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Evaluation of *Ficus glomerata* extract as potential anticancer agents and prevents the genetic toxicity induced by benzo(a)pyrene in male mice

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Abstract: The present study is designed to assess the *in vitro* effect of *Ficus glomerata* Roxb methanol extract (Branches)against liver, lung,breast and colon human tumour cell lines beside evaluated the protective effects at different five doses against benzo(a) pyrene(BaP) inducedin adult albino male mice. Natural phenolic compounds are known to possess potent antioxidant activity, offering numerous health benefits. Several plants are found to have phenolic compounds in which *Ficus glomerata*is one of these promising plants. The results indicated that the methanol extract of *F. glomerata* has strong antiproliferated activity against HEPG2 and MCF7 cell lines equivalent to the anticancer drug doxorubicin. Concerning to *in vivo* studies, no morphological toxic symptoms were observed in the treated group, while the levels of Alanine transaminase (ALT) and Aspartate transaminase (AST)in BaP group were significantly higher than those in treated groups. Furthermore, there is a significant increase ($P \leq 0.01$) in the gene expression of cancer related genes (CYP1A1,CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53) in liver tissues of male mice and increased the rate of DNA damage in mice blood cells in BaP group. Pre-treatment with the methanol extract (*Ficus glomerata*) has a significant liver and blood cells protectionby inhibiting gene expression alteration parameters and rate of DNA damage and decreasedALT and AST levels induced by BaP in male mice at 1000 mg $kg^{-1}$of extract.
Keywords: *Ficus glomerata*, Benzo(a)pyrene, ALT and AST, Cancer relate genes, Genetic toxicity, Mice.

Introduction

Natural products are playing important role in discovering the drugs for their potential to prevent as well as treat cancer due to their ability to target multiple molecular pathways. More than 60% of currently used anti-cancer drugs are originally derived from natural sources such as plants, marine organisms and microorganisms. One of such plant have used in folk medicine is *Ficus glomerata*. Several chemical constituents have been isolated from the *Ficus glomerata* plant. The stem bark showed the presence of two leucoanthocyanins: leucocyanidin-3-O-β-glucopyranoside, leucopelarogonidin-3-O-α-Lrhamnopyranoside, β-sitosterol, unidentified long chain ketone, ceryl behenate, lupeol, its acetate, α- amyrin acetate. From the trunk bark, lupeol, β-sitosterol and stigmasterol were isolated. Fruit of *Ficus racemosa* Linn. contains glauanol, hentriacontane, β-sitosterol, gluanol acetate, glucose, tiglic acid, and esters of taraxasterol, lupeol acetate, friedelin, higher hydrocarbons and other phytosterol. A new tetracyclic triterpene glauanol acetate, which is characterized as 13α, 14β, 17 βH, 20 αH-lanosta-8, 22-diene-3 β-acetate and racemosic acid were isolated from the leaves. A thermo stable aspartic protease was isolated from the latex of the plant. The stem bark and fruit

also showed presence of glauanol acetate [1]. The leaf of this plant also contains sterols, triterpenoids in Petroleum ether extract and alkaloids, tannins and flavonoids in ethanolic extract.

Ficus glomerata (Roxb) which has been used in traditional system of medicine for treating diabetes, liver diseases, piles, asthma, leprosy and diarrhea[2]. The hepatoprotective activity of leaves of Ficus glomerata has been reported [3]. Leaves shows anti-bacterial activity [4], stem bark shows anti-tussive potential[5],anti-diuretic activity [6], anti-pyretic potential [7], anti-inflammatory activity of the leaves, bark and unripe fruit[4,8], hypoglycemic activity of roots, leaves and fruit[9,10,11] and anti-filarial activity of the fruits[12].

Benzo(a)pyrene (BaP) is the most potent carcinogen; it is embryo toxic and teratogenic in animals[13]. The level of BaP may be a good marker for carcinogenic potential contamination in an environmental sample [14]. BaP is metabolized by the liver microsomal mixed function oxidase system to highly reactive compounds that can bind to specific target sites of DNA, which is of critical importance in the initiation of BaP-induced carcinogenesis [15].

The oxidative damage of biological molecules is an important event in the development of a variety of human disorders that result from overwhelming the biological defence system against oxidative stress, drugs and carcinogens. The intake in the human diet of antioxidant compounds, or compounds that ameliorate or enhance the biological antioxidant mechanisms, can prevent and in some cases help in the treatment of some oxidative-related disorders and carcinogenic events [16].

