



Faculty of Science  
Zoology Department

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

**A Thesis**

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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.*

*My special dedicate to my son and my daughter for my Preoccupation most of time they were needed me beside them.*

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## ABSTRACT

**Abd El-Samie, Hanaa Fathy**

### **Evaluation of Anticancer Activity of Fenugreek Seed Aqueous Extract and Diosgenin in Chemically Induced Liver Carcinoma in Male Albino Rats**

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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 9<sup>th</sup> 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  $\alpha$ -fetoprotein, and proinflammatory cytokines (interleukin-1 $\beta$  and tumour necrosis factor- $\alpha$ ), 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

$\gamma$ -GT	Gamma-glutamyl transferase
4-OH-CP	4-Hydroxycyclophosphamide
4-OHIle	4-Hydroxyisoleucine
AAP	4-Aminophenazone
ALAT	Alanine aminotransferase
ALP	Alkaline phosphatase
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 <sub>450</sub>
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 peroxidase
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 <sub>2</sub> PO <sub>4</sub>	Potassium dihydrogen phosphate
LPO	Lipid peroxidation
mg	Milligram
MgCl <sub>2</sub>	Mercuric chloride
MGMT	O6-methylguanine-DNA methyl-transferase
min	Minute
ml	Millilitre



MN	Micronucleus test
MnPCEs	Micronucleated polychromatic erythrocytes
Na <sub>2</sub> HPO <sub>4</sub>	Disodium hydrogen phosphate
NADPH	Reduced form of nicotinamide adenine dinucleotide 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

## LIST OF TABLES

**Table's Number & Title** **Page**

### **Materials and Methods**

<b>1</b>	Different trials to induce experimental HCC in male albino rats in the present study	28
<b>2</b>	Different groups and doses treatments	31

### **Results**

<b>3</b>	Effects of FSAE and DIOS with/without CP on the body weight change (g) in HCC rat model	54
<b>4</b>	Effects of FSAE and DIOS with/without CP on relative liver weight (g/100g b.w) in HCC rat model	55
<b>5</b>	Effects of FSAE and DIOS with/without CP on bone marrow cell density ( $10^6$ cells) in HCC rat model	59
<b>6</b>	Effects of FSAE and DIOS with/without CP on thymus cell density ( $10^6$ cells/g thymus) in HCC rat model	60
<b>7</b>	Effects of FSAE and DIOS with/without CP on clotting time (sec) in HCC rat model	63
<b>8</b>	Effects of FSAE and DIOS with/without CP on prothrombin time (sec) in HCC rat model	64
<b>9</b>	Effects of FSAE and DIOS with/without CP on serum total protein, albumin and globulins concentrations (g/dl) and albumin/globulins ratio in HCC rat model	68
<b>10</b>	Effects of FSAE and DIOS with/without CP on serum $\alpha$ -fetoprotein concentration (ng/ml) in HCC rat model	72
<b>11</b>	Effects of FSAE and DIOS with/without CP on	76

	serum bilirubin (total, direct and indirect) concentration (mg/dl) in HCC rat model	
<b>12</b>	Effects of FSAE and DIOS with/without CP on the activity (IU/L) of serum enzymatic markers of tissue injury (ALAT, ASAT, ALP and $\gamma$ -GT) in HCC rat model	80
<b>13</b>	Effects of FSAE and DIOS with/without CP on hepatic non-enzymic and enzymic glutathione system in HCC rat model	84
<b>14</b>	Effects of FSAE and DIOS with/without CP on hepatic catalase enzyme (CAT) activity (U/g protein) in HCC rat model	88
<b>15</b>	Effects of FSAE and DIOS with/without CP on hepatic superoxide dismutase enzyme (SOD) activity (U/g protein) in HCC rat model	89
<b>16</b>	Effects of FSAE and DIOS with/without CP on serum anti-inflammatory and pro-inflammatory cytokines concentrations (pg/ml) in HCC rat model	93
<b>17</b>	Effects of FSAE and DIOS with/without CP on the count of bone marrow MnPCEs in HCC rat model	105
<b>18</b>	Effects of FSAE and DIOS with/without CP on hepatic DNA fragmentation (%) in HCC rat model	109
<b>19</b>	Effects of FSAE and DIOS with/without CP on the expression of hepatic caspase-3, caspase-9 and p53 in HCC rat model	113

