidant and nutritional properties including betalains such as betacyanins and betaxanthins. Additionally, previous studies have suggested that compounds from prickly pear may possess hypoglycemic and hypolipidemic properties.

Inflammation and oxidative stress are underlying and concurring conditions associated with multiple chronic diseases including cancer, obesity, and diabetes. In this study, antioxidant activities were assessed using assays for reactive oxygen species generation and anti-oxidant capacity (ORAC). Inflammation was assessed with quantification and

analysis of mRNA and proteins from cytokines, and other proteins associated with inflammatory pathways, specifically the NF-κB dependent pathway. Prickly pear extract provided a protective effect when inflammation was induced with LPS as evidenced by reduced production of cellular mRNA coding for TNF-α, IL-6 and VCAM-1. HPLC analysis has revealed high levels of polyphenols and betalains, that possess anti-oxidant properties in each of the prickly pear extracts assessed. It may be concluded that prickly pear fruit is a viable source of anti-oxidative and anti-inflammatory compounds, but that further investigation is suggested to establish the mechanisms by which these effects are observed and quantify the bioavailability of these compounds in products suitable for human consumption.

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The research performed for this project would not have been possible without the support of the students and post-graduate researchers of the Texas A&M University Nutrition and Food Science Department in Dr. Susanne Talcott's laboratory including Dr. Giuliana Noratto, Alex Bass, Andrea Roque, Shirley Arbizu, Enrique Garcia, and Hunter Couch, or without additional advising from Dr. Stephen Talcott, Associate Professor of the Texas A&M University Nutrition and Food Science Department.

NOMENCLATURE

spp.	*Species pluralis*
NF-κB	Nuclear Factor Kappa-light-chain-enhancer of B Cells
ROS	Reactive Oxygen Species
DMSO	Dimethyl Sulfoxide
GAE	Gallic Acid Equivalent
LPS	Lipopolysaccharide
SD	Standard Deviation
SE	Standard Error
VCAM-1	Vascular Adhesion Molecule 1
TNF-alpha	Tumor Necrosis Factor Alpha
HPLC-MS	High Performance Liquid Chromatography – Mass Spectroscopy
ORAC	Oxygen Radical Absorbance Capacity

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CHAPTER I

INTRODUCTION

Prickly pear is a long-domesticated cactus crop that is important in agricultural economies throughout arid and semiarid parts of the world. The center of cultivation for prickly pear is central Mexico.[1] Secondary plant compounds found in consumable fruits and vegetables have been found to possess anti-inflammatory and anti-oxidant properties.[2] Prickly pear fruit has been shown to contain potentially anti-inflammatory compounds with anti-oxidant and nutritional properties. These bioactive compounds include the betalains, and their subgroups betacyanins and betaxanthins.[3]

Inflammation and oxidative stress often occur together as underlying conditions associated with multiple chronic diseases including cancer, obesity, and diabetes.[4] The complex immune response elicited by pro-inflammatory stimuli such as foreign bodies and naturally occurring pro-oxidative metabolites exists to eliminate the irritant and provide defense and healing for normal cells. As the human vascular system plays an important role in regulating systemic inflammatory responses, better understanding of its mechanisms and interactions with compounds derived from the diet could offer insight into methods for reducing the rate of chronic diseases.

This thesis follows the style of *Journal of Agricultural and Food Chemistry*.

Objective

Primary objective

The objective of this project was to investigate the anti-inflammatory and anti-oxidant activities of extracts derived from prickly pear, *Opuntia spp.,* in human vascular endothelial cells as a model for cardiovascular disease with a focus on Nf-κB-related pathways. Additionally, the potentially bioactive polyphenolic compounds present in prickly pear fruit were identified. Lipopolysaccharide was used to induce inflammation in the vascular cells. Inflammation stimulation and attenuation of this inflammation by the extracts was assessed via stimulation of a central pro-inflammatory transcription factor NF-κB and genes for inflammatory cytokines.[5] Specifically, the results of these experiments will be used to explore potential implications of prickly pear fruit consumption in the prevention cardiovascular disease-associated inflammation.

Objective 1: (Matthew Feltmann)

Chemical characterization of prickly pear extracts was be performed by HPLC-MS, and anti-oxidant capacity of the extracts using the ORAC assay.

Objective 2: (Julia Nelson and Benjamin Walker)

Anti-inflammatory effects of prickly pear extracts in HUVEC cells will be assessed using cell proliferation, gene expression and protein expression of inflammation-associated factors.