Chemoprevention offers a novel approach to control the incidence of several types of cancer. The search for new chemopreventive and antitumor agents that are more effective and less toxic has kindled great interest in phytochemicals [17]. A recent international report concluded that high dietary intake of alkaloids and flavonoids reduce further risk of developing cancer. The main mechanism responsible for this reduced risk is the strong antioxidant effect of these substances [18]. Several kinds of flavonoid and dietary nutrient found in onion, grapes, green vegetables have been shown to possess potent antioxidant and antiproliferative effects against various malignant cellsand also has a potent cytochrome P450 inhibitor [19]. Flavonoid has a wide spectrum of anticancer properties including inhibition of the growth of cells derived from human cancers such as those of stomach, colon, prostate and breast [20,21,22,23]. Additionally, it suppresses the growth and development of uterine cervical cancer, melanomasand intestinal tumours I n whole mice[24,25,26].

However, there is no scientific claims has been made regarding the protective activity of *Ficus glomerata* on the gene expression changes, DNA damage and biochemical toxicity induced by benzo(a) pyren. In view of this, in the present investigation an attempt will be made to study of antioxidant activity of *Ficus glomerata* extract in male mice.

Material and Methods

Collection of Plants

To carry out this study *Ficus glomerata* Roxb (Branches) (Family Moraceae) was collected from Orman Garden, Egypt. This plant was identified by Therese Labib and the nomenclaturefollows [27].

Extract preparation of *Ficus glomerata*

The extract of *Ficus glomerata* Roxb was carried out on a small quantity of plant in which it was sufficient to yield about 50 g dry weight, was collected for preliminary bio-screening. Routine protection of natural plant constituents from denaturation during the extraction and concentration procedures was assured during the preparation of crude extracts [28]. Whole plants or plant parts were dried in a solar oven at 40°C, ground and extracted with methanol at ambient temperature by percolation. Extracts were filtered and methanol was evaporated to dryness under reduced pressure and totally freed from water by freeze drying, and stored under freezing at −20°C until used.

Cytotoxic effect on human cell line (HePG 2 – MCF 7 – HCT 116&A549)

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [29].

Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II (Baker, SG403INT, Sanford, ME, USA). Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h under 5% CO_2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of the sample to give a final concentration of 100-50-25-12.5-6.25-3.125-0.78 and 1.56 µg/ml. Cells were suspended in RPMI 1640 medium for HepG2 and HCT116&DMEM for MCF 7 and A549, 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine in 96-well flat bottom microplate at 37 ºC under 5% CO_2. After 48 h of incubation, the medium was aspirated, 40µl MTT salt (2.5µg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200µl of 10% Sodium dodecyl sulphate (SDS) in deionized water were added to each well and incubated overnight at 37ºC. A positive control at 100µg/ml was used (a known cytotoxic natural agent which gives 100% lethality under the same conditions) [30].

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was calculated between the samples and the negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO was used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

(1- (Reading of extract / Reading of negative control)) x 100

A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program

Acute toxicity test

This is a qualitative and quantitative single dose toxicity test for the bioactive agent by oraladministration in rats or mice. The qualitative study shall include the toxic symptoms and signs observed, and post-mortum findings for prediction of the probable cause of death. The quantitative study shall include determination of the LD-50 value by the known 72-hour test. The study will comprise an evaluation of:

(a) Any toxic symptoms and signs, as well as growth changes.
(b) Hematological parameters (blood picture).
(c) Physiological tests: serum bilirubin, serum transaminases, alkaline phosphatase, urea nitrogen, serum creatinine.

Animals

Adult albino male mice (n=80) weighing 20-25 g were obtained from the Animal House Colony of the NRC. The animals were kept individually in a wire bottomed cages at room temperature (25 ± 2ºC) under 12 h dark-light cycles. They were maintained on a standard laboratory diet and water *ad libitum.* The animals were allowed to acclimatize their new conditions for one week before commencing experiment, and then they were allocated into eight groups (10 mice/ group).

Experimental design

Selection of the doses of benzo[a]pyrene and *Ficus glomerata* extract and the route of administration were selected based on their citation in the literature. The animals were acclimatized for a period of one week and were classified into the following groups: group (1) untreated control animals for 4 weeks treatment period; group (2) animals treated with dimethyl sulfoxide (DMSO) for 4 weeks; groups (3-7) animals were orally treated with *Ficus glomerata* extract (100, 250, 500, 1000 and 2000 mg kg^{-1} b.w) for 4 weeks; group (8) animal treated with a single dose of 50 mg/kg B.w. of benzo[a]pyrene dissolved in dimethylsulfoxide (DMSO). At the end of the experimental period, 5 animals were killed and fasting blood samples were withdrawn from the retro-orbital venous plexus under diethyl ether anaesthesia. Blood samples were received in EDTA containing tubes for the comet assay. Afterwards, the other 5 animals were killed and the liver samples were removed and stored at -80 ºC for gene expression analysis.

