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

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By

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

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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).
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°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 α₂MG, 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 anticancer 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

Oleh

ARIYO MOVAHEDI

Februari 2014

Pengerusi: Profesor Fauziah Othman, PhD
Faculty: Perubatan dan Sains Kesihatan

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 diikorbankan bagi mendapatkan data garis dasar kajian. Tikus disimpan di dalam bilik menunggu selama seminggu lagi mengadaptasi sebelum disuntikan kanser. Selepas itu, 10 daripada 90 tikus tersebut diikorbankan 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 dikaitkan 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 dipisahkan secara rawak kepada lima kumpulan, kumpulan kawalan (NC), Orthosiphon stamineus (OS), Teucrium polium (TP), Berberis 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 dipisahkan semalaman dan kemudiannya diikorbankan 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 alcalin 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 α2MG, 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.

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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 histopathology part of this study in a highly qualified way.

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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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>ix</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxi</td>
</tr>
</tbody>
</table>

## CHAPTER

1 **INTRODUCTION**

1.1 Study Objectives

1.1.1 General Objective

1.1.2 Specific Objectives

2 **LITERATURE REVIEW**

2.1 Cancer

2.1.1 Definition

2.1.2 Prognosis

2.1.3 Epidemiology

2.1.4 Liver Cancer

2.2 Cancer Pathogenesis

2.2.1 Apoptosis

2.2.2 Necrosis

2.2.3 Cell Proliferation

2.3 Carcinogenesis

2.3.1 Hepatocarcinogenesis

2.4 Carcinogenic Factors

2.4.1 Carcinogenic Chemicals

2.4.1.1 N-nitroso Compounds

2.4.1.2 Diethyl Nitrosamine

2.4.1.3 2-Acetylaminofluorene

2.5 Histology Examination

2.5.1 Light Microscopy

2.5.2 Fluorescent in situ Hybridization

2.5.3 Response of Cells to Injury

2.5.4 Acute and Chronic Hepatitis

2.5.5 Steatosis

2.5.6 Lesion Scoring

2.5.7 Lesion Scoring and Hepatocarcinogenesis

2.6 Biochemical Factors and Cancer

2.6.1 Fasting Blood Glucose

2.6.2 Serum Lipid Profile

2.6.3 Lipase

2.6.4 Alkaline phosphatase
2.6.5. C-Reactive Protein 28
2.6.6. Alpha-Fetoprotein Tumor Marker 29
2.6.7. Alanine Aminotransferase 30
2.6.8. Aspartate Aminotransferase 31
2.6.9. AST/ALT Ratio 32
2.6.10. Gamma Glutamyl Transferase 32
2.6.11. Tumor necrosis Factor Alpha 33
2.6.12. Homocysteine 34
2.6.13. Glucocorticoid/Corticosteroid Binding Globulin 35
2.6.14. Interleukin-6 37
2.6.15. Serum Total Antioxidant Status 38
2.6.16. Alpha 2 Macroglobulin 39
2.6.17. Serum Bilirubin 39
2.6.18. Serum Lactate Dehydrogenase 40
2.7. Preventions and Treatments of Cancer 41
  2.7.1. Prevention 41
  2.7.2. Treatment 42
2.8. Herbs in Combating Cancer 43
  2.8.1. Vitamins and Cancer 43
  2.8.2. Minerals and Cancer 47
  2.8.3. Antioxidant Nutrients and Cancer 51
  2.8.4. Fibers and Cancer 52
  2.8.5. Phytochemical Compounds 53
2.9. Berberis vulgaris 55
2.10. Teucrium polium 57
2.11. Orthosiphon stamineus 59
2.12. Standardization of Herbs in Medicinal Study 62
2.13. Decoction 63

