UNIVERSITI PUTRA MALAYSIA

COMPARATIVE SYNERGISTIC EFFECTS OF *ORTHOSIPHON STAMINEUS BENTH*, *TEUCRIUM POLIUM L.*, AND *BERBERIS VULGARIS L.* ON BIOCHEMICAL MARKERS, GLUCOCORTICOID RECEPTORS, AND HISTOLOGY OF HEPATOCARCINOGENIC RATS

ARIYO MOVAHEDI

FPSK(p) 2014 11

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By

ARIYO MOVAHEDI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

February 2014

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On behalf of my dear lovely parents, I would like humbly to dedicate this thesis to the best and finest women and queen of all worlds, Seyyedeh Fatemeh Al-Zahra (SA), daughter of the best and greatest man, whom Allah has created all the universe and the seven skies because of him, the holy prophet Hazrat Muhammad (SA).

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

COMPARATIVE SYNERGISTIC EFFECTS OF *ORTHOSIPHON STAMINEUS BENTH, TEUCRIUM POLIUM L.* , AND *BERBERIS VULGARIS L.* ON BIOCHEMICAL MARKERS, GLUCOCORTICOID RECEPTORS, AND HISTOLOGY OF HEPATOCARCINOGENIC RATS

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February 2014

Chairman: Professor Fauziah Othman, PhD

Faculty: Medicine and Health Sciences

Nutrition is an important element in the prevention and treatment of cancer. Herbs and fruits have been used for a long time as an alternative remedy in various diseases including cancer. In the present study, decoction of *Orthosiphon stamineus, Teucrium polium,* and *Berberis vulgaris* and a combination of all three herbs as synergistic group were studied on hepatocarcinogenic rats to investigate the possible cancer preventive/suppressor effect by these plants. Ninety male Sprague Dawley rats (age: 8±1 weeks, weight: 248.1±7.21g) were purchased from UPM Veterinary Faculty and were housed in individual plastic bottom cages and maintained in a room at 22^{o} C temperature with a 12h light/dark cycle. All rats had free access to the standard rat food pellet and drinking water during the study. Rats were kept for one week as adaptation prior to cancer induction. After that, 10 of 90 rats were sacrificed to obtain the baseline data and the rest were induced with cancer by means of intraperitoneal injection of 200mg/kg diethyl nitrosamine (DEN) dissolved in normal saline. This was then followed by 2 weeks feeding on hepatocarcinogenesis promoter diet made from a mixture of standard rat diet with 2-acetylaminofluorene (0.02% AAF). After this period, the leftover rats (n=60) were weighed again and were randomly separated into five groups of equal animal numbers, i.e., control (NC), *O. stamineus* (OS), *T. polium* (TP), *B. vulgaris* (BV) and the synergistic (SY). Rats were force-feed with the decoction of the herb (0.7ml/100g BW) for 7 months based on their respective herbal treatment group. After the treatment period, the rats were fasted overnight and sacrificed for serum and histology analyses of their livers. All data were analyzed using one way ANOVA followed by Dunkan's multiple range post hoc test. Differences between groups were considered significantly different when the P value was less than 0.05.

Based on the present study BV decoction showed significantly higher level of total phenolic and flavonoids contents as well as antioxidant activity as compared to other groups ($p<0.05$). The SY decoction group also showed significantly higher level of TPC and TFC as compared to decoction of both OS and TP ($p<0.05$). Even though, decoction of TP showed significantly lower level of both TPC and TFC, it showed high level of DPPH scavenging activity similar to OS decoction but lower than decoction of BV and SY.

Despite the insignificant difference of body weight between the different groups, liver weight of control group was significantly higher as compared to the other groups ($p<0.05$). Biochemical assay on alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) showed significantly lower level of these markers in BV group as compared to control and other treatment groups ($p<0.05$). BV groups showed significantly higher total serum lipase and total antioxidant status (TAS) than other groups as well ($p<0.05$), which showed the possible of beneficial effect of BV on the above mentioned markers. Although SY group also showed significantly higher level of TAS as compared to both control and OS groups ($p<0.05$), this level of TAS did not show any positive effect on the above mentioned biochemical markers.

