Ain Shams University
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Evaluation of Anticancer Activity of Fenugreek Seed Aqueous Extract and Diosgenin in Chemically Induced Liver Carcinoma in Male Albino Rats

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By
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DEDICATION

I would like to dedicate this thesis and all my efforts done the last years to achieve my degree to my precious family, especially my mother, husband and sisters for nursing me with love and support me and teaching me to believe in myself to achieve my dreams.

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ABSTRACT

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Key Words: Albino rats; Antihepatocarcinogenesis activity; Cyclophosphamide; Diethylnitrosamine; Diosgenin; Immunological cytokines; Oxidative stress and apoptosis; *Trigonella foenum-graecum*.

Hepatocellular carcinoma (HCC) is an aggressive cancer. Chronic hepatic inflammation and oxidative stress increases the risk for HCC. The present study evaluated and compared the anticarcinogenesis activity of fenugreek seed (*Trigonella foenum-graecum* L.) aqueous extract (FSAE, 6 g/kg body weight, orally and daily for 14 weeks) and diosgenin (one of fenugreek seeds active components, 36 mg/kg body weight, orally and daily for 14 weeks) in presence/absence of cyclophosphamide (CP, 20 mg/kg body weight, intraperitoneal injection, twice/week for 6 consecutive weeks starting from the 9th week) in experimentally-induced hepatocellular carcinoma (HCC) in male Wistar albino rats by diethylnitrosamine (100 mg/kg body weight, intraperitoneal injection, once every 2 weeks for 14 consecutive weeks). The results showed that FSAE and diosgenin in presence/absence of CP significantly (P<0.05-0.001) reduced the relative liver weight, the concentration of serum α-fetoprotein, and proinflammatory cytokines (interleukin-1β and tumour necrosis factor-α), hepatic glutathione-S-transferase activity, the count of bone marrow micronucleated polychromatic erythrocytes and DNA fragmentation, and improved liver histopathology (in presence of CP only) and functions, as well as downregulated the expression of hepatic caspase-3, caspase-9 and p53 in the HCC

rat model. In addition, FSAE and diosgenin in presence/absence of CP significantly (P<0.05-0.001) increased the body weight, thymus and bone marrow cells density, the concentration of serum albumin and globulins (in presence of CP only), total proteins and the anti-inflammatory cytokine (interleukin-10), hepatic reduced glutathione concentration, and the activity of hepatic antioxidant enzymes (catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase) in the HCC rat model. Generally, the antihepatocarcinogenesis activity of CP plus either FSAE or diosgenin was more than that of CP alone. In conclusion, both FSAE and diosgenin reduced hepatocarcinogenesis and augmented the therapeutic activity of CP in the HCC rat model. Possible mechanisms for the anticarcinogenesis activities of FSAE and DIOS shown in the present study in the HCC rat model are their ability to modulate hepatic inflammation, oxidative stress, genotoxicity and apoptosis.

LIST OF ABBREVIATIONS

γ-GT	Gamma-glutamyl transferase
4-OH-CP	4-Hydroxycyclophosphamide
4-OHIle	4-Hydroxyisoleucine
AAP	4-Aminophenazone
ALAT	Alanine aminotransferase
ALP	Alkaline phosphatise
AOM	Azoxymethane
ASAT	Aspartate aminotransferase
Bp	Base pair
b.w	Body weight
CAT	Catalase
cDNA	Complementary deoxyribonucleic acid
CDNB	1-Chloro-2,4-dinitrobenzene
CP	Cyclophosphamide
CYP450	Cytochrome P_{450}
DAD	Diode array detector
DEN	Diethylnitrosamine
DHBS	3,5-Dichloro-2-hydroxybenzene sulfonic acid
DIOS	Diosgenin
dl	Decilitre
DMH	1,2-Dimethyl-hydrazine
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates

DPA	Diphenylamine
DTNB	5,5`Dithiobis (2-nitrobenzoic acid)
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
g	Gram
FSAE	Fenugreek seed aqueous extract
GADPH	Glyceraldehyde-3-phosphate dehydrogenase
GPx	Glutathione peroxidise
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione-S-transferase
HCC	Hepatocellular carcinoma
HPLC	High-performance liquid chromatography
HRP	Horseradish peroxidase
i.p	Intraperitoneal
IL	Interleukin
IU	International unit
KH_2PO_4	Potassium dihydrogen phosphate
LPO	Lipid peroxidation
mg	Milligram
$MgCl_2$	Mercuric chloride
MGMT	O6-methylguanine-DNA methyl-transferase
min	Minute
ml	Millilitre

MN	Micronucleus test
MnPCEs	Micronucleated polychromatic erythrocytes
Na_2HPO_4	Disodium hydrogen phosphate
NADPH	Reduced form of nicotinamide adenine di-nucleotide phosphate
NBT	Nitroblue tetrazolium
NF	Nuclear factor
ng	Nanogram
NK	Natural killer
pg	Picogram
PMS	Phenazine methosulphate
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Reverse transcriptase-polymerase chain reaction
sec	Second
SEM	Standard error of mean
SOD	Superoxide dismutase
TBE	Tris-borate-EDTA
TE	Tris HCl-EDTA
Th	T-helper
TMB	Tetramethylbenzidine
TNF	Tumour necrosis factor
U	Unit

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