3 MATERIAL AND METHODS 65
3.1. Experimental Design 65
3.2. Animals 65
3.3. Induction of Cancer 66
3.4. Animal Grouping 67
3.5. Force-Feed Calculation 67
3.6. Protocols for Standardization of Herbs 68
3.7. Preparation of Herb’s Decoction 68
3.8. Determination of Total Phenolic Content 68
3.9. Determination of Total Flavonoids 69
3.10. Assay of DPPH Scavenging Activity 69
3.11. HPLC Assay 70
3.12. Termination of Experiment 70
3.13. Biochemical Assays 70
3.14. Histology 71
  3.14.1. Light Microscopy of Liver Cells 71
  3.14.2. Frozen Section Staining 72
  3.14.3. Lesion Scoring 72
  3.14.4. Determination of Glucocorticoid Liver Cell Receptors by Fluorescent in situ Hybridization 73
3.15. Data Analysis 73
4  RESULTS  
4.1. Decoctions Characteristics  
4.1.1. Total Phenolic Content  
4.1.2. Total Flavonoid Content  
4.1.3. DPPH Scavenging Activities  
4.1.4. HPLC Analysis  
4.1.5. Heavy Metal Analysis  
4.2. Mortality Rate of Rats  
4.3. Body Weight Profile of Rats  
4.4. Liver Weight and Relative Liver Weight of Rats  
4.5. Biochemical Assays Results  
4.5.1. Fasting Blood Glucose  
4.5.2. Serum Lipid Profile  
4.5.3. Serum Lipase  
4.5.4. Common Liver Function Tests  
4.5.5. Serum Corticosteroid Binding Globulin  
4.5.6. Serum Homocysteine  
4.5.7. Serum Tumor Necrosis Factor Alpha  
4.5.8. Serum Interlukin-6  
4.5.9. Serum Alpha 2 Macroglobulin  
4.5.10. Serum Alpha Fetoprotein  
4.5.11. Serum C-Reactive Protein  
4.5.12. Serum Total Antioxidant Status  
4.5.13. Serum Gamma Glutamyl Transferase  
4.5.14. Serum Lactate Dehydrogenase  
4.6. Morphology and Histology Results  
4.6.1. Liver Gross Appearance  
4.6.2. Light Microscopy Results  
4.6.3. Lesion Scoring  
4.6.4. Confocal Microscopy of Fluorescent in situ Hybridization  