Gene expression analysis

I. RNA isolation

To carry out the expression of liver cancer related genes, total RNA was isolated from liver tissues of male mice by the standard TRIzol® Reagent extraction method (Invitrogen, Germany). RNA was dissolved in diethylpyrocarbonate (DEPC) -treated water by passing solution a few times through a pipette tip. Total RNA was treated with 1 U of RQ1 RNAse-free DNAse (Invitrogen, Germany) to digest DNA residues, re-suspended in DEPC-treated water. Purity of total RNA was assessed by the 260/280 nm ratio (between 1.8 and 2.1). Additionally, the integrity was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. Aliquots were used immediately for reverse transcription (RT) otherwise, it was kept under -80ºC until use.

II. Reverse transcription reaction

To synthesis cDNA copies of mice tissues, the complete Poly(A)$^+$ RNA isolated from male mice was reverse transcribed into cDNA in a total volume of 20 µl using RevertAidTM First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). An amount of total RNA (5µg) was used with a reaction mixture, termed as master mix (MM). The MM was consisted of 50 mM MgCl2, 5x reverse transcription (RT) buffer (50 mMKCl; 10 mMTris-HCl; pH 8.3; 10 mM of each dNTP, 50 µM oligo-dT primer, 20 U ribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNase activity) and 50 U M- MuLV reverse transcriptase. The RT reaction was carried out at 25 °C for 10 min, followed by 1 h at 42 °C, and the reaction was stopped by heating for 5 min at 99 °C. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through quantitative real time-polymerase chain reaction (qRT-PCR).

III. qRT-PCR assay

The Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was carried out usingcDNA copies of male mice.PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd., Germany), 0.5 µL 0.2 µM sense primers, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of CDNA template. The reaction program was allocated to 3 steps. The first step was at 95.0°C for 3 min. Second step consisted of 40 cycles in which each cycle divided into 3 steps: (a) at 95.0°C for 15 Sec; (b) at 55.0°C for 30 Sec; and (c) at 72.0°C for 30 Sec. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 Sec up to 95.0°C. At the end of each qRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers**.** Each experiment included a distilled water control.

The quantitative values of RT-PCR (qRT-PCR) of liver cancer related genes (CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53) were normalized on the bases of ß-actin expression. The primer sequences of liver cancer related genes are listed in Table 1.At the end of each qRT-PCR a melting curve analysis was performed at 95.0 °C to check the quality of the used primers.

Table 1: Sequences of primers for Real-Time PCR assay.

Gene	Primer Forward	Primer reverse
CYP1A1	CGGCCCCGGCTCTCT	CGGAAGGTCTCCAGGATGAA
CYP1A2	AGCTTC TCC TGG CCT CTG C	GGACTTTTCAGGCCTTTGGG
CYP3A4	CAG GAG GAAATT GAT GGTT TT	GTCAAGATACTCCATCTGTAGCAC AGT
CYP2B6	TTAGGGAAGCGGATTTGTCTT G	GGAGGATGGTGG TGA AGAAGAG
CD59	CTG TGG ACA ATC ACA ATG GGA ATC CAA GGA	GGT GTT GACTTA GGG ATG AAG
hTRET	CGG AAG AGT GTC TGG AGC AA	GGA TGA AGC GGA GTC TGG A
P53	TCA GAT CCT AGC GTC GAG CCC	GGG TGT GGA ATC AAC CCA CAG
ß-actin	CTG GCA CCC AGC ACA ATG	GCC GAT CCA CAC GGA GTA CT

Gene expression calculation

First the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formulae:[31]:

$Ef = 10^{-1/slope}$
Efficiency (%) = (Ef – 1) x 100
The relative quantification of the target to the reference was determined by using the
ΔCT method if E for the target (CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53) and the reference primers (β-Actin) are the same [31].
$Ratio_{(reference/\ target\ gene)} = Ef^{CT(reference) - CT(target)}$.