Both BV and TP groups showed significantly lower values of α_2MG, alpha fetoprotein, homocysteine, interleukin 6, lactate dehydrogenase, gamma-glutamyl transpeptidase, and tumor necrosis factor α as compared to other treatment groups ($p<0.05$). Both BV and TP groups showed significantly higher values of corticosteroid-binding globulin ($p<0.05$).

Light microscopy histological evaluation illustrated that there were significant changes in the lesion score of BV and TP in portal and lobular region compared to OS, SY and control groups. Fluorescence *in situ* hybridization evaluation of glucocorticoid receptors (GR) showed significantly higher activity of GRs in both BV and TP groups.

The present findings showed advantages and disadvantages of the synergistic effects of herbal decoction in cancer prevention or suppression. On the other hand, unlike few previous studies on *O. stamineus,* even though it showed anticancer properties, the present study could not support strongly the past findings. Overall, the present results showed that the decoction of both *B. vulgaris* and *T. polium* has high anti-cancer activity, which might be due to their either high antioxidant activity or their compounds. Due to these anticancer properties, daily intake of these herbs to prevent cancer in healthy individuals or to suppress and decrease cancer development among patients who are suffering from liver cancer could be recommended.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PERBANDINGAN SINERGISTIK *ORTHOSIPHON STAMINEUS BENTH, TEUCRIUM POLIUM L.* , DAN *BERBERIS VULGARIS L.* KE ATAS PENANDA-PENANDA BIOKIMIA, RESEPTOR GLUKOKORTIKOID DAN HISTOLOGI TIKUS HEPATOKARSINOGENIK

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Nutrisi merupakan elemen penting di dalam pencegahan dan rawatan kanser. Herba dan buah-buahan telah lama digunakan sebagai penawar alternatif dalam perbagai jenis penyakit termasuk kanser. Dalam kajian terbaru ini, air rebusan *Orthosiphon stamineus, Teucrium polium* dan *Berberis vulgaris* dan suatu kombinasi ketiga-ketiga herba tersebut sebagai kumpulan sinergistik telah dikaji ke atas tikus jantan hepatokarsinogenik bagi menyelidiki kemungkinan kesan pencegahan/penindasan kanser oleh tumbuh-tumbuhan tersebut. Sembilan puluh ekor tikus Sprague Dawley jantan (umur: 8±1 minggu, berat: 248.1±7.21g) telah diperolehi dari Fakulti Veterinar, UPM dan ditempatkan di dalam sangkar individu berdasar plastik dan diselenggara dalam sebuah bilik pada suhu 22ºC dengan kitaran 12 jam terang/malam. Kesemua tikus mempunyai akses bebas ke atas pelet makanan piawai dan air minuman semasa kajian. Tikus disimpan di dalam bilik menunggu selama seminggu bagi mengadaptasi sebelum rangsangan kanser. Selepas itu, 10 daripada 90 tikus tersebut dikorbankan bagi mendapatkan data garis dasar dan tikus selebihnya dirangsang dengan kanser dengan cara suntikan intraperitonium 200mg/kg dietil nitrosamina (DEN) yang dilarutkan di dalam salin normal. Ini kemudiannya diikuti dengan pemberian diet penggalak hepatokarsinogenesis yang dibuat daripada campuran diet tikus piawai dan 2-asetilaminofluorin selama 2 minggu (0.02% AAF). Selepas itu, tikus ditimbang sekali lagi dan dan dipisahkan secara rawak kepada lima kumpulan dengan jumlah tikus yang sama rata, iaitu kumpulan kawalan (NC), *O. stamineus* (OS), *T. polium* (TP), *B. vulgaris* (BV) dan sinergistik (SY). Tikus disuap paksa dengan rebusan herba (0.7ml/100g BW) selama 7 bulan berdasarkan kumpulan rawatan herba masing-masing. Selepas jangkamasa rawatan, tikus dipuasakan semalaman dan kemudiannya dikorbankan bagi analisis serum dan histologi hati. Kesemua data dianalisa menggunakan ANOVA satu hala dan diikuti dengan ujian

pos hoc julat berganda Duncan. Perbezaan antara kumpulan dianggap signifikan apabila nilai P kurang daripada 0.05.