CHAPTER II

METHODS

Plant material and extraction

Pulp from prickly pear cactus fruit (*Oppuntia spp*) was generously donated by S&P Marketing, Inc.. Other commercially and readily available fruit juices and compounds with proven and presumed bioactive polyphenolic components were collected and similarly processed for comparison with prickly pear performance. Fruit pulp and skin were obtained after juice pressing and seed removal. Secondary plant compounds were extracted with 100% methanol (0.01% 12N hydrochloric acid) (1:1 ratio). Preparation of the methanolic extracts was performed as described by Cardona et al., 2009.[6] Briefly, methanolic extracts were filtered through Whatman No. 1 filter paper, and solvent was removed under reduced pressure at 45˚C. The aqueous extract was loaded on the C_{18} cartridge pre-conditioned with 50 mL of methanol and 50 mL of nanopure water. After washing with 50 mL of water to eliminate organic acids and sugars, polyphenols bound to the matrix were eluted with 50 mL 100% methanol and evaporated using a speedvac (Savant, Thermo Scientific Inc, Pittsburgh, PA). The dried polyphenolics were stored at -80°C under 100% nitrogen. For cell culture assays, dried polyphenolics were re-dissolved in DMSO to a known concentration based on total phenolics content, quantified against a gallic acid standard, and expressed as mg of gallic acid equivalent (GAE)/L. All polyphenolic extracts re-dissolved in DMSO were normalized to a final

concentration of 0.2% DMSO in culture medium when applied to the cells; a control with 0.2% DMSO was included in all assays.

Chemicals, antibodies, and reagents

The Folin-Ciocalteu reagent, dichlorofluorescein diacetate (DCFH-DA), and lipopolysaccharide (LPS) were purchased from Fisher Scientific (Pittsburgh, PA). Dimethyl sulfoxide (DMSO) and Triton X-100 were obtained from Sigma Aldrich (St Louis, MO). HPLC grade solvents and hydrogen peroxide were purchased from VWR International (Bristol, CT). Bradford reagent was obtained from BioRad (Hercules, CA), and antibodies against NF-kB p65 and were obtained from Cell Signaling Technology (Beverly, MA).

Chemical analysis

Polyphenolics in each extract were identified and quantified by HPLC at 280 nm. Polyphenolic profiles were obtained using a Waters 2695 alliance HPLC system equipped with Waters 996 photodiode array detector. Mass spectrometric analysis was performed to further aid in polyphenolic identification using a Thermon Finnigan LCQ Deca XP Max MS*n* ion trap mass spectrometer system. The MS was operated in negative ion mode and fitted to an atmospheric pressure electrospray ionization (ESI) source with sheath gas (N2) set to 60 units/min, auxiliary gas (N2) at 5 units/min, spray voltage at 3.5 kV, capillary temperature at 250 °C, capillary voltage at 1.5 V, and tube lens offset at 0 V. Mass spectra were obtained in the full scan mode (*m*/*z* 200–2000).

Anti-oxidant capacity and total phenolics

Anti-oxidant capacity was measured using the ORAC (Oxygen Radical Absorbance Capacity) assay as previously described[7] and adapted to a 96-well FLUOstar Omega plate reader (BMG Labtech Inc., Durham, NC). Total soluble phenolic (TSP) concentration (measure of total metal ion reducing capacity) was measured by the Folin–Ciocalteu assay.

Cell line

Human umbilical vein endothelial cells (HUVEC) were obtained from ScienCell Research Laboratories (Carlsbad, CA) and cultured using ECM medium supplemented with 5% of fetal bovine serum, 1% of endothelial growth supplement (ECGS) and 1% of penicillin/streptomycin solution (ScienceCell, Carlsbad, CA). Cells were maintained at 37 ºC with a humidified 5% CO_2 atmosphere.

Cell proliferation

Cells (1.5×10^4) were seeded in a 24-well plate and incubated for 24 h to allow cell attachment before exposure to varying concentrations of prickly pear polyphenolics. The number of cells present at treatment (0-time value) was quantified with an electronic cell counter (Z1™ Series, Beckman Coulter, Inc), and medium was replaced containing the prickly pear polyphenolics dissolved in DMSO. The difference in number of cells between final incubation time (24h and 48h) and 0-time represents net growth.