Determination of DNA damage using comet assay

The comet assay was carried out on peripheral blood lympocytes from male mice which were isolated by centrifugation (15min, 280 g) in a density gradient of Gradisol L (Aqua Medica, Lodz, Poland). The concentration of the cells was adjusted to (1-3) x 10^5 cells/ ml by adding RPMI 1640 without glutamine to the single cell suspension. A freshly prepared suspension of cells in 0.75% low melting point agarose (Sigma Chemicals) dissolved in phosphate buffer saline (PBS; sigma chemicals) was cast onto microscope slides precoated with 0.5% normal melting agarose. The cells were then lysed for 1h at 4°C in a buffer consisting of 2.5M NaCl, 100 mMEDTA, 1% Triton X-100, 10mM Tris, pH10. After the lysis, DNA was allowed to unwind for 40 min in electrophoretic solution consisting of 300mM NaOH, 1mM EDTA, pH>13. Electrophoresis was conducted at 4°C for 30 min at electric field strength 0.73 V/cm (30mA). The slides were then neutralized with 0.4M Tris, pH 7.5, stained with 2ug/ml ethidium bromide (Sigma Chemicals) and covered with cover slips. The slides were examined at 200 x magnification fluorescence microscope (Nikon Tokyo, Japan) to a COHU 4910 video camera (Cohu, Inc., San Diego, CA, USA) equipped with a UV filter block consist an excitation filter (359nm) and barrier filter (461nm) and connected to a personal computer –based image analysis system, Lucia-Comet v.4.51. Fifty images were randomly selected from each sample and the comet tial DNA was measured [32]. Endogenous DNA damage measured as the mean comet tail DNA of peripheral blood lymphocytes of five mice groups (10 mice each). The number of cells scored for each animal was 100 [32].

Statistical analysis

The data of biochemical parametersm gene expression and DNA damage were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System [33], followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean±SEM. All statements of significance were based on probability of $P< 0.05$.

Results

Cytotoxic effect on human cell line (HePG 2 – MCF 7 – HCT 116 &A549)

The results indicated that the methanol extract of the branches of *Ficus glomerata* hasstrongly antiproliferated activity against HEPG2 and MCF7 cell line equivalent to the anticancer drug doxorubicin. While the antiproliferated activity of this extract was a moderate effect on A549 and HCT116 cell line (Table 2).

Table 2: IC_{50} of Ficus glomerata extract on 4 cell lines

Cell line	IC_{50}
HEPG2	23.6
Doxorubicin	21.6
HCT116	58.4
Doxorubicin	37.6
A549	59.5
Doxorubicin	28.3
MCF7	28.8
Doxorubicin	26.1

A.2. Acute toxicity study:

The Acute oral toxicity test was performed as per OECDguidelines. All the animals were randomly distributed intoone control group and five treated groups, containing 6animals per group. Groups 1, 2, 3,4 and 5 were orally administered 100, 250, 500, 1000 and 2000 mg/kg body weight methanolic extract (Table 3). The control groupreceived vehicle alone (DMSO). The animals were observedcontinuously for the first 24 hours and 7 days for any signs ofbehavioral changes, toxicity, mortality and body weight.

Table 3: Lethal dose LD50 of *Ficus glomerata* methanolic extract

Dose (mg / kg	Number of mice	Number of dead mice	Z	d	(Z)x(d)
100	6	0	0	100	0
250	6	0	0	150	0
500	6	0	0	250	0
1000	6	2	1	500	500
2000	6	3	2.5	1000	2500

LD50 of *Ficus glomerata*methanol extract = 1500mg/kg

A.2.3 Biochemical Analysis

Table 4 shows the changes of biochemical parameters in the serum of mice treated by *Ficus glomerata*. In the treated mice, there are no significant changes in the serum levels of Creatinine in all treated groups. Regarding ALT values the results explored that all levels in treating groups in the normal range. While the serum level of AST rise in treated groups, the same results were obtained for bilirubin.

Table4. Changes of biochemical parameters in the serum of mice treated by promising extracts

Extract	ALT (U/I)	AST(U/I)	Creat. (mg/dl)	BUN. (mg/dl)
Ficus glomerata	54	486	0.4	45
Control	48	359	0.5	21
Normal values	17-77(U/I)	54-298(U/I)	0.2-0.9 mg/dl	8-33 mg/dl

General Sign and Behavioural Analysis

The toxic effect of *Ficus glomerata* methanolic extract on the appearance and the general behavioral pattern of mice are shown no toxic symptoms were observed in any treated animals, which lived up to 7 days after the administration of methanol extract at different doses (100, 250, 500,1000 and 2000 mg/kg body weight).The behavioral patterns of animals were observed first 6 h and followed by 7 days after the administration and the animals in both vehicle treated and extract-treated groups were normal and did not display significant changes in behavior, skin effects, breathing, impairment in food intake and water consumption and hair loss up to 1000 mg/kg body weight. While most of the animal were dead at dose of 3000mg/kg body weigh.

