



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF BERBERIS VULGARIS (L.) FRUIT EXTRACT ON
ANTIOXIDANT ENZYME ACTIVITIES, α -FETOPROTEIN CONTENT AND
HISTOLOGY OF HEPATOCARCINOGENIC RATS**

GHOLAMREZA MOTALLEB

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By

GHOLAMREZA MOTALLEB

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Master of Science**

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ANTIOXIDANT ENZYME ACTIVITIES, α -FETOPROTEIN CONTENT AND
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GHOLAMREZA MOTALLEB

May 2006

Chairman: Associate Professor Fauziah Othman, PhD

Faculty: Medicine and Health Sciences

The chemopreventive agent of *Berberis vulgaris* fruit extract in hepatocarcinogenesis female Sprague Dawley rats was studied to investigate the possible cancer preventive effect of the plant. Total antioxidant activity and phenolic content of BFE extracts were measured.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay was used to determine antioxidant properties of barberry fruit by measuring the decrease in absorbance at 517 nm. In distilled water, BFE showed $82.52 \pm 0.64\%$ free radical scavenging activity with an $EC_{50} = 0.65$. In ethanol, BFE extract showed $73.62 \pm 1.88\%$ free radical scavenging activity with an $EC = 0.658$, BHT $67.50 \pm 0.53\%$ ($EC = 0.612$) and Vitamin C $88.56 \pm 0.43\%$ ($EC = 0.252$), respectively. Meanwhile BFE in 80% methanol had the highest phenolic content ($28000 \pm 500 \text{ mg/100g}$) followed by extract in water ($10000 \pm 400 \text{ mg/100g}$). The



Histological evaluation illustrated that there were significant changes in the lesion score of 50 and 100mg/kg/bodyweight BFE in the DAB₅₀ and DAB₁₀₀ in portal and lobular region compared to DEN/AAF control group. However, the liver of DAB₂₅ showed significant changes in the lesion score only in the portal region not in the lobular region.

The liver enzymes activity measured were xenobiotic metabolizing enzymes: gamma glutamyl transpeptidase (GGT), and glutathione S-transferase (GST). Alpha feto protein(AFP) level was measured as a liver tumour marker. The results indicated that there were significant differences ($p < 0.05$) in the activities of GGT and GST between DEN/AAF rats and normal rats. In liver cancer rats treated with *Berberis vulgaris*, the activities of GST and GGT were significantly lower ($p < 0.05$) compared with the DEN/AAF group. The findings showed that BFE could reduce the activity of liver enzymes of rats during hepatocarcinogenesis.

The results showed that the DEN/AAF group had the least increase of body weight compared to other groups. The normal control and normal control treated with BFE had a significantly lower the liver weight to body weight ($p < 0.05$) ratio compared with the DEN/AAF group. The DEN/AAF groups treated with BFE had a lower liver weight to body weight ($p < 0.05$) ratio compared with the DEN/AAF group (except DEN/AAF treated with BFE₂₅).

The DEN/AAF group showed the highest level of AFP (2.014 ± 1.013 IU/ml). Level of AFP in serum of rats in control, BFE groups (NB₂₅, NB₅₀, NB₁₀₀) and in DEN rats treated

with BFE at different doses (DAB₂₅, DAB₅₀ and DAB₁₀₀) were significantly lower ($p < 0.05$) compared with the DEN/AAF group. DEN/AAF control treated with BFE showed significantly decrease in serum AFP concentration (as a tumor marker) compared with DEN/AAF control group ($p < 0.05$). Meanwhile, the RT-PCR analysis of hepatocytes illustrated the AFP gene expression in DEN/AAF group only.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Master of Sains

KESAN EKSTRAK BUAH *BERBERIS VULGARIS* (L.) KE ATAS AKTIVITI ENZIM ANTIOKSIDA, KANDUNGAN α -FETOPROTEIN AND HISTOLOGI DIDALAM TIKUS HEPATOKARSINOGENIK

Oleh

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Mei 2006

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Kesan anti-kanser *Berberis vulgaris* semasa pengaruhan hepatokarsinogenesis keatas tikus betina Sprague Dawley telah dikaji kemungkinan kesannya. Kesan aktiviti antioksidan dan kandungan phenolic ekstrak BFE telah ditentu nilai.