Generation of reactive oxygen species (ROS)

The dichlorofluorescein diacetate (DCFH-DA) assay was used to determine the intracellular generation of ROS by lipopolysaccharide as described by Wang *et al*.[8] HUVEC ($6x10^4$/mL) were seeded in a 96-well plate. Following 24 h incubation, cells were pre-treated for 60min with different concentrations (5-50 mg GAE/L) of prickly pear polyphenolics. Cells were then washed with phosphate buffer solution pH 7.0 (PBS) and incubated with 5 μM DCFH-DA for 30 min at 37°C. After incubation, cells were centrifuged at low speed (1000 rpm) for 3min and then were gently washed with PBS. The cells were then stimulated with 25 mM lipopolysaccharide and the fluorescence intensity was measured after 30 min using a fluorescent microplate reader (BMG Labtech Inc., Durham, NC) at 485 nm excitation and 538 nm emission; relative fluorescence units (RFU) were normalized to control cells not treated with polyphenolics.

mRNA analysis

Cells (3 x 105) were seeded onto a 12-well plate and incubated for 24 h to allow cell attachment. Cells were pre-treated with varying concentrations of prickly pear polyphenolics for 30 min and stimulated with lipopolysaccharide (1μg/mL) for 3h before mRNA extraction and analysis. Total RNA was isolated according to the manufacturer's recommended protocol using the mirVanaTM extraction kit (Applied Biosystems, Foster City, Ca) and samples were evaluated for nucleic acid quality and quantity using the NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Isolated RNA was used to synthesize cDNA using a Reverse Transcription Kit (Invitrogen Corp., Grand Island, NY) according to the manufacturer's protocol. qRT-PCR was carried out with the SYBR Green PCR Master Mix from Applied Biosystems (Foster City, Ca) on an ABI Prism 7900 Sequence Detection System (Applied Biosystems Inc, Foster City, CA). Primers were designed using Primer Express software (Applied Biosystems, Foster City, CA). Each primer was homology-searched by an NCBI BLAST search to ensure that it was specific for the target mRNA transcript. The pairs of forward and reverse primers were purchased from Integrated DNA Technologies, Inc. (San Diego, CA). Product specificity was examined by dissociation curve analysis. The sequences of the primers used were: NF-kB (forward: 5'-TGG GAA TGG TGA GGT CAC TCT-3'; reverse: 5'- TCC TGA ACT CCA GCA CTC TCT TC-3'); IL-6 (forward: 5'-AGG GCT CTT CGG CAA ATG TA-3'; reverse: 5'-GAA GGA ATG CCC ATT AAC AAC AA-3'); IL-8: (forward: 5'-CAC CGG AAG GAA CCA TCT CA-3'; reverse: 5'-AGA GCC ACG GCC AGC TT-3'); TNF-a: (forward: F: 5´-AGAGTTTGCCGTCCAAGCA-3´; R: 5´-TGGTAGAGGACGGACACAGACA-3´).

Western-blot analysis

Cells (1 x 10^6) were seeded onto a 10 cm culture plate in medium and incubated for 24 h to allow cell attachment. Cells were treated with prickly pear polyphenolics at 5 and 10 ppm extract added to cell medium. After 24 h incubation, medium was discarded and cells were washed with PBS and removed by scraping using PBS. After centrifugation, cell pellets were lysed with non-denaturing buffer (10 mM Tris-HCl, 10 mM NaH_2PO_4,

130 mM NaCl, 1% (v/v) Triton X-100, 10 mM sodium pyrophosphate, (pH 7.5), and 1% proteinase inhibitor cocktail (Sigma,-Aldrich) for 30 min in ice. Solid cellular debris was removed by centrifugation at 10,000 rpm for 10min at 4°C. The supernatant was collected and stored at -80°C. Protein content was determined using the Bradford reagent (Bio-Rad, Hercules, CA) following the manufacturer's protocol. For each lane, 60µg of protein was diluted with Laemmli's loading buffer, boiled for 5 min, loaded on an acrylamide gel (10%) and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis at 100V for ~2h. Proteins were transferred by wet blotting onto 0.2 µm PVDF membrane (Bio-Rad, Hercules, CA). Membranes were blocked using 5% milk in 0.1% PBS-Tween (PBS-T) for 30 min and incubated with primary antibodies (1:1000) in 3% bovine serum albumin in PBS-T overnight at 4°C with gentle shaking, followed by incubation with the secondary antibody (1:2000) in 5% milk PBS-T for 2 h. Reactive bands were visualized with a luminal reagent (Santa Cruz Biotechnology, Inc. Santa Cruz, CA) after 1 min of reaction.

Statistical analysis

Quantitative data represent mean values with the respective standard deviation (SD) or standard error of the mean (SE) corresponding to 3 or more replicates. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS version 15.0 (SPSS Inc., Chicago, IL). Duncan pairwise comparisons were used to establish statistically significant differences.

CHAPTER III

RESULTS

Comparison of fruit extracts

The concentration of total soluble phenolics and ORAC values were determined. The content of total soluble phenolics of prickly pear extracts was within the same concentration-range of other fruit extracts prepared by various methods, but had a lower average concentration relative to other fruits as given in μg GAE/mL. The comparison of the total amount of fruit/extract/juice that would have to be consumed in order to obtain a comparable concentration in a human body at 5mg/L plasma-concentrations shows that the amount to be consumed varies significantly between extracts. This is assuming an absorption-rate of 5% which was found for anthocyanins and other flavonoids. It is also assumed that there are no interfering compounds, metabolisms or other factors involved and therefore represents a solely theoretical comparison of extracts based on polyphenolic content and anti-inflammatory quality for each extract.