Expression analysis of CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53 genes

The expression of liver related genes were determined using several concentrations of the *Ficus glomerata* extract (100,250,500, 1000 and 2000 mg/kg b.w.) and benzo[a]pyrene.The expression of liver cancer related genes (CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53) in male mice are summarized in Figures 1-7.

The current results revealed that the expression levels of CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53 genes were highly increased with benzo[a]pyrene treatment compared with control or dimethyl sulfoxide (DMSO) and other groups (Figures 1-7).

In contrary, treatment of male mice with low doses of the *Ficus glomerata*extract (100 and 250 mg/kg b.w.) increased slightly the expression of CYP1A1, CYP1A2 and CYP3A4 genes compared with the control group (Figures 1-3). However, the expression levels of CYP2B6, CD59, hTRET and P53 genes increased

significantly with low dose of the *Ficus glomerata* extract (250 mg/kg b.w.) compared with control and DMSO groups (Figures 4-7 and Table 2), but these level were significantly lower compared with male mice treated with benzo[a]pyrene (Figures 4-7).

On the other hand, treatment of male mice with high dose of the *Ficus glomerata* extract (500-2000 mg/kg b.w.) did not increase significantly the expression of CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53 genes compared with the control group (Figures 1-7), however, these level were significantly lower compared with male mice treated with benzo[a]pyrene (Figures 1-7). Whereas, the expression levels of CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53 genes in the groups treated with high dose of the *Ficus glomerata* extract (500-2000 mg/kg b.w.) were relatively similar to those in control and DMSO groups and lower than those in benzo[a]pyrene treated mice.

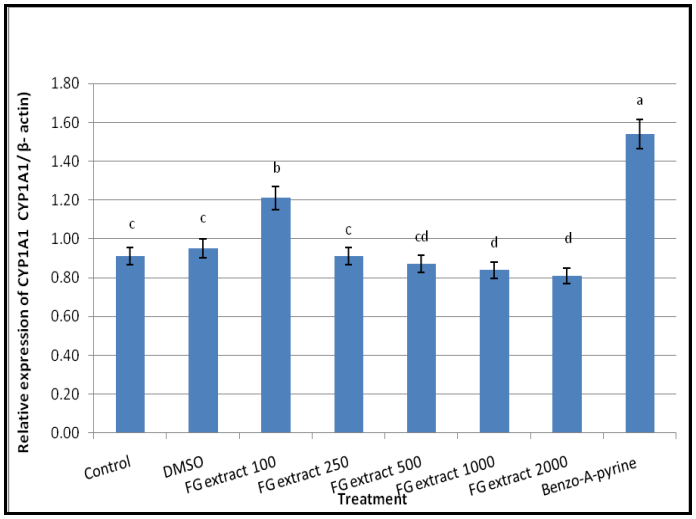

Figure 1: The relative expression of CYP1A1 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficusglomerata*extract. Mean values in the same column with different superscript differ significantly (*P*< 0.05).

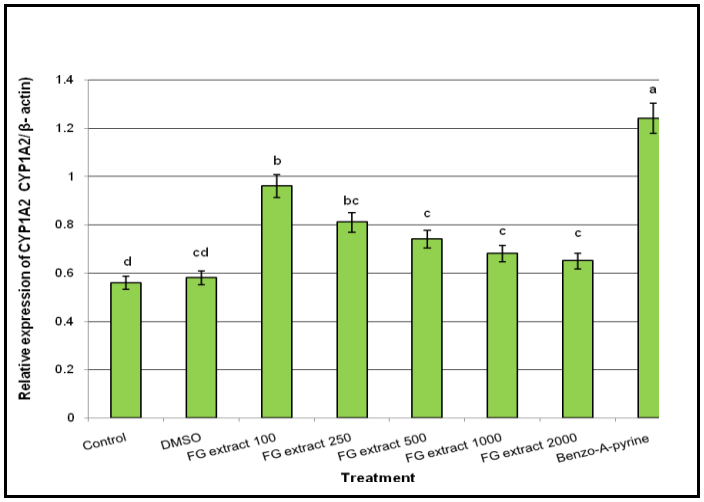

Figure 2: The relative expression of CYP1A2 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficusglomerata*extract. Mean values in the same column with different superscript differ significantly (*P*< 0.05).

