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The Effects of Wormwood *(Artemisia monosperma A.*) Oil Extract on The Risks of Colon Cancer Induced in Rats

Thesis

Submitted for Faculty of Women, Ain Shams University
In partial Fulfillment For the Master Science Degree in Biochemistry and Nutrition

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2016

بسم الله الرحمن الرحيم

صدق الله العظيم

ACKNOWLEDGEMENT

First and foremost, thanks to **ALLAH** for most merciful and most gracious who gave me the ability to carry out this work.

I would like to express my sincere gratitude and great thanks to **Prof. Dr. Mona Ahmed Sadek**, Prof. of Biochemistry and Nutrition, Biochemistry and Nutrition Department, Faculty of Women, Ain Shams University, not only for suggesting, planning the point of research and valuable supervision, but also for her great help, guidance, appropriate choice of the research topic, continuous encouragement, she tided me over many difficulties throughout this work. No words seen to be sufficient to describe, to her owe much.

I would like to express heartily thankful to **Dr. Gehan salah El-Din Moram**, assistant prof. of Nutrition, Biochemistry and Nutrition Department, Faculty of Women, Ain Shams University, for helping me, sincere advice and supporting me during all steps of this work.

My deep thanks with love to **Dr. Heba Adel Abd El-Hamid Barakat**, assistant prof. of Biochemistry and Nutrition, Biochemistry and Nutrition Department, Faculty of Women, Ain Shams University, for helping me and valuable advice to me during all steps of this work.

I am thankful to **Prof. Dr. Mohamed Ibrahim Aref,** Prof. of Clinical Pathology, Department of Immunology, Faculty of Medicine, Al-Azhar University, for the help that he offered in the histopathological examination carried out in this study

With great pleasure, I would like to express my sincere gratitude to **the staff members** of Biochemistry and Nutrition Department, Women's College, Ain Shams University, for their encouragement to carry out this work. I also like to express my thanks with love to my friends for their help and support throughout this work.

Last but not least, infinite thanks to **My family** for their continuous support, encouragement and everything else…….

Soha Nassar

ABSTRACT

Some herbal plants play a nutraceutical role to improve health and eliminate risks which brave quality of life of both healthy individuals and patients. Oil extract of *Artemisia monosperma A.* (wormwood) used as traditional medicine for reducing risks of a variety of lethal diseases. The main goal of this study was to identify the bioactive chemical compounds of wormwood oil extract and tested the effects of oral doses on colon cancer through different biological and biochemical measurements in rats in addition of the histopathological changes in colon tissue sections of malignant rats compare to healthy group.

The results of wormwood oil (WO) extract chemical analysis revealed that each 100 gram of the prepared oil extract contained 237 ± 2.3 mg as gallic acid equivalent for total phenols, 3.06 ± 0.01 mg as catechin equivalent for flavonoids, 11.34 ± 0.68 mg of carotenoids, 1.95 ± 0.06 mg as alkaloids, and polyunsaturated fatty acid represent 66.16 % of fatty acids. The tested oil extract also showed high free radical scavenging activity of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radicals by about 95.6 %. The biological trial showed that oral consumption of WO extract tested dose before and after colon cancer induction caused significant enhancement in nutritional parameters; food intake, change in body weight, feed efficiency ratio and feed conversion ratio (***P<0.05***) as compared with malignant group. In addition hematological parameters; hemoglobin, hematocrit, RBC's count, WBC's count and lymphocytes percentage showed significant enhancement in treated group with WO extract as compared with malignant group. There were noticeable improvements in blood indices as well. Further, WO administration to rats before and after colon cancer induction caused a significant improvement in some tumor and inflammatory markers as alpha-fetoprotein (AFP) (83.6 %), cancer antigen-19.9 (CA-19.9) (76.8 %), carcinoembryonic antigen (CEA) (70.4 %), tumor necrosis factor-α (TNF-α) (61.6 %), alkaline phosphatase (ALP) activity (11.6 %), interleukin-6 (IL-6) (57.28 %), c-reactive protein (CRP) (91.8 %) and erythrocyte sedimentation rate (ESR) (60.54 %) compared with malignant rats (***p<0.05***). Significant improvements were also found in the levels of blood proteins, albumin, globulins, albumin/globulins (A/G) ratio, iron and ferritin in malignant rats by oral doses of the oil extract when compared with the malignant group. Treatment of malignant rats with WO as pre and post oral doses caused suppression of oxidative stress status as significant reduction in the levels of serum and colonic malondialdehyde (MDA) and nitric oxide (NO) by about 71.44 %, 71.37 %, 53.59 % and 53.95 %, respectively in comparison with malignant group accompanied by increased levels of blood

and tissue reduced glutathione (GSH) and serum total anti-oxidant capacity (TAC) by 116.7 %, 33.69 % and 119.27 %, respectively compared to malignant group, with supporting the activities of erythrocyte superoxide dismutase (SOD) and serum catalase (CAT) by 27.56 % and 67.06 %, respectively when compared with the control malignant group ***(P<0.05).*** Histopathological examination of the colon tissue confirmed the results of the biochemical analysis showing the anti-tumor effects of WO extract.