Cell culture

Human Umbilical Vein Endothelial Cells (HUVEC) were cultured in endothelial cell medium (ECM) containing 5% fetal bovine serum, 1% endothelial cell growth supplement, 1% non-essential amino acids, 100 units/mL penicillin G, 100 μg/mL streptomycin, 1.25 μg/mL amphotericin B, and 10 mM sodium pyruvate (Gibco BRL Life Technology, Grand Island, NY). Cells were incubated at 37°C and 5% CO2, and

Table 1. Extract Identification, Soluble Phenolic Content (mg/L as gallic acid), and ORAC Measurements (µmol Trolox equivalents/mL).

TAMU #	Sample ID	Total Soluble Phenolics **C18 Extract**	Total Soluble Phenolics **Juice Equiv.**	ORAC **C18 Extract**	ORAC **Juice Equiv.**
1	Prickly Pear Concentrate (11G07SP.NOG.ASA10334-B335)	5,028	503	57.36	5.74
2	Frozen Prickly Pear Juice Concentrate 36°Brix (10D09SP.DNU.7B702)	15,112	1,511	1034.39	103.44
3	Mangosteen Juice (11G07SP.XNG.V110252)	12,377	1,238	132.64	13.26
4	Nopal Juice (11G07SP.ULM.SCR033)	11,290	1,129	353.52	35.35
5	Aseptic Prickly Pear Puree Non-Acidified (10J29SP.LMM.973.10P173)	5,658	707	80.92	10.12
6	Aseptic Prickly Pear Puree (11E10SP.LMM.973.10P191)	2,736	547	43.94	8.79
7	Mona Vie-Active Juice (Lot: P12081013) Best by 01/12	10,148	1,015	156.24	15.62
8	PomWonderful Juice, 100% pomegranate Retail purchase date: July 2011	14,605	1,537	169.70	17.86
9	Frozen Prickly Pear Juice (ProSun)	4,068	488	67.89	8.15

Table 2. Total Soluble Phenolics (mg/L as gallic acid), and Projected Amount of Juice/Concentrate needed for human consumption targeting a systemic concentration as used in vitro *Not considering metabolism, accumulation, tissue concentrations other than blood, or intestinal effects.

TAMU #	Sample ID	Total Soluble Phenolics **Juice Equiv., mg/L**	Amount (ml) of juice/concentrate used for 5mg/L in vitro	Amount of juice/concentrate to be consumed to obtain in vitro concentration in vivo at assumed 5% absorption*
1	Prickly Pear Concentrate (11G07SP.NOG.ASA10334-B 335)	503	14.51204	1451.204
2	Frozen Prickly Pear Juice Concentrate 36°Brix (10D09SP.DNU.7B702)	1,511	2.81897	281.897
3	Mangosteen Juice (11G07SP.XNG.V110252)	1,238	5.15717	515.717
4	Nopal Juice (11G07SP.ULM.SCR033)	1,129	8.250232	825.0232
5	Aseptic Prickly Pear Puree Non-Acidified (10J29SP.LMM.973.10P173)	707	8.681983	868.1983
6	Aseptic Prickly Pear Puree (11E10SP.LMM.973.10P191)	547	11.25278	1125.278
7	Mona Vie-Active Juice (Lot: P12081013) Best by 01/12	1,015	6.533438	653.3438
8	PomWonderful Juice, 100% pomegranate Retail purchase date: July 2011	1,537	3.123481	312.3481
9	Frozen Prickly Pear Juice (ProSun)	488	13.19351	1319.351

utilized between passages 4-12. Cells were incubated with extracts at concentrations which did not reduce proliferation of cells or show any other cytotoxic effects when compared to control culture in DMSO, medium of extraction preservation. Cell proliferation was not significantly changed by the concentrations used for the following assays (data not shown).