5  DISCUSSION  
5.1. Decoctions Characteristics  
5.1.1. Total Phenolic, Flavonoid Content, and DPPH Scavenging Activities  
5.1.2. Heavy Metals and Main Compounds  
5.2. Mortality Rate of Rats  
5.3. Body Weight Profile, Liver, and Liver/BW Ratio of Rats  
5.4. Blood Parameters Evaluation  
5.4.1. Fasting Blood Glucose  
5.4.2. Lipid Profile and Serum Lipase  
5.4.3. Common Liver Function Tests  
5.4.4. Serum Corticosteroid binding globulin  
5.4.5. Serum Homocysteine  
5.4.6. Serum Tumor Necrosis Factor Alpha  
5.4.7. Serum Interlukin-6  
5.4.8. Serum Alpha 2 Macroglobulin  
5.4.9. Serum Alpha Fetoprotein  
5.4.10. Serum C-Reactive Protein
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Study protocol and treatment groups</td>
<td>67</td>
</tr>
<tr>
<td>4.1 Comparison the DPPH scavenging activity of different decoctions with standards</td>
<td>77</td>
</tr>
<tr>
<td>4.2 Frequency of Different types of liver disorders in treated and control group of rats</td>
<td>106</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Age-standardized incidence rates of primary liver cancer per 100,000 population at risk.</td>
<td>8</td>
</tr>
<tr>
<td>2.2</td>
<td>Morphological Changes During Apoptosis and Necrosis</td>
<td>12</td>
</tr>
<tr>
<td>2.3</td>
<td>Response of Cells to Injury</td>
<td>20</td>
</tr>
<tr>
<td>2.4</td>
<td>The Different Preneoplastic Lesions and Their Proposed Location in the Multistep Process of Human Hepatocellular Carcinoma (HCC) Development</td>
<td>23</td>
</tr>
<tr>
<td>2.5</td>
<td>Flower, Fruit and Dried Fruit of <em>Berberis vulgaris</em> in Iran.</td>
<td>57</td>
</tr>
<tr>
<td>2.6</td>
<td>Different types of <em>Teucrium polium.</em></td>
<td>59</td>
</tr>
<tr>
<td>2.7</td>
<td>Leaves and Different flowers of <em>Orthosiphon stamineus.</em></td>
<td>61</td>
</tr>
<tr>
<td>3.1</td>
<td>Flowchart of Study</td>
<td>66</td>
</tr>
<tr>
<td>4.1</td>
<td>Comparison the Total Phenolic Content of Different Decoctions</td>
<td>76</td>
</tr>
<tr>
<td>4.2</td>
<td>Comparison the Total Flavonoids Content of Different Herbal Decoctions</td>
<td>76</td>
</tr>
<tr>
<td>4.3</td>
<td>Antioxidant Activity of Different Decoctions Compared to Standards Based on DPPH Antioxidant Scavenging</td>
<td>78</td>
</tr>
<tr>
<td>4.4</td>
<td>HPLC Analyzing of Berberine (UV Chromatogram)</td>
<td>79</td>
</tr>
<tr>
<td>4.5</td>
<td>HPLC Analyzing of Apigenin and Rutin (UV Chromatogram)</td>
<td>80</td>
</tr>
<tr>
<td>4.6</td>
<td>HPLC Analyzing of Sinesetin (UV Chromatogram)</td>
<td>81</td>
</tr>
<tr>
<td>4.7</td>
<td>SEM Result of Heavy Elements Analysis of <em>B. vulgaris</em> Decoction</td>
<td>81</td>
</tr>
<tr>
<td>4.8</td>
<td>SEM Result of Heavy Elements Analysis of <em>T. polium</em> Decoction</td>
<td>82</td>
</tr>
<tr>
<td>4.9</td>
<td>SEM Result of Heavy Elements Analysis of <em>O. stamineus</em> Decoction</td>
<td>82</td>
</tr>
<tr>
<td>4.10</td>
<td>Effect of Different Treatment on Mortality Rate of Rats</td>
<td>83</td>
</tr>
<tr>
<td>4.11</td>
<td>Effect of Different Treatments on Body Weight Profile of Rats as Compared to Control Group</td>
<td>84</td>
</tr>
<tr>
<td>4.12</td>
<td>Effect of Different Treatments on Liver Weight of Rats as Compared to Control and Baseline</td>
<td>85</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>4.13</td>
<td>Effect of Different Treatments on Relative Liver Weight Ratio of Rats as Compared to Control and Baseline</td>
<td>85</td>
</tr>
<tr>
<td>4.14</td>
<td>Effect of Different Treatments on Fasting Blood Glucose of Rats as Compared to Baseline and Control Group</td>
<td>86</td>
</tr>
<tr>
<td>4.15</td>
<td>Effect of Different Treatments on Serum Total Cholesterol of Rats as Compared to Baseline and Control Group</td>
<td>87</td>
</tr>
<tr>
<td>4.16</td>
<td>Effect of Different Treatments on Serum Low Density Lipoprotein of Rats as Compared to Baseline and Control Group</td>
<td>88</td>
</tr>
<tr>
<td>4.17</td>
<td>Effect of Different Treatments on Serum High Density Lipoprotein of Rats as Compared to Baseline and Control Group</td>
<td>88</td>
</tr>
<tr>
<td>4.18</td>
<td>Effect of Different Treatments on Serum Triglyceride of Rats as Compared to Baseline and Control Group</td>
<td>89</td>
</tr>
<tr>
<td>4.19</td>
<td>Effect of Different Treatments on Serum Lipase of Rats as Compared to Baseline and Control Group</td>
<td>90</td>
</tr>
<tr>
<td>4.20</td>
<td>Effect of Different Treatments on Serum ALP of Rats as Compared to Baseline and Control Group</td>
<td>91</td>
</tr>
<tr>
<td>4.21</td>
<td>Effect of Different Treatments on Serum AST of Rats as Compared to Baseline and Control Group</td>
<td>92</td>
</tr>
<tr>
<td>4.22</td>
<td>Effect of Different Treatments on Serum ALT of Rats as Compared to Baseline and Control Group</td>
<td>93</td>
</tr>
<tr>
<td>4.23</td>
<td>Effect of Different Treatments on AST/ALT Ratio of Rats as Compared to Baseline and Control Group</td>
<td>93</td>
</tr>
<tr>
<td>4.24</td>
<td>Effect of Different Treatments on Serum Total Bilirubin of Rats as Compared to Baseline and Control Group</td>
<td>94</td>
</tr>
<tr>
<td>4.25</td>
<td>Effect of Different Treatments on Serum CBG of Rats as Compared to Baseline and Control Group</td>
<td>95</td>
</tr>
<tr>
<td>4.26</td>
<td>Effect of Different Treatments on Serum HCY of Rats as Compared to Baseline and Control Group</td>
<td>96</td>
</tr>
<tr>
<td>4.27</td>
<td>Effect of Different Treatments on Serum TNF-α of Rats as Compared to Baseline and Control Group</td>
<td>97</td>
</tr>
<tr>
<td>4.28</td>
<td>Effect of Different Treatments on Serum IL-6 of Rats as Compared to Baseline and Control Group</td>
<td>98</td>
</tr>
<tr>
<td>4.29</td>
<td>Effect of Different Treatments on Serum α2MG of Rats as Compared to Baseline and Control Group</td>
<td>99</td>
</tr>
<tr>
<td>4.30</td>
<td>Effect of Different Treatments on Serum AFP of Rats as Compared to Baseline and Control Group</td>
<td>100</td>
</tr>
<tr>
<td>4.31</td>
<td>Effect of Different Treatments on Serum CRP of Rats as</td>
<td>101</td>
</tr>
</tbody>
</table>
Compared to Baseline and Control Group