LIST OF ABBREVIATIONS

8-OH-G	8- hydroxyl guanine
8-OHdG	8-hydroxy guanine aducts
A	Albumin
A/G ratio	Albumin/globulins ratio
AAP	4-aminophenazone
ABC	Avidin-biotin-peroxidase complex
ACF	Aberrant crypt foci
ADP	Adenosine diphosphate polymerase
AFP	Alpha-Fetoprotein
AIN-93	American Institute of Nutrition-93
Alp	Alkaline phosphatase enzyme
AP-1	Activator protin-1
BAX	B-cell leukemia-2-associated X protein
BCG	Bromocrsol green
BCL-2	B-cell leukemia-2 gene
BMI	Body mass index
B. wt.	Body weight
CA-19.9	Cancer antigen-19.9
CAT	Catalase enzyme
CD	Crohn's Disease
CE	Catechin equivalent
CEA	Carcinoembrionic antigen
Conc.	Concentration
COX	Cyclooxygenase
CRC	Colorectal cancer
CRP	C-reactive protein
D.H_2O	Distilled water
DHA	Dihydroartemisinin
DHBS	Dichloro-2-hydroxybenzene sulfonic acid
DMMR	Defective mismatch repair
DNA	Deoxyribonuclic acid
DNCB	Dinitrochlorobenzene

DPPH	1,1-diphenyl-2-picryl hydrazyl radical
DTNB	5-5'Dithio-bis-2 nitrobenzoic acid
DW	Dried weight
EC	Endothelial cell
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
ESR	Erythrocyte sedimentation rate
FA	Fatty acid
FAA	Free fatty acids
FCR	Feed conversion ratio
FER	Feed efficiency ratio
G	Globulins
GADD153	Growth arrest and DNA damage-inducible gene 153
GAE	Gallic acid equivelant
GIT	Gastrointestinal tract
GPX	Glutathione peroxidase enzyme
GRP78	Glucose-regulated protein 78
GSH	Reduced glutathione
GSSG	Glutathione disulfide
GST	Glutathione-S- transferase enzyme
H_2O_2	Hydrogen peroxide
Hb	Hemoglobin
HcT	Hematocrit
HIF-1	Hypoxia-inducible factor 1
HLA	Human leukocyte antigen
HPLC-MS	High performance liquid chromatography mass
hr.	Hour
HRP	Horseradish peroxidase
IBD	Inflammatory bowel disease
IFN-γ	Interferon-γ
iNOS	Inducible nitric oxide synthase
IL	Interleukin
IR	Ionizing radiation

LOX	Lipoxygenase enzyme
LPL	Lipoprotein lipase enzyme
LS	Lynch syndrome
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDA	Malodialdehyde
MIP-1α	Macrophage inflammatory protein-1 alpha
MMR genes	Mismatch repair genes
MRNA	Messenger ribonucleic acd
MSI	Microsatellite instability
M.WT	Molecular weight
NADP	Nicotinamide adenine dinucleotide phosphate
NBT	Nitroblue tetrazolium
NEDA	N-(1-naphthyl)- ethylenediamine
NF-κB	Nuclear factors kappa B
NIH	National Institute of Health
NK cell	Natural killer cell
NO	Nitric oxide
NOS	Nitric oxide synthase enzyme
O2-	Superoxide anion
O.D.	Optical density
O.D.T	Optical density of test
PARR	Poly (ADP-ribose) polymerase enzyme
PBS	Phosphate buffered saline
PLA2	Phospholipase A2
PMS	Phenazine methosulphate
PRE	Prebiotics
PRO	Probiotics
RBCs	Red blood cells
RE	Rutin equivalent
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rpm	Round per minute
S.D.	Stander deviation

SI	Saturation index
SOD	Superoxide dismutase enzyme
SPSS	Statistical Package for Social Science
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TFR 1	Transferrin receptor 1
TMB	Tetramethylbenzedine
TNF-α	Tumor necrosis factor-α
UV	Ultraviolet
VEGF	Vascular epithelial growth factors
VI	Volume index
WAT	White adipose tissue
WBCs	White blood cells
WO	Wormwood oil

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