Reactive oxygen species

Reactive oxygen species (ROS) are small oxygen-containing molecules, including oxygen ions, free radicals, and peroxides, that can be highly reactive. ROS are part of normal metabolism and acute inflammatory response. However, under conditions of oxidative stress, ROS can dramatically increase and contribute to cell damage, mutations, long-term inflammation which may be involved in the induction of cardiovascular disease and cancer. Under normal conditions, cells are able to defend themselves against ROS with anti-oxidant enzymes such as superoxide dismutases, catalases, and glutathione peroxidases. Anti-oxidant vitamins and polyphenolic compounds can help cells to prevent ROS induced damage. The dichlorofluorescein (DCF) assay was used to assess the extent of ROS generation. Cells were treated with extracts for 3h and incubated with DCF diacetate (DCFH-DA) substrate Fluorescence was determined for 30min after incubation with hydrogen peroxide using a BMG Labtech FLUOstar fluorescent microplate reader (485 nm excitation and 538 nm emission. ROS were generated through lipopolysaccharide (LPS) which induced inflammation in cells. The process of inflammation includes the generation of

intracellular ROS. All extracts significantly decreased the concentration of LPS-induced ROS in cells, where extracts 4, 6, and 9, (from the varieties nopal and prickly pear) decreased ROS even below levels observed in control cells (Figure 1).

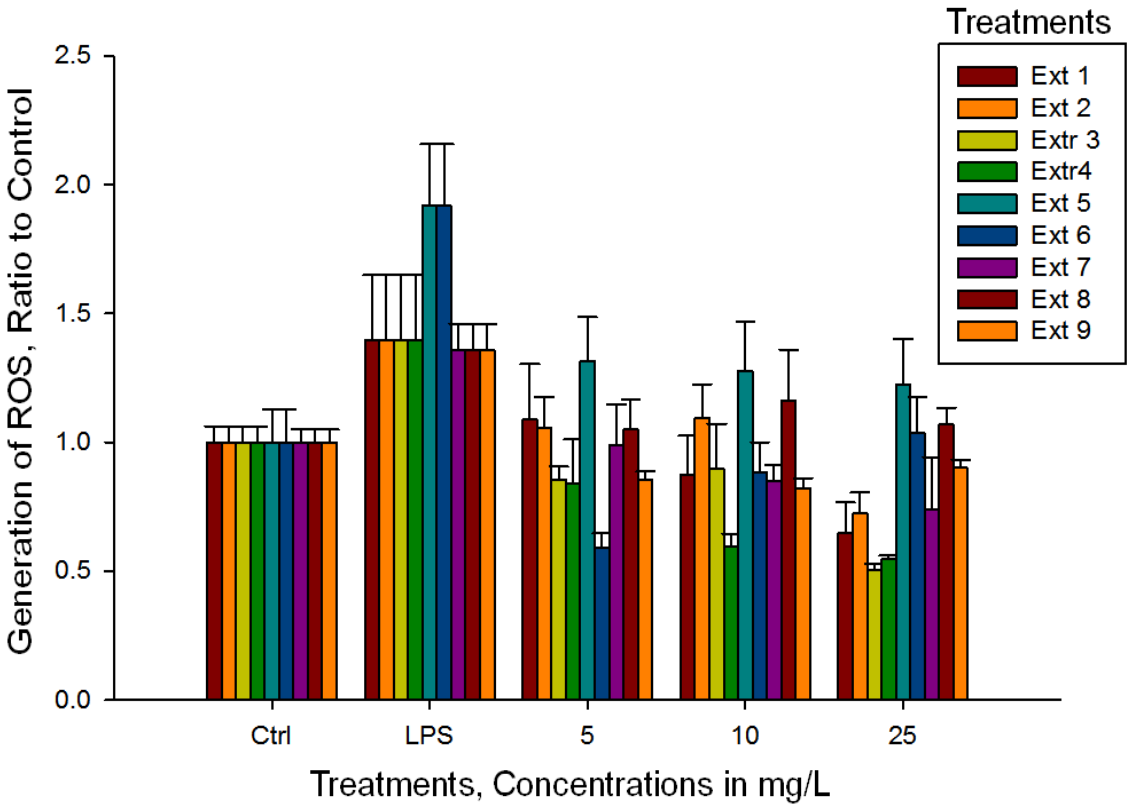

Figure 1. Generation of ROS

mRNA analysis

Messenger ribonucleic acid (mRNA) encode for proteins within a cell. mRNA contains the information which is specific for creation of different cellular proteins. By analyzing