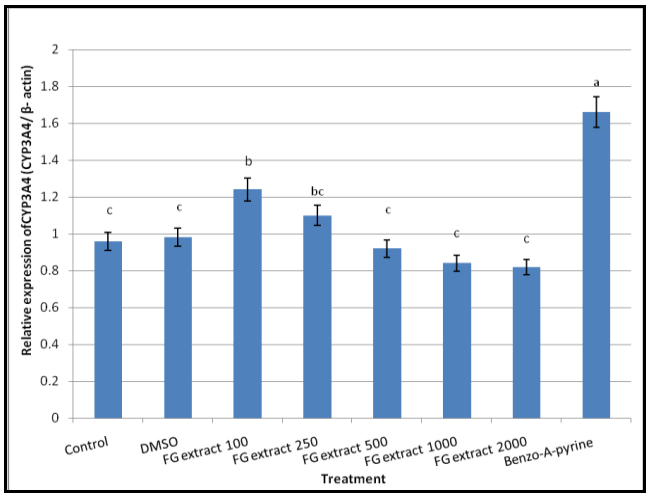

Figure 3: The relative expression of CYP3A4 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficusglomerata*extract. Mean values in the same column with different superscript differ significantly (*P*< 0.05).

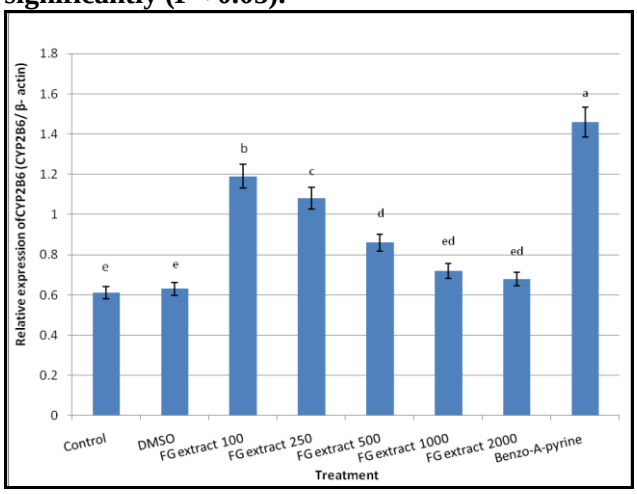

Figure 4: The relative expression of CYP2B6 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficusglomerata*extract. Mean values in the same column with different superscript differ significantly (*P*< 0.05).

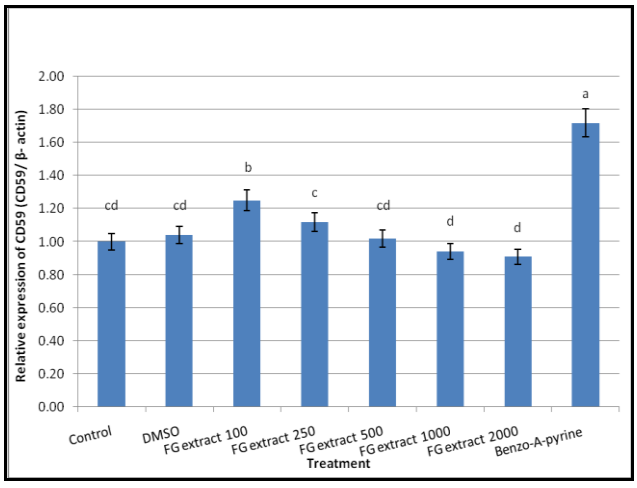

Figure 5: The relative expression of CD59 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficusglomerata*extract. Mean values in the same column with different superscript differ significantly (*P*< 0.05).

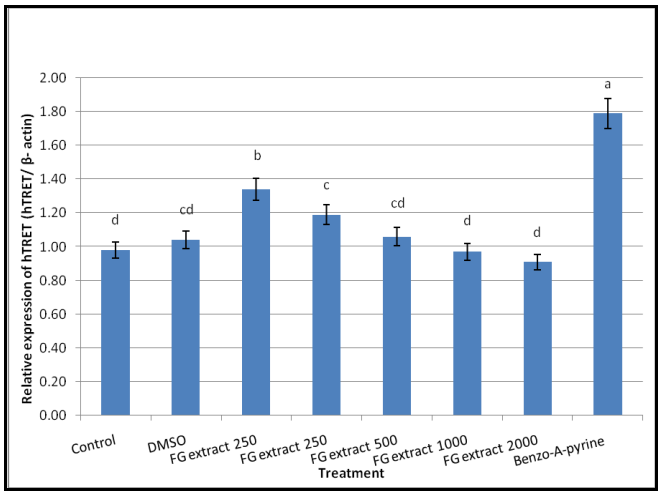

Figure 6: The relative expression of hTRETgene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficusglomerata*extract. Mean values in the same column with different superscript differ significantly (*P*< 0.05).