Berdasarkan kajian terbaru ini, air rebusan BV menunjukkan kandungan keseluruhan flavonoid dan fenol yang lebih tinggi secara signifikan berserta aktiviti antioksida berbanding kumpulan-kumpulan lain ($P<0.05$). Kumpulan air rebusan SY juga menunjukkan tahap TPC dan TFC yang lebih tinggi secara signifikan berbanding air rebusan kedua-dua OS dan TP ($P<0.05$). Walaupun air rebusan TP menunjukkan tahap TPC dan TFC yang lebih rendah secara signifikan, ia menunjukkan tahap aktiviti pencarian DPPH yang tinggi menyamai air rebusan OS tetapi lebih rendah dari air rebusan BV dan SY.

Walaupun tiada perbezaan berat badan yang signifikan antara kumpulan-kumpulan yang berlainan, berat hati dalam kumpulan kawalan adalah lebih tinggi secara signifikan berbanding kumpulan-kumpulan lain ($p<0.05$). Asai biokimia ke atas alkalin fosfatase (ALP), aspartat aminotransferase (AST), dan alanin aminotransferase menunjukkan tahap yang lebih rendah bagi penanda-penanda ini dalam kumpulan BV berbanding kumpulan kawalan dan kumpulan rawatan yang lain ($p<0.05$). Kumpulan BV juga menunjukkan lipase serum dan status antioksida keseluruhan yang lebih tinggi secara signifikan berbanding kumpulan-kumpulan lain ($p<0.05$), di mana ianya menunjukkan kemungkinan kesan bermanfaat BV ke atas penanda-penanda yang tersebut di atas. Walaupun kumpulan SY juga menunjukkan tahap TAS yang lebih tinggi secara signifikan berbanding kedua-dua kumpulan kawalan dan OS ($p<0.05$), tahap TAS ini tidak menunjukkan sebarang kesan positif ke atas penanda-penanda biokimia di atas.

Kedua-dua kumpulan BV dan TP menunjukkan nilai α_2MG, alfa fetoprotein, homosistin, interleukin 6, laktat dehidrogenase, gama-glutamiltranspeptidase, dan faktor nekrosis tumor α yang lebih rendah secara signifikan berbanding rawatan lain ($p<0.05$). Kedua-dua kumpulan BV dan TP menunjukkan nilai globulin pengikat-kortikosteroid yang lebih tinggi secara signifikan ($p<0.05$).

Penilaian histologi mikroskopi rendah menunjukkan terdapat perubahan yang signifikan pada skor lesi BV dan TP di dalam kawasan portal dan lobular berbanding kumpulan OS, SY dan kawalan. Penilaian hibridisasi *in situ* pendarfluor reseptor glukokortikoid menunjukkan aktiviti GRs yang lebih tinggi secara signifikan di dalam kedua-dua kumpulan BV dan TP.

Penemuan terbaru ini menunjukkan kelebihan dan keburukan kesan sinergistik air rebusan herba dalam pencegahan atau penindasan kanser. Sebaliknya, tidak seperti beberapa kajian terdahulu, kajian terhadap *O. Stamineus*, walaupun menunjukkan ciri-ciri antikanser, kajian terbaru ini tidak dapat menyokong penemuan-penemuan sebelumnya. Secara keseluruhannya, penemuan terbaru ini menunjukkan air rebusan kedua-dua *B. vulgaris* dan *T. polium* mempunyai aktiviti antikanser yang tinggi, yang mungkin disebabkan oleh samada aktiviti antioksida yang tinggi atau sebatian-sebatian mereka. Berdasarkan ciri-ciri antikanser ini, pengambilan setiap hari herba-herba ini bagi mencegah kanser dalam individu sihat atau bagi menindas dan mnegurangkan perkembangan kanser dalam pesakit yang mengalami kanser hati adalah disarankan.