## LIST OF FIGURES

### Figure's Number & Title

### Page

#### Literature Review

<b>1</b>	Chemical structure of diosgenin	8
<b>2</b>	Basic structures of sapogenins: <b>(a)</b> a triterpenoid and <b>(b)</b> a steroid	9
<b>3</b>	Extrinsic and intrinsic caspase activation cascades	15
<b>4</b>	Chemical structure of DEN	18
<b>5</b>	Biotransformation of DEN and mechanism of DNA adduct formation	19
<b>6</b>	Chemical structure of cyclophosphamide	21

#### Materials and Methods

<b>7</b>	Chromatograms of diosgenin standard <b>(a)</b> and fenugreek seeds alcoholic extract <b>(b)</b>	27
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#### Results

<b>8</b>	Effects of FSAE and DIOS with/without CP on the body weight change (g) and relative liver weight (g/100g b.w) in HCC rat model	56
<b>9</b>	Effects of FSAE and DIOS with/without CP on cell density of central lymphoid organs (bone marrow and thymus) in HCC rat model	61
<b>10</b>	Effects of FSAE and DIOS with/without CP on clotting time (sec) and prothrombin time (sec) in HCC rat model	65
<b>11</b>	Effects of FSAE and DIOS with/without CP on serum total protein, albumin and globulins concentrations (g/dl) and albumin/globulins ratio in HCC rat model	69

<b>12</b>	Effects of FSAE and DIOS with/without CP on serum $\alpha$ -fetoprotein concentration (ng/ml) in HCC rat model	73
<b>13</b>	Effects of FSAE and DIOS with/without CP on serum bilirubin (total, direct and indirect) concentration (mg/dl) in HCC rat model	77
<b>14</b>	Effects of FSAE and DIOS with/without CP on the activity (IU/L) of serum enzymatic markers of tissue injury (ALAT, ASAT, ALP and $\gamma$ -GT) in HCC rat model	81
<b>15</b>	Effects of FSAE and DIOS with/without CP on hepatic non-enzymic (GSH) and enzymic (GPx, GR and GST) glutathione system in HCC rat model	85
<b>16</b>	Effects of FSAE and DIOS with/without CP on hepatic catalase (CAT) and superoxide dismutase (SOD) activities (U/g protein) in HCC rat model	90
<b>17</b>	Effects of FSAE and DIOS with/without CP on serum anti-inflammatory (IL-10) and pro-inflammatory (IL-1 $\beta$ and TNF- $\alpha$ ) cytokines concentrations (pg/ml) in HCC rat model	94
<b>18</b>	A photograph of liver histopathology of the control group and the CP-treated group	97
<b>19</b>	A photograph of liver histopathology of the FSAE-treated group and the DIOS-treated group	98
<b>20</b>	A photograph of liver histopathology of the HCC group that received vehicle	99
<b>21</b>	A photograph of liver histopathology of the HCC group that received CP	100
<b>22</b>	A photograph of liver histopathology of the HCC groups that received either FSAE or DIOS	101
<b>23</b>	A photograph of liver histopathology of the HCC	102

	groups that received either FSAE plus CP or DIOS plus CP	
<b>24</b>	Photos of the bone marrow micronucleated polychromatic erythrocytes (MnPCEs)	104
<b>25</b>	Effects of FSAE and DIOS with/without CP on the count of bone marrow MnPCEs in HCC rat model	106
<b>26</b>	Agarose gel for hepatic DNA fragmentation	108
<b>27</b>	Effects of FSAE and DIOS with/without CP on hepatic DNA fragmentation (%) in HCC rat model	110
<b>28</b>	Reverse transcriptase polymerase chain reaction (RT-PCR) products of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), caspase-3, caspase-9, and p53	112
<b>29</b>	Effects of FSAE and DIOS with/without CP on the expression of hepatic caspase-3, caspase-9 and p53 in HCC rat model	114

### **Discussion**

<b>30</b>	Antihepatocarcinogenesis mechanisms of fenugreek seed aqueous extract (FSAE) and diosgenin (DIOS) in DEN-treated rats	129
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# CONTENTS

	Page
<b>Introduction .....</b>	<b>1</b>
<b>Literature Review .....</b>	<b>6</b>
<b>Materials and Methods .....</b>	<b>24</b>
<b>Results .....</b>	<b>52</b>
<b>Discussion .....</b>	<b>116</b>
<b>Summary .....</b>	<b>130</b>
<b>References .....</b>	<b>133</b>
<b>Arabic Summary</b>	