Grape Sourced Bioactives: A Potential New Treatment Strategy for Intestinal Mucositis and Colon Cancer

BY

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Abbreviations

°C	Celsius Degrees
AIF	Apoptosis Inducing Factor
ANOVA	Analysis of Variance
bwt	Bodyweight
CD90	Cumulative dose at 90 min
cm	Centimetre
CO_2	Carbon Dioxide
DMACA	p-Dimethylaminocinnamaldehyde
DMEM	Dulbecco's Modified Eagle's Minimum Essential Medium
DMSO	Dimethylsulfoxide
DP	Degree of Polymerization
DPBS	Dulbecco's Phosphate Buffer Saline
DSS	Dextran Sulphate Sodium
EGCG	Epigallocatechin Gallate
FC	Folin-Ciocalteau
FCS	Fetal Calf Serum
FRAP	Ferric Reducing Antioxidant Power
g	Gram
GA	Gallic Acid
g/kg	Gram per Kilogram
g/L	Grams per Litre
GPC	Gel Permeation Chromatography
GSE	Grape Seed Extract

h	Hour
HPLC	High Pressure Liquid Chromatography
IBD	Inflammatory Bowel Disease
IC_{50}	50% Inhibitory Concentration
IL-1β	Interleukin-1 Beta
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IRMS	Isotope Ratio Mass Spectrometry
JI	Junction of Jejunum and Ileum
kg	Kilograms
KJ	Kilojoules
L	Litre
LD_{50}	Lethal Dose 50%
mDP	Mean Degree of Polymerization
mg	Milligram
mg/kg	Milligam per Kilogram
mg/L	Milligram per Litre
min	Minute
mL	Millilitre
mM	Millimolar
MTT	3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyl-tetrazolium bromide)
MTX	Methotrexate
MPO	Myeloperoxidase
NF-κB	Nuclear Factor-kappa B

ODS	Overall Histological Disease Severity Score
PA	Proanthocyanidin
PARP	Poly-ADP-Ribose Polymerase
pH	Measurement of Acidity
PI3-K	Phosphoinositide 3-Kinase
QTY	Quantity
r^2	Coefficient of Determination
ROS	Reactive Oxygen Species
RP-HPLC	Reverse Phase High Liquid Pressure Chromatography
SBT	Sucrose Breath Test
SEM	Standard Error of Mean
TNBS	2,4,6-Trinitrobenzene Sulfonic Acid
TNF-α	Tumour Necrosis Factor-Alpha
TPTZ	Tri[2-pyridyl]-s-triazine
U	Units
wt	Weight
v/v	Volume per Volume
w/v	Weight per Volume
5-FU	5-Fluorouracil
µg	Microgram
µg/mL	Microgram per Millilitre
µL	Microlitre
µm	Micrometre
µM	Micromolar

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Declaration

I, Ker Yeaw Cheah, certify that this work contains no material which has been accepted for the award of any other degree of diploma in any university of other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Abstract:

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Abstract

Mucositis is a serious condition involving inflammation and ulceration to the lining of gastrointestinal tract that results from cancer chemotherapy. Grape seeds represent a rich source of proanthocyanidins (PAs) which have been reported to be strong antioxidant and chemopreventative agents. This thesis will examine in detail the potential for grape seed PAs to act as novel therapeutic adjunct in cancer treatment.

Previously, Cheah *et al.* (2009) demonstrated that grape seed extract (GSE; 400mg/kg) improved the parameters of intestinal damage in rats with experimentally-induced mucositis. However, its optimal dose and dose responsiveness remained undetermined. In the current study, the effects of increasing GSE doses (400, 600 and 1000mg/kg) on the severity of intestinal mucositis were investigated in a rat model. GSE at higher doses (600 and 1000mg/kg) were more effective than a lower dose (400mg/kg) at ameliorating intestinal injury induced by the chemotherapy agent, 5-Fluorouracil (5-FU) in the proximal small intestine. In addition, no deleterious effects of GSE at these doses were apparent in healthy animals.

The promising effects of GSE in a model of mucositis, and its anti-cancer activity, provided the impetus to further investigate its potential impact on the effectiveness of chemotherapy against colon cancer cells. It was decided to characterise the link between the chemical structures of the main polyphenolic compounds within GSE (PAs), and polyphenolic compounds (epigallocatechin, gallic acid (GA), resveratrol and catechin), which have all been reported to display growth inhibition on cancer cells. At a lower dose, GSE (25µg/mL) and GA (10µg/mL) significantly enhanced the capacity for 5-FU to reduce Caco-2 cell proliferation by 20-26%. Treatment with polyphenols alone, (with the exception of

catechin) at higher doses, exerted more potent growth inhibitory activities compared to 5-FU alone.

Due to the protective effects of GSE against mucositis and the positive findings that GSE and GA reduced the viability of colon cancer cells, it was important to identify the bioactive compounds in GSE responsible for these effects. Six different PA fractions, with increasing mean degree of polymerization (mDP), were isolated from Cabernet Sauvignon grape seeds. GSE, which contained a mixture of oligomers and polymers of PAs, was included as a positive control. This study reported that smaller grape seeds PAs (mDP 2-6) were more effective chemotherapeutic agents than 5-FU alone against colon cancer and exerted greater cytotoxic activity on Caco-2 cells than the crude GSE.

As the health promoting properties of grape seeds are attributed to their PA content and GSE used in parts of this study were derived from multiple grape varieties, it was important to determine the PA profiles of grape seeds derived from different varieties and from different provenance. This study reported the difference in polyphenolic content, not only between grape varietals, but also between geographical regions. Despite Chardonnay and Tannat seeds from Nuriootpa had the highest total polyphenol and flavan-3-ol content, they were the most effective antioxidant agents. In conclusion, grape seeds are a rich source of PA (especially mDP 2-6) and may represent promising therapeutic adjuncts to conventional chemotherapy, which ameliorates mucositis.