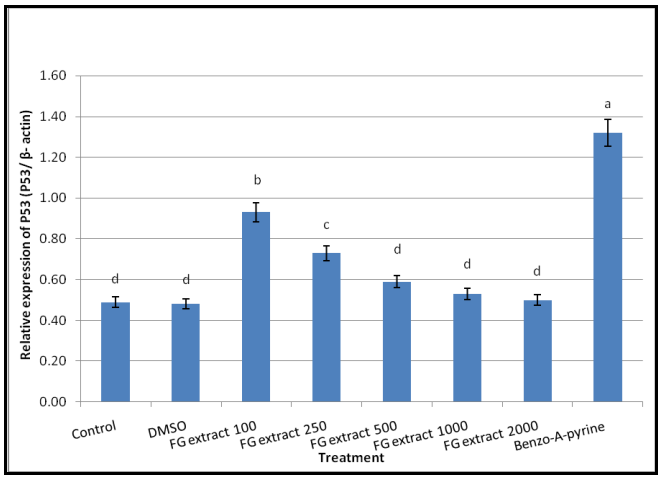

Figure 7: The relative expression of P53gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficusglomerata*extract. Mean values in the same column with different superscript differ significantly (*P*< 0.05).

Determination of DNA Damage

The present results of the comet assay revealed that treatment of male mice with different concentrations of the *Ficus glomerata* extracts induced different rats of DNA damage (Table 5, Figure 8).

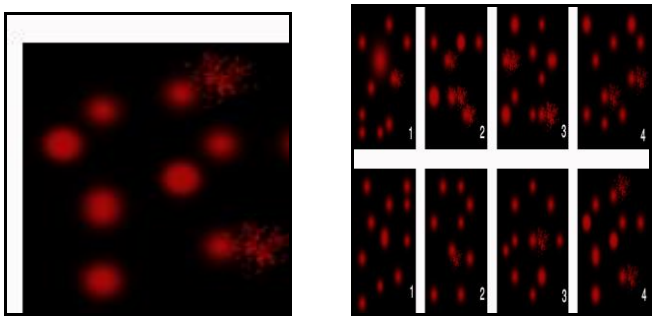

Figure 8:DNA damage in male mice treated with Benzo-A-pyrine and/or *Ficus glomerata*extract. Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.

The percentages of DNA damage in male mice treated DMSO solution induced a low rate of DNA damage which was relatively similar to control male mice (Table 5). However, the rate of DNA damage in male mice treated with low dose of the *Ficus glomerata* extract induced a high rate of DNA damage which was 7.4% compared with 4.8% in control mice (Table 5).

In contrary, treatment of male mice with high dose of the *Ficus glomerata* extract induced lower rate of DNA damage than that found in mice treated with the low doseof the *Ficus glomerata* extract. Whereas, the rate of DNA damage was 5.2% and 4.4% in male mice treated with high doses of the *Ficus glomerata* extract compared with 7.4% in mice treated with low dose of the *Ficus glomerata* extract (Table 5).

On the other hand, treatment of male mice with benzo[a]pyrene induced very higher rate of DNA damage than that found in control mice and other groups. Whereas, the rate of DNA damage was 16.4%in male mice treated with benzo[a]pyrene compared with 4.8% in control mice (Table 5).

Table 5: Visual score of DNA damage in male mice treated with Benzo-A-pyrine and/ or *Ficus glomerata* extract using comet assay.

Treatment	Number of animals	No. of cells		Class[¥] of comet				DNA damaged cells (%)
		Analyzed(*)	Total comets	0	1	2	3	
Control	5	500	24	476	19	5	0	4.8
DMSO	5	500	27	473	20	7	0	5.4
FG extract 100	5	500	37	463	21	9	7	7.4
FG extract 250	5	500	34	466	19	9	6	6.8
FG extract 500	5	500	28	472	14	7	7	5.6
FG extract 1000	5	500	26	474	14	7	5	5.2
Extract 2000	5	500	22	478	12	6	4	4.4
Benzo-A-pyrine	5	500	82	418	26	25	31	16.4

[¥]: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.(*): No of cells analyzed were 100 per an animal.