Ekstrak ini telah disediakan dengan air suling, ethanol dan methanol (80%). Essei 1, 1-dipenyl-2-picrylhydrazyl (DPPH) telah digunakan untuk menentukan kandungan antioksidan buah berberry dengan mengukur pengurangan serapan pada 517nm. Dengan air suling, ekstrak BFE menunjukkan $82.52 \pm 0.64\%$ aktiviti radikal bebas. Dengan ethanol, ekstrak BFE menunjukkan $73.62 \pm 1.88\%$ aktiviti radikal bebas, BHT $67.50 \pm 0.53\%$ dan vitamin C $88.56 \pm 0.43\%$.

Ekstrak buah *Berberis vulagris* menunjukkan aktiviti radikal bebas yang tinggi didalam air suling dengan 82.52% dan $EC_{50} = 0.64\text{mg/ml}$. Sementara itu BFE didalam 80% methanol mempunyai kandungan phenolic yang paling tinggi diikuti ekstrak dengan air. Analisis dengan ANOVA menunjukkan terdapat perbezaan ketara ($p \leq 0.05$) purata aktiviti radikal bebas buah barberry didalam air dan methanol.

Empat puluh lapan ekor Sprague Dawley betina (120-230g) berusia 6-8 minggu dibahagikan kepada 8 kumpulan: NC(kumpulan normal), NB₂₅ (kumpulan tikus dirawat dengan 25mg/kg/berat badan BFE), NB₅₀(kumpulan tikus dirawat dengan 50 mg/kg/berat badan BFE), NB₁₀₀ (kumpulan tikus dirawat dengan 100mg/kg/berat badan BFE), DAC(kumpulan tikus teraruh karsinogenesis dengan DEN/AAF), DAB₂₅ (DEN/AAF kumpulan tikus teraruh kanser hepar dirawat dengan 25 mg/kg/berat badan BFE), DAB₅₀ (DEN/AAF kumpulan tikus teraruh kanser hepar dirawat dengan 50mg/kg/berat badan BFE) dan DAB₁₀₀ (kumpulan tikus teraruh kanser hepar dirawat dengan 100mg/kg/berat badan BFE) telah digunakan untuk menilai kandungan anti-kanser tumbuhan tersebut.

Keterukan neoplasia tersebut telah dikaji dengan penilaian histologi, profail berat badan dan hepar dan penanda kanser hepar. Penilaian histologi menunjukkan organisasi sel normal telah hilang apabila karsinogen dimasukkan kedalam badan. Pemerhatian skor lesion mikroskopi menunjukkan perubahan yang signifikan ($p \leq 0.05$) antara DEN/AAF dan kumpulan normal terkawal.

Penilaian histologi menggambarkan bahawa terdapat perubahan ketara dalam skor lesion ke atas tikus di rawat dengan 50 dan 100mg/kg/berat badan BFE di dalam DAB₅₀ dan DAB₁₀₀ keatas bahagian portal dan lobul berbanding dengan kumpulan tikus kanser hepar. Walau bagaimanapun, hepar tikus kumpulan DAB₂₅ menunjukkan perubahan ketara di dalam skor lesion pada bahagian portal tetapi bukan pada bahagian lobul.

Aktiviti enzim metubolisme xenobiotik gamma glutamyl transpeptidase(GGT), dan Glutathione S-transferase(GST), aras alfa fetoprotein(AFP) telah diukur sebagai penanda hepatokarsinogenesis. Keputusan menunjukkan terdapat perubahan ketara ($p \leq 0.05$) di dalam aktiviti GGT, GST berbanding kumpulan terkawal dan kumpulan DEN/AAF.

Di dalam kumpulan kanser dirawat *Berberis vulgaris*, aktiviti GST, GGT adalah ketara rendah ($p \leq 0.05$) berbanding kumpulan DEN/AAF. Penemuan ini menunjukkan bahawa BFE boleh merendahkan aktiviti enzim hepar tikus semasa hepatokarsinogenesis.