4.32 Effect of Different Treatment on Level of TAS in Treated Rats as Compared to Control and Baseline Group 102

4.33 Effect of Different Treatments on Serum GGT of Rats as Compared to Baseline and Control Group 103

4.34 Effect of Different Treatments on Serum LDH of Rats as Compared to Baseline and Control Group 103

4.35 Normal Liver Appearance of Baseline Group 104

4.36 Common Appearance of Most Treated Rats 105

4.37 Multi Nodular Fatty Hepatic Adenoma in Synergistic Treated Group 105

4.38 Hepatic Adenoma in Control Group of Rats 106

4.39 Light Micrograph of Normal Liver Cell at the Lobular Region of Baseline Group. 107

4.40 Light Micrograph of Normal Liver Cell at the Lobular Region of Baseline Group. 107

4.41 Light Micrograph of Hepatitis at the Lobular Region of Control Group. 109

4.42 Light Micrograph of Hepatitis at the Lobular Region of *T. polium* Treated Group. 109

4.43 Light Micrograph of Hepatitis at the Lobular Region of *B. vulgaris* Treated Group. 110

4.44 Light Micrograph of Hepatitis at the Lobular Region of Synergistic Treated Group. 110

4.45 Light Micrograph of Hepatitis at the Lobular Region of *O. stamineus* Treated Group. 111

4.46 Light Micrograph of Steatohepatitis at the Centrilobular Region of Control Group. 111

4.47 Light Micrograph of Steatosis at the Centrilobular Region of *T. polium* Treated Group. 112

4.48 Light Micrograph of Steatosis at the Lobular Region of *O. stamineus* Treated Group. 112

4.49 Light Micrograph of Hepatitis with Pre-cirrhotic Stage of Primary Biliary Cirrhosis in Control Group. 113

4.50 Light Micrograph of Hepatocellular Carcinoma in Control Group. 113

4.51 Light Micrograph of Hepatocellular Carcinoma in Synergistic Group. 114
4.52 Light Micrograph of Hepatocellular Carcinoma in *O. stamineus* Treated Group.

4.53 Light Micrograph of Hepatocellular Carcinoma in *T. polium* Treated Group.

4.54 Light Micrograph of Severe Cell Degeneration in Control Group.

4.55 Light Micrograph of Hepatocellular Carcinoma in Synergistic Treated Group.

4.56 Effect of Different Treatments on Mean Lesion Score of Rats Liver Tissue

4.57 Fluorescent *in situ* Hybridization Micrograph of Lobular Region of Protein Expression of Glucocorticoid Receptors in Cytoplasm All Groups.