ACKNOWLEDGEMENTS

I am honored to start my acknowledgments by quoting a great word of wisdom of Amiralmomenin Imam Ali (AS) in acknowledging almighty Allah. "Praise is due to Allah whose worth cannot be described by speakers, whose bounties cannot be counted by calculators and whose claim (to obedience) cannot be satisfied by those who attempt to do so, whom the height of intellectual courage cannot appreciate, and the divings of understanding cannot reach; He for whose description no limit has been laid down, no eulogy exists, no time is ordained and no duration is fixed".

Imam Sajjad (AS) said "Among the most grateful of people to Allah are those who are most grateful to other people". It is my honor to thank all people who supported me and were involved in one way or another in the preparation of this thesis. I would like to express my sincere gratitude to my supervisor, Prof. Dr. Fauziah Othman for her insightful comments, meticulous supervision, constant encouragement, and kind cooperation in all steps of my study. I am also grateful to the support and mentoring of my co-supervisors, Prof. Dr. Asmah Rahmat, for her grate supports and guides who always acts as scholarly as motherly for all the students. Special thanks to Assoc. Prof. Dr. Rusliza Basir who was always great and kind support with constructive perfect comments with her fastidious eyes on all aspects. I also want to thank Dr. Seyyed Muhammad Charafeddine for his great professional supports and guides which facilitated the histopathlogy part of this study in a highly qualified way.

No words can express my gratefulness to my lovely parents who have accepted and tolerated all these years of my post graduate studies in different countries, and not only let their son chase his dreams but also always did great encouragements. Your support and prayers were essential for this work in all aspects. Thank you for being my role models. I want to thank my lovely sisters Leonoreh and Hanieh, also my brother Matthew and auntie Azam whom their love and support has been and will continue to be my inspiration, and I am so blessed to have such a caring and supporting family.

My endless appreciation goes to my lovely friends and semi family, the Charafeddine family in my lovely Lebanon, Ayatollah Seyyed Charaffedine and his great kind wife who cares and treats me like her son, also Seyyedeh Fatemeh, Dr. Mageda Zahra, and Seyyed Ali. You were always a great support in all steps.

I would show my gratitude to my great friends and supporters during my rough times, Seyyedeh Robab Sadr and her honorable family, Seyyed Louay, Seyyedeh Farideh, Leili and Lina as well.

I also want to thank Mrs. Melanie Matar for all her favor and kind efforts in reviewing my literature review, also Dr. Maya Bassil from Lebenese American University, and Dr. Dalia Khoury from University of Toronto, in helping me to have access to full text resources. I would like to acknowledge the support of my lab mates and friends: Dr. Mahin Salimi, Amira, Leila, Tayyebeh, Marzieh, and Najla.

I would also like to acknowledge and thank the staff of Laboratories of Nutrition, Biochemistry, Anatomy, Stem cell, Chemical pathology, Immunology, University Agriculture Park of UPM, Histopathology, the Microcopy unit of the Institute of Bioscience, and Animal house at FMHS/FPSK to make this study possible.

I offer my regards and blessings to all of those who supported me in any respect during the completion of the project. In addition, I wish to thank the many people who I am unable to mention in deep for their support during difficult times.

I certify that an Examination Committee has met on 11.Feb.2014 to conduct the final examination of Ariyo Movahedi on his Doctor of Philosophy thesis entitled "Comparative Synergistic Effects of *Orthosiphon Stamineus Benth*, *Teucrium Polium L.* , and *Berberis Vulgaris L.* on Biochemical Markers, Glucocorticoid Receptors, and Histology of Hepatocarcinogenic Rats", in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