mRNA and quantifying results, the gene expression of anti- or pro-inflammatory proteins can be determined. Inflammation was induced with LPS (2 microg/L) for 3h after pre-incubation of cells with extracts for 21h. Data are shown for extract concentrations of 5 mg/L. The short-term induction of inflammation by NF-κB is mainly modulated through phosphorylation and translocation of subunits; however, the constitutive gene expression of the p65 unit can be indicative of prolonged inflammation. Extract 4, nopal juice, was the only extract that significantly decreased the constitutive expression of mRNA of the p65 unit of NF-κB (Figure 2). Extracts 2, 4, 5, 6, 7, 8, and 9 significantly decreased the expression of NF-κB -dependent gene TNF-alpha (Figure 3). Extracts 1-7 significantly reduced the gene-expression of IL-6, an NF-κB-dependent interleukin involved in inflammatory signaling between cells (Figure 4). IL-6 is involved in stimulating the production of neutrophils in the bone marrow and growth of different immune cells and induces angiogenesis and attracts macrophages into inflamed tissue. In contrast, only extract 7, Mona-Vie active juice, significantly decreased the gene expression of the inflammatory interleukin IL-8 (Figure 5), which is involved in attracting neutrophil blood cells to infiltrate inflamed tissue. All extracts significantly decreased the expression of cellular adhesion molecule VCAM-1 which also is regulated through NF-κB. Extracts 3, 4, and 6 were more efficient compared to the other extracts (Figure 6), and this is at the same total polyphenolic equivalent concentration. This indicates that the combination of bioactive secondary plant compounds is more effective in the mitigation of inflammation in these extracts.