4.58 Fluorescent *in situ* Hybridization Micrograph of Lobular Region of Baseline Group.

4.59 Fluorescent *in situ* Hybridization Micrograph of Lobular Region of Control Group.

4.60 Fluorescent *in situ* Hybridization Micrograph of Lobular Region of Synergistic Group.

4.61 Fluorescent *in situ* Hybridization Micrograph of Lobular Region of *O. stamineus* Group.

4.62 Fluorescent *in situ* Hybridization Micrograph of Lobular Region of *T. polium* Group.

4.63 Fluorescent *in situ* Hybridization Micrograph of Lobular Region of *B. vulgaris* Group.

4.64 Fluorescent *in situ* Hybridization Micrograph of Lobular Region of *B. vulgaris* Group.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-AAF</td>
<td>2-Acetylaminofluore</td>
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<tr>
<td>AAR</td>
<td>AST/ALT Ratio</td>
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<tr>
<td>ACS</td>
<td>American Cancer Society</td>
</tr>
<tr>
<td>ACTB</td>
<td>Actin Beta</td>
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<tr>
<td>AFP</td>
<td>Alpha-Fetoprotein</td>
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<tr>
<td>AI</td>
<td>Adequate Intake</td>
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<tr>
<td>AIN</td>
<td>American Institute of Nutrition</td>
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<tr>
<td>AKT (PKB)</td>
<td>Protein Kinase B</td>
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<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
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<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
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<tr>
<td>ASR</td>
<td>Age-Standardised Rate</td>
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<tr>
<td>AST</td>
<td>Aspartate Aminotransferase</td>
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<tr>
<td>ATBC</td>
<td>α-Tocopherol B-Carotene (ATBC) Cancer Prevention Study</td>
</tr>
<tr>
<td>BCL-2</td>
<td>B-Cell Lymphoma 2</td>
</tr>
<tr>
<td>BCLC</td>
<td>Barcelona Clinic Liver Cancer</td>
</tr>
<tr>
<td>BHA</td>
<td>Butylated Hydroxyanisole</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BRAF</td>
<td>Birth Rapidly Accelerated Fibrosarcoma, is a human gene that makes a protein called B-Raf</td>
</tr>
<tr>
<td>BV</td>
<td><em>B. vulgaris</em></td>
</tr>
<tr>
<td>C</td>
<td>Cilicius</td>
</tr>
<tr>
<td>CAM</td>
<td>Complementary or Alternative Medicine</td>
</tr>
<tr>
<td>CARET</td>
<td>β-Carotene and Retinol Efficacy Trial</td>
</tr>
<tr>
<td>CCGPS</td>
<td>Copenhagen General Population Study</td>
</tr>
<tr>
<td>CCHS</td>
<td>Copenhagen City Heart Study</td>
</tr>
<tr>
<td>CGB</td>
<td>Corticosteroid Binding Globulin</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Intervals</td>
</tr>
<tr>
<td>CLIP</td>
<td>Cancer of The Liver Italian Program</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>CPD</td>
<td>Cyclobutane Pyrimidine Dimers</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>DAPI</td>
<td>4',6-Diamidino-2-Phenylindole</td>
</tr>
<tr>
<td>DENA</td>
<td>Diethylnitrosamine</td>
</tr>
<tr>
<td>DL</td>
<td>Decilitre</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-Diphenyl-1-Picrylhydrazyl</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
</tbody>
</table>
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-CoA  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
IC50  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  Methylene tetrahydrofolate 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-κdB  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 Temperature
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
α₂MG  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 2:1. The regions of high incidence are Eastern and Southeastern Asia, Middle and Western Africa, also Melanesia and Micronesia/Polyynesia (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 in incidence and mortality across different geographic regions in different types of cancer, especially 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; Maheshwari, 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 for chronic disorders (glucose and lipid profile). In order to investigate the public myth on eating good foods, specifically herbs together might boost the strength of the 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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