2-AAF	2-Acetylaminofluore
AAR	AST/ALT Ratio
ACS	American Cancer Society
ACTB	Actin Beta
AFP	Alpha-Fetoprotein
AI	Adequate Intake
AIN	American Institute of Nutrition
AKT (PKB)	Protein Kinase B
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ASR	Age-Standardised Rate
AST	Aspartate Aminotransferase
ATBC	α-Tocopherol B-Carotene (ATBC) Cancer Prevention Study
BCL-2	B-Cell Lymphoma 2
BCLC	Barcelona Clinic Liver Cancer
BHA	Butylated Hydroxyanisole
BMI	Body Mass Index
BRAF	Birth Rapidly Accelerated Fibrosarcoma, is a human gene that makes a protein called B-Raf
BV	*B. vulgaris*
C	Cilicius
CAM	Complementary or Alternative Medicine
CARET	β-Carotene and Retinol Efficacy Trial
CCGPS	Copenhagen General Population Study
CCHS	Copenhagen City Heart Study
CGB	Corticosteroid Binding Globulin
CI	Confidence Intervals
CLIP	Cancer of The Liver Italian Program
cm	Centimeter
COX	Cyclooxygenase
CPD	Cyclobutane Pyrimidine Dimers
CRP	C-Reactive Protein
CYP	Cytochrome P450
DAPI	4',6-Diamidino-2-Phenylindole
DENA	Diethylnitrosamine
DL	Decilitre
DNA	Deoxyribonucleic Acid
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay

EPIC	European Prospective Investigation Into Cancer
ERK	Extracellular-Signal-Regulated Kinases
FBS	Fasting Blood Sugar (Glucose)
FISH	Fluorescence *In situ* Hybridization
FMCHES	Finnish Mobile Clinic Health Examination Survey
g	Gram
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GC	Glucocorticoid
GGT	Gamma Glutamyl Transferase
GR	Glucocorticoid Receptor
H&E	Hematoxylin and Eosin
HBSAG	Hepatitis B Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HCY	Homocysteine
HDL	High Density Lipoprotein
HEAL	Health, Eating, Activity, and Lifestyle
HGDN	High-Grade Dysplastic Nodule
HIF	Hypoxia Inducible Factor
HL	Hepatic Lipase
HMG-CO A	3-Hydroxy-3-Methylglutaryl-Coenzyme A
HPFS	Health Professionals Follow-Up Study
HPLC	High-Performance Liquid Chromatography
HR	Hazard Rate
I.P.	Intraperitoneal Injection
IARC	International Agency For Research On Cancer
IC_{50}	Half Maximal Inhibitory Concentration
IL6	Interleukin-6
IOM	Institute of Medicine
IUPAC	International Union of Pure and Applied Chemistry
JAK-STAT	Janus Kinase-Signal Transducer and Activator of Transcription
JPHC	Japan Public Health Center-Based Prospective Study
K	Kilo
Kg	Kilogram
KDa	Kilo Dalton
K-RAS	Kirsten Rat Sarcoma
L	Litre
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
LGDN	Low-Grade Dysplastic Nodule
MAPK	Mitogen-Activated Protein Kinase
ME-CAN	Metabolic Syndrome and Cancer Project

mg	Miligram
ml	Mililitre
mmol/L	Milimol Per Litre
mRNA	Messenger RNA
MTHFR	Methylenetetrahydrofolate Reductase
MYC	Myelocytomatosis, A Regulator Gene That Codes For A Transcription Factor
n	Nano / Number
NASH	Non-Alcoholic Steatohepatitis
NCI	National Cancer Institute
NCR	National Cancer Registry
NDMA	N-Nitrosodimethylamine
NDR	National Death Registry
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NFLD	Non-Alcoholic Fatty Liver Disease
NHS	Nurse's Health Study
NHNES	National Health and Nutrition Examination Survey
NIH	National Institutes of Health
nm	Nano Meter
NMSC	Non-Melanoma Skin Cancer
NOC	N-Nitroso Compounds
NPC	Nutritional Prevention of Cancer
OD	Optical Density
OS	*O. Stamineus*
p	Pico
P-450	Officially Abbreviated As CYP
P53	Protein 53
PBS	Phosphate Buffered Saline
PGE	Prostaglandin E
PI3K	Phosphatidylinositol 3-Kinase
PKC	Protein Kinase C
PLCO	Prostate, Lung, Colorectal, and Ovarian
PSC	Primary Sclerosing Cholangitis
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RPM	Revolution Per Minute
RT	Room Temprature
SCFA	Short-Chain Fatty Acids
SELECT	Selenium and Vitamin E Cancer Prevention Trial
SEM	Scanning Electron Microscopy Standard Error of Mean
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase