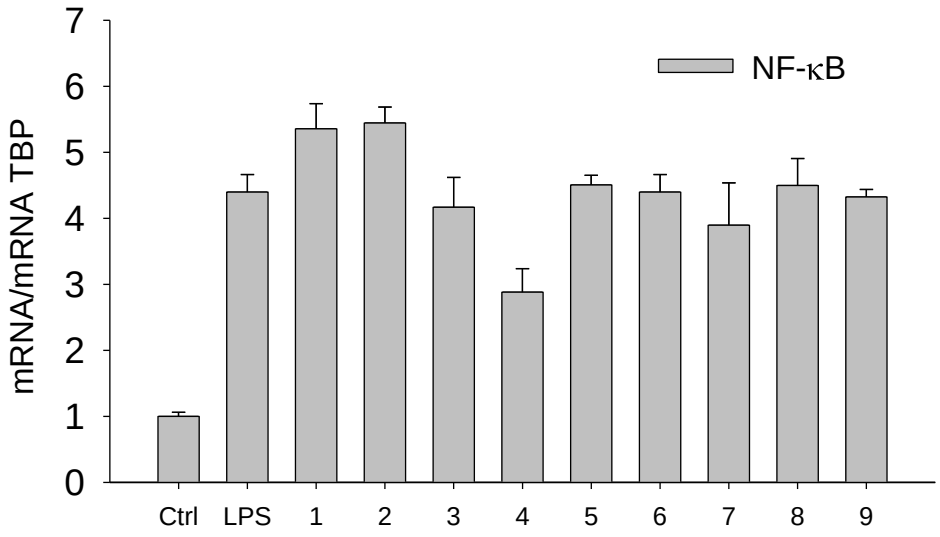

Figure 2. Production of NF-kB

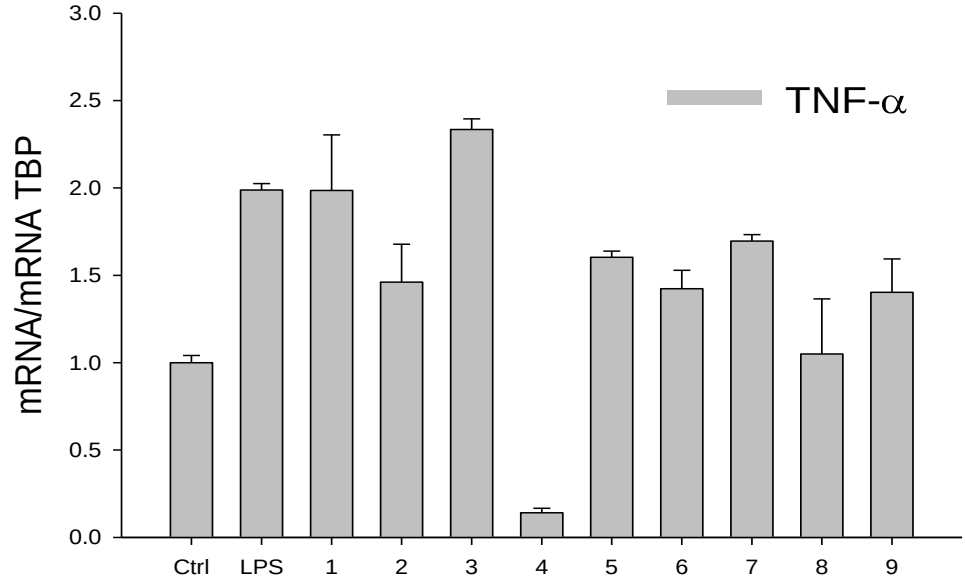

Figure 3. Production of TNF-a

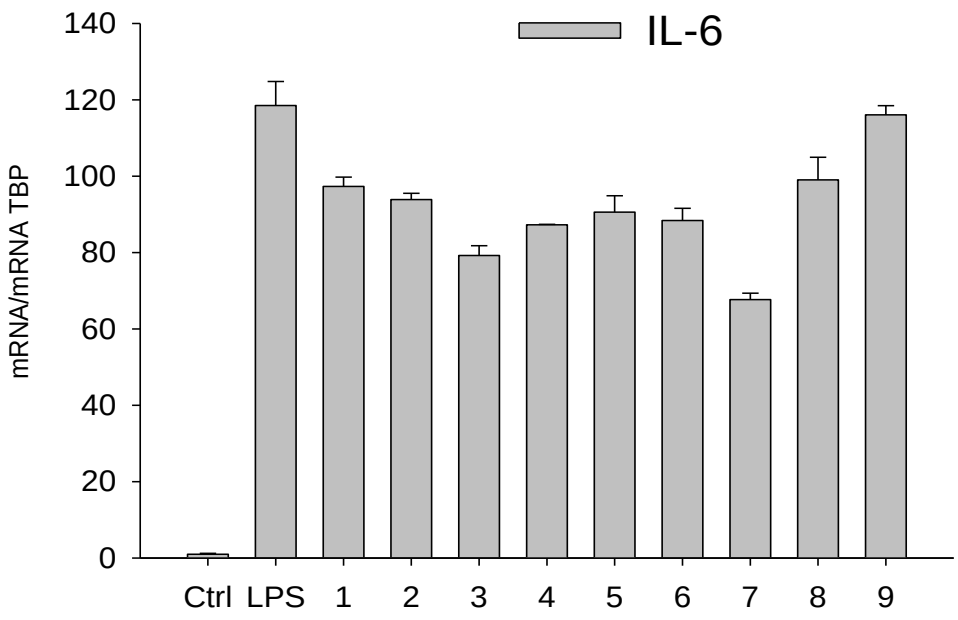

Figure 4. Production of IL-6

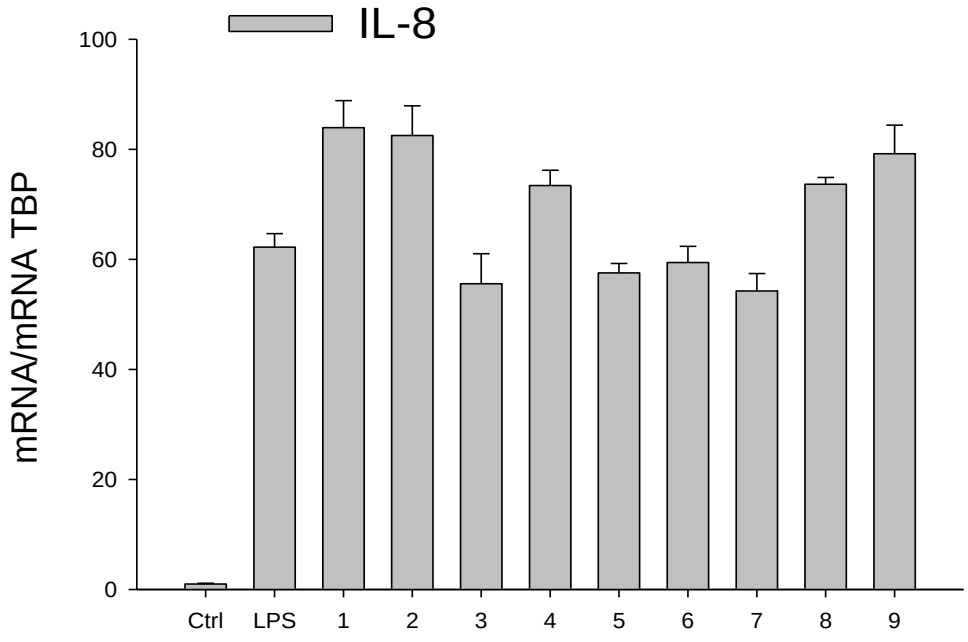

Figure 5. Production of IL-8

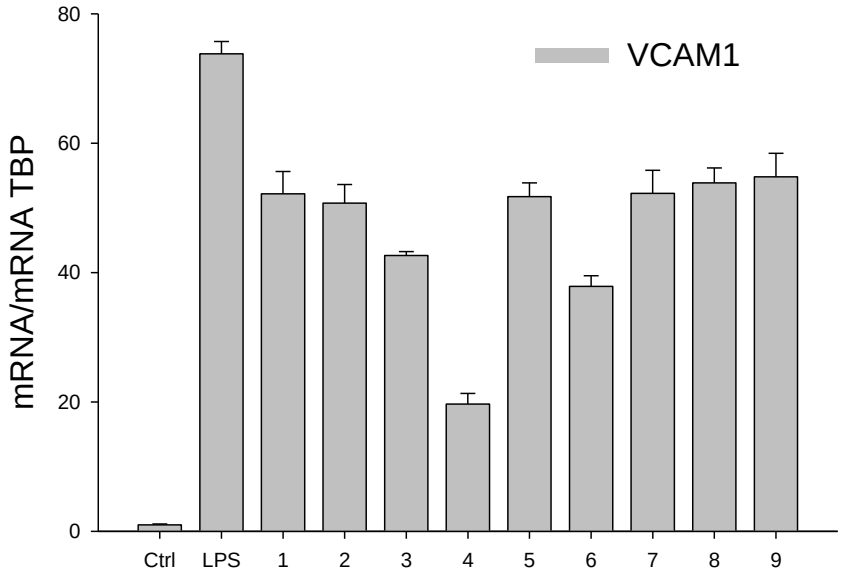

Figure 6. Production of VCAM 1

Protein synthesis

HUVEC cells were seeded onto 6 well plates and incubated for 24 h in normal medium. Then, DMSO was added to control cells and prickly pear extracts 5 and 9 were added to experimental wells with concentrations of 5 and 10 ppm. After 24 h incubation, medium was discarded and cells were washed with PBS and removed by scraping. Protein extraction and quantification was performed. Gel electrophoresis was conducted and secondary antibody for NF-κB was applied to the membrane. Western blot analysis of extracts 5 and 9, non-acidified aseptic prickly pear puree and frozen prickly pear juice, respectively, revealed dose dependent reduction in the expression of phosphorylated NF-κB p65-(Figure 7).

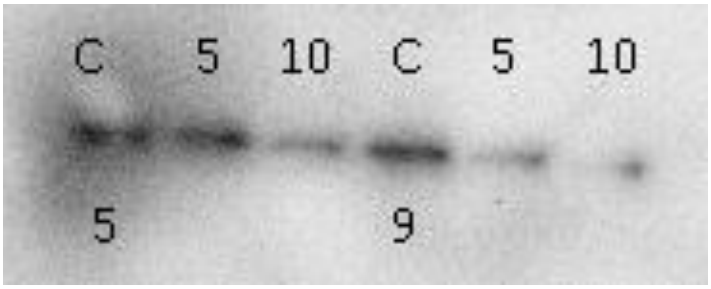

Figure 7. Western Blot Analysis of P-p65

The labels above indicate concentration where C=control and concentrations are in mg/L GAE. The labels below the protein bands indicate the sample number.

CHAPTER IV

CONCLUSIONS

Recent research has emphasized the anti-inflammatory and antioxidant properties of bioactive components in fruits and vegetables including polyphenolics and betalains. Inflammatory processes induce oxidative stress and lipid peroxidation, generating excess reactive oxygen species.[5] As chronic inflammation is associated with an increased risk of various health complications, the identification of dietary compounds with potential to reduce excessive inflammatory activity is crucial to the development of improved methods for addressing the prevention and treatment of these conditions. This study identified several fruit extracts that possess antioxidant and anti-inflammatory properties, where the investigated extract from nopal seemed to have the highest potency, but also extracts from prickly pear were very effective in the mitigation of oxidative stress and inflammation.