Keputusan ini menunjukkan peningkatan berat badan yang kurang dalam kumpulan terkawal DEN/AAF berbanding dengan kumpulan yang lain. Kumpulan normal terkawal dan kumpulan BFE menunjukkan penurunan nisbah berat hepar keatas berat badan yang rendah berbanding dengan kumpulan DEN/AAF. Kumpulan DEN/AAF yang dirawat dengan BFE mempunyai nisbah yang rendah ($p \leq 0.05$) berbanding dengan kumpulan DEN/AAF (kecuali DEN/AAF dirawat dengan BFE₂₅).

Kumpulan DEN/AAF menunjukkan aras AFP yang tinggi (2.014 ± 1.01 IU/ml). Manakala arus AFP serum pada kumpulan NC, NB₂₅, NB₅₀, NB₁₀₀, DAB₂₅, DAB₅₀ dan DAB₁₀₀ adalah ketara rendah ($p \leq 0.05$) berbanding dengan kumpulan DEN/AAF. Kumpulan kawalan DEN/AAF yang dirawat dengan BFE menunjukkan penurunan ketara dalam kepekatan serum AFP (sebagai penanda kanser) berbanding dengan DEN/AAF kumpulan terkawal ($p \leq 0.05$). Sementara itu, Analisis RT-PCR ke atas sel hepatosit menggambarkan ekpresi gen AFP hanya hadir dalam kumpulan DEN/AAF sahaja.



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GHOLAMREZA MOTALLEB

July 2006



I certify that an Examination Committee has met on 29 May 2006 to conduct the final examination of Gholamreza Motaleb on his Master of Science thesis entitled "Effects of *Berberis vulgaris* (L.) Fruit Extract on Antioxidant Enzyme Activities, α -Fetoprotein Content and Histology of Hepatocarcinogenic Rats" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



GHOLAMREZA MOTALLEB

Date: 10 of Jun 2006

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LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS

AAF	2-Acetylaminofluorene
AHF	Altered hepatic foci
AFP	α -feto protein
AMV	Avian myeloblastosis virus
ARE	Antioxidant response elements
AHH	Aryl hydrocarbon hydroxylase
BBI	bis-benzylisoquinoline
BHT	Botylated hydroxy toluene
BHA	Botylated hydroxyanisole
CDNB	1-chloro-2, 4-dinitrobenzene
cDNA	complementary Deoxyribonucleic acid
DNA	Deoxyribonucleic acid
DEN	Diethylnitrosamine
DEPC	Diethylpyrocarbonate
dNTP	deoxy nucleotide triphosphate
DPPH	1,1 diphenyl-2-picrylhydrazyl
ELISA	Enzyme-linked immunosorbent assay
EDTA	Ethylenediamine tetra-acetic acid
GGT	Gamma-glutamyl transpeptidase
GSH	Glutathione
GST	Glutathione S-transferase



GST-P	Glutathione S-transferase placental
GCT	Germ cell tumours
Hb	Hepatoblastoma
HC	Hepatocarcinogenesis
HCC	Hepatocellular carcinoma
H&E	Hematoxylin and eosin
IARC	International agency for research on cancer
iNOS	inducible NO synthase
MoAbs	Monoclonal antibodies
MHC-II	Major histocompatibility complex class II
NOC	N-Nitroso compounds
NOCP	NOC Precursors
N-ras	Proteins with GTPase activity
P53	53-kilodalton tumour suppressor protein p53
PAHs	Polycyclic aromatic hydrocarbons
PUFA	Polyunsaturated fatty acid
PH	Partial hepatectomy
ras T24	The oncoprotein Ras, a 21 kDa guanine nucleotide-binding protein is encoded by a member (Harvey-, Kirsten-, and Neural-ras) of the ras proto-oncogene family
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RT	Reverse transcriptase
mRNA	messenger ribonucleic acid

PCR	Polymerase chain reaction
RLUs	Relative light units
ROS	Reactive oxygen species
SA	Serum albumin
SV40	Simian virus 40
Tfl	Thermos flavus
XRE	Xenobiotic response element
YSVE	Yolk sac visceral endoderm
Alb	Albumin
C/EBP	CCAAT/enhancer-binding protein
HNF	hepatocyte nuclear factor