SIRS	Systematic Inflammatory Response Syndrome
STZ	Streptozotocin
SVR	Sustained Virological Response
SY	Synergistic
TAS	Total Antioxidant Status
TC	Total Cholesterol
TG	Triglyceride
TNF-α	Tumor Necrosis Factor Alpha
TNM	Tumor Node Metastasis
TP	*T. Polium*
TRIB3	Tribbles Homolog 3
TSC	Total Serum Cholesterol
UK	United Kingdom
US	United States
UV	Ultraviolet
VITAL	Vitamins and Lifestyle
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization
x	Times
α_2MG	Alpha 2 Microglobulin
µ	Micro

CHAPTER I

INTRODUCTION

One of the most vital health risks in our present time is chronic diseases. Chronic diseases are diseases of long period and generally slow in progression, such as heart disease, stroke, cancer, chronic respiratory diseases and diabetes, which by far are the leading cause of mortality throughout the world, representing 60% of all deaths (WHO, 2010). Chronic diseases account for 70% of all losses in the U.S., which is 1.7 million per year. These diseases also cause major limitations in daily life for almost 1 out of 10 Americans or about 25 million people. Among chronic diseases, cancer has an especial place. Human beings and other animals have had cancer throughout documented history (ACS, 2013d). Cancer is a generic term for a large set of diseases that can affect any part of the body and is one of the dreadful diseases among the chronic disorders. Cancer is a leading cause of death worldwide. It accounted for 7.4 million deaths (around 13% of all deaths) in 2004. Lung, stomach, colorectal, liver, and breast cancer cause the most cancer deaths every year (WHO, 2010). Liver cancer is the fifth most common cancer in men (523000 cases, 7.9% of the total) and the seventh in women (226000 cases, 6.5% of the total), and most of the burden is in developing countries, where almost 85% of the cases occur mainly in men with the overall male: female ratio around 2:4. The regions of high incidence are Eastern and Southeastern Asia, Middle and Western Africa, also Melanesia and Micronesia/Polynesia (particularly in men). Low rates are estimated in developed regions, with the exception of Southern Europe where the incidence in men (ASR 10.5 per 100,000) is significantly higher than in other developed regions (GloboCan, 2008). There were an estimated 694000 deaths from liver cancer in 2008 (477000 in men, 217000 in women), and because of its high fatality (overall ratio of mortality to incidence of 0.93), liver cancer is the third most common cause of death from cancer worldwide. The geographical distribution of the mortality rates is similar to that observed for occurrence (GloboCan, 2008).

Fortunately, above 30% of cancer deaths can be averted (WHO, 2013) and one of the most important factors, which can influence as well as prevent cancer, is food. More than 25,000 different bioactive compounds are thought to occur in the foods consumed by human beings. More than 500 of these compounds have already been identified as possible modifier of the cancer process and others will likely to surface. This diverse array of dietary constituents may modify, either positively or negatively, cancer risk and tumor activity (Milner, 2008). These bioactive food components may arise from plants (*phytochemicals*), animal sources (*zoochemicals*), or mushrooms (*fungochemicals*) or from the metabolism of food components by bacteria within the gastrointestinal tract (*bacterochemicals*) (Guarner & Malagelada, 2003; Heerdt, Houston, Anthony, & Augenlicht, 1998; Moquin, Blackman, Mitty, & Flores, 2009; Wasser, 2002).