Discussion

Tumor is one of the most formidable health burdens in the world and it remains the second leading cause of death after heart disorders. Liver cancer is one of the most common malignancies affecting in men in the worldwide today, the lifetime risk being approximately 10% [34,35]. Benzo[a]pyreneis considered as one of the most potent carcinogen and toxic drug in embryosas well as caused teratogenic effects in animals [13]. Therefore, BaP may be considered as a good marker for contamination with carcinogenic potent in the environment[14]. Our current results revealed that work BaP induced over expression of several liver cancer related genes (CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53) and enhanced the rates of DNA damage in male mice which considered as high expecatation for tumour progression.

It has beed known that tumour progression are related to increasing the reactive oxygen species (ROS). ROS are generated by exogenous and endogenous factors such as during hyperglycemia ensued by oxidative stress [36,37]. Oxidative stress induces DNA damage and when DNA damage exceeds the cellular capacity to repair it, the accumulation of errors can overwhelm the cell resulting in cell death or the fixation of genome mutations that can be transmitted to future cell generations. Additionally, mutations can promote genome instability and directly lead to various human diseases such as cancer, neurological abnormalities, immunodeficiency, and premature aging [38]. In addition, several studies reported that free radicals and reactive oxygen species are responsible for initiating and regulate the progression of many diseases like liver diseases[34,35]. In agreement with our results, the current results showed that the liver tissues of male mice

exposed to BaP showed damaging effects due to formation of mutganic compounds. In this regard, protection of these organs using natural products is the main objective of the current study.

Antioxidants are closely related to their bio-functionalities, such as the reduction of cellular abnormalities like DNA damage, mutagenesis, carcinogenesis and which is also associated with free radical propagation in biological systems [39]**.** The current study revealed that different doses of methanol extract of *Ficus glomerata*were capable of scavenging hydroxyl and may have a stronger hydroxyl radical scavenging activity. The present study reveals that *Ficus glomerata*extract decreased significantly the rate of the DNA damage in male mice exposed to BaP. The current results indicate that methanol extracts of *Ficus glomerata*had a prominent effect on hydroxyl radical/and or super oxide scavenging. These results were in agreement with those of Sundari, who reported that methanol fractions decreased the DNA damage in human peripheral blood lymphocytes exposed to UVB-irradiation [40]. In addition, the study revealed that natural products such as cactus cladode extract decreased the DNA fragmentation induced by benzene exposure in male rats [41].Moreove, the current study showed that *F. glomerata* extract reduced the oxidationinduced DNA damage caused by BaP, where the expression alterations in the liver cancer related genes and the rate of DNA damage were markedly inhibeted by *F. glomerata* extracttreatment. In the same line, Irfanfound that hepatcellular carcinoma induced by aracetamol and CCl_4 treated in albino rats was suppressed by treatment with *Ficus glomerata* extract [42].

The present study revealed that treatment with *F. glomerata*reduced activity of ALT and AST in the serum of male mice probably due to increase the antioxidants and may serve as a significant reflection of the anti-oxidant activity [43]. Interestingly, Irfan described the compounds with reducing power of oxidation as electron donors and can reduce the oxidized intermediates of processes of lipid peroxidation[42]. Therefore, these compounds could be act as primary and secondary anti-oxidants. These compoundsare considered as antioxidantssuch as polyphenols (flavonoids). Irfan found that flavonoids of plants have strong antioxidant activity on several oxidation pathways such as on lipid peroxidation exhibiting scavenging activity on hydroxyl radicals [42].

Therefore, the current study demonstrates that the protective activity *Ficus glomerata*on DNA damage and gene expression alterations of liver related genes could be attributed to the inhibition of peroxidation pathway. In addition, it very important for the plant breeders and food producers to identify new genotypes of herbs, fruits and vegetables which are rich in active ingredients promising for antioxidant activities. In this regards, our study give additional support for this view in which several medicinal active ingredients are considered as promising sources for protection against variety of oxidants and consequently inhibits a lot of diseases.

In conclusion, protective role of the methanol extract of Ficus glomerata against BaP was observed as nhibition of the expression alterations of live cancer related genes, DNA damage and suppress the biochemical alterations. The observed antioxidant and hepatoprotective activity of Ficus glomerata may be due to the presence of flavanoids. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

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