HPLC analysis has revealed high levels of polyphenols, betalains and flavonoids, that possess anti-oxidant properties in each of the prickly pear extracts assessed. The total anti-oxidant capacities of the prickly pear extracts are comparable to those of some products already on the market, such as Mona-Vie and PomWonderful juice, and are reportedly more than two times greater than those measured for pear, apple, and white grape.[9]

Anti-oxidative properties of prickly pear extracts are clearly demonstrated by the generation of LPS-induced ROS. Significant reduction was observed in ROS production among those cells protected with prickly pear extract as compared to control cells. The results obtained are comparable to those identified for products currently marketed for their anti-oxidant properties. Subsequent analysis confirmed that prickly pear extract provided a protective effect when inflammation was induced with LPS as evidenced by reduced production of cellular mRNA coding for TNF-α, IL-6 and VCAM-1. Reduced expression of these pro-inflammatory cytokines reflects potential for decreasing the stimulation of immune related functions associated with the acute phase reactions, apoptosis, and production of various inflammation stimulants. Protein assessment indicated decreased the activating phosphorylation of the NF-κB p65 unit that plays a significant role in regulation of the cellular response to increased stress (such as that induced by LPS, ROS and TNF-α) though DNA transcription of genes that control cell growth cycles and apoptosis. It may be concluded that prickly pear fruit is a viable source of anti-oxidative and anti-inflammatory bioactive compounds, but that further investigation is suggested to establish the mechanisms by which these effects are observed and quantify the bioavailability and also toxicity of these compounds in products derived from the investigated fruit varieties.

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