Epidemiological studies have shown noticeable variations incidence and mortality across different geographic regions in different type of cancer specially prostate cancer leading to the rising interest in the role of nutrition in prostate cancer risk. There is also a large body of evidence that a diverse diet, rich in vegetables, can reduce the risk of prostate cancer (Chang *et al.,* 2009). Based on scientific research, some of the most important food factors which could prevent or help patients in order to better cope with cancer are antioxidants, flavonoids, omega 3 fatty acids, and dietary fibers (Park, Brinton, Subar, Hollenbeck, & Schatzkin, 2009; Ravasco, 2009; Strouch *et al.,* 2011; Zhang *et al.,* 2011). Most of these components could be found in fruits and vegetables. A growing body of epidemiological and preclinical evidence points to culinary herbs and spices as minor dietary constituents with multiple anticancer characteristics (Kaefer & Milner, 2008). In general, plants are nature's remedies and have been used as food and medicinal purposes since ancient times. There are herbs for almost every human affliction (Ali Khan & Khanum, 2005). Herbal traditions have been passed down and refined with scientific understanding, providing information to help in health maintenance. Herbs act on the blood, metabolism, and all cells. Thus, they are capable of bringing the body into harmony and health; herbs are considered food for the body. They are valuable sources of natural medicine, vitamins and minerals that have a remarkable history of curative effects, when used in the proper way. Moreover, not only specific components of herbs might have anticancer capabilities, but also the herbs that are useful for certain ailments usually contain vitamins and minerals and specific biochemical constituents that are also helpful in those ailments (Moquin *et al.,* 2009).

Among possible herbs and fruits with anticancer properties *Orthosiphon stamineus* from Malaysia and two Iranian ones *Teucrium polium* and *Berberis vulgaris* have shown promising effects (Kandouz *et al.,* 2010; Maheswari, 2008; Motalleb, Hanachi, Fauziah, & Asmah, 2008). In this study it was hypothesized that these herbs could suppress liver cancer development in carcinogenic rats. Therefore, anticancer properties of these herbs were examined and compared for their possible anticancer characteristics such as effects on common liver cancer blood markers, as well as serum glucocorticoid level and liver glucocorticoid receptors, also common blood parameters risk factors for chronic disorders (glucose and lipid profile). In order to investigate the public myth on eating good foods specifically herbs together might boost strength of body to cure disease, for the first time the possible synergistic effect of combination of these herbs to prevent or treat hepatocarcinoma was studied as well.

1.1. Study Objectives

1.1.1. General Objective

To investigate and compare the anti-cancer capabilities of *Orthosiphon stamineus*, *Teucrium polium* and *Berberis vulgaris* on hepatocarcinogenic rats.

1.1.2. Specific Objectives

a. To evaluate the total phenolic, total flavonoid, and total antioxidant content of these herbs.
b. To analyze the main well-known active components of *Orthosiphon stamineus*, *Teucrium polium* and *Berberis vulgaris* and their combination.
c. To investigate and compare the effects of *Orthosiphon stamineus, Teucrium polium* and *Berberis vulgaris* and their combination on serum level of cancer markers (Alkaline phosphatase (ALP), C-Reactive Protein (CRP), Alpha-Fetoprotein Tumor Marker (AFP), Alanine Aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/SGOT), Gamma Glutamyl Transpeptidase (GGT), Tumor necrosis factor alpha (TNF-α), Homocysteine (HCY), Interleukin-6 (IL6), Total antioxidant status (TAS), Alpha 2 microglobulin (α2MG), Bilirubin-total, Lactate dehydrogenase (LDH) and serum Lipase) in hepatocarcinogenic Rats.
d. To study and compare the effects of these three herbs and their combination on blood glucose and lipid profile (TG, HDL, LDL, Cholesterol) in hepatocarcinogenic rats.
e. To inspect and compare the effects of these herbs and their combination on corticosteroid binding globulin (CBG) in hepatocarcinogenic rats.
f. To investigate and compare the effects of these herbs and their combination on liver glucocorticoid receptors in hepatocarcinogenic rats.
g. To examine and compare the effects of those herbs and their combination on weight gain, liver weight and liver/body weight ratio in hepatocarcinogenic rats.

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