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PROTECTIVE EFFECTS OF *FICUS GLOMERATA* EXTRACT AGAINST BENZO(A)PYRENE-INDUCED GENE EXPRESSION ALTERATIONS AND DNA DAMAGE IN MALE MICE

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ABSTRACT

Ficus glomerata Roxb (Branches) is a rich source of phenolic compounds known to possess potential antioxidant activity offering numerous health benefits. The present study evaluated the protective effects of methanol extract of *Ficus glomerata* at two doses (FG_{250}; 250 mg kg^{-1} and FG_{500}; 500 mg kg^{-1}) against benzo(a)pyrene (BaP) induced gene expression changes and DNA damage in male mice. Fifty adult albino male mice allocated in five groups were used in this study. Benzo(a)pyrene (BaP) administration resulted in significant increase ($P \leq 0.01$) in the gene expression of liver cancer-related genes (CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53) in hepatic tissues of male mice. In addition, (BaP) increased the rate of DNA damage in mice blood cells. Extract pretreatment restored genetic alteration parameters toward normalization. FR_{250} and FR_{500} decreased the gene expression alterations and rate of DNA damage induced by BaP in male mice. Extract pretreatment, 500 mg kg^{-1}, was more effective in reducing the genetic toxicity than the extract, 250 mg kg^{-1}. BaP exposure resulted in pronounced oxidative stress, and administration of *Ficus glomerata* extract offers significant liver and blood cells protection by inhibiting the changes of gene expression and damage of the DNA through scavenging free radicals.

Keywords: Benzo(a)pyrene, *Ficus glomerata* extract, Gene expression, DNA damage, Oxidative stress

INTRODUCTION

Benzo(a)pyrene (BaP) is the most potent carcinogen; it is embryo toxic and teratogenic in animals [1]. The level of BaP may be a good marker for carcinogenic potent contamination in an environmental sample [2]. 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 [3].

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 defense 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 [4].

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 [5]. 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 [6]. Several kinds of flavonoid and dietary nutrient found in onion, grapes, green vegetables etc. has been shown to possess potent antioxidant and antiproliferative effects against various malignant cells [7] is also a potent cytochrome P450 inhibitor. It has a wide spectrum of anticancer properties

including inhibition of the growth of cells derived from human cancers such as those of stomach [8] colon [9] prostate [10] and breast [11]. Additionally, it suppresses the growth and development of uterine cervical cancer [12] melanomas [13] and intestinal tumors [14] in whole mice. These antioxidants terminates chain radical reactions by donating hydrogen atoms to the peroxy radical forming of a new molecules which in turn reacts with free radicals thus terminating the propagating chain [15]. It is known to protect against oxidative damage by quenching free radicals and oxygen species or enhancing antioxidant enzymes [16]. These enzymes reduce carcinogen-DNA interaction by providing a large nucleophilic pool for the electrophilic carcinogen.

One of such plant having the antioxidants properties is *Ficus glomerata* (Roxb) which has been used in traditional system of medicine for treating diabetes, liver diseases, piles, asthma, leprosy and diarrhea [17]. The hepatoprotective activity of leaves of *Ficus glomerata* has been reported [18]. Leaves shows anti-bacterial activity [19], stem bark shows anti-tussive potential [20], anti-diuretic activity [21], anti-pyretic potential [22], anti-inflammatory activity of the leaves, bark and unripe fruit [23,24], hypoglycemic activity of roots, leaves and fruit [25, 26] and anti-filarial activity of the fruits [27].

However, there is no scientific claims has been made regarding the protective activity of *Ficus glomerata* on the gene expression changes and DNA damage. In view of this fact, the present study evaluated the antioxidant activity of *Ficus glomerata* extract preventing the genetic toxicity in male mice.

MATERIAL AND METHODS

Plant material: *Ficus glomerata* Roxb (Branches) (Family Moraceae) was collected from King Abdulaziz University Garden, Jeddah, Saudi Arabia. This plant was identified by botanic experts and the nomenclature follows Huxley et al. [28].

Preparation of extracts: A small quantity of each plant, sufficient to yield about 50 g dry weight, was collected for preliminary bio-screening. Routine protection of natural plant constituents from denaturation or artifact formation during the extraction and concentration procedures was assured during the preparation of crude extracts. 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.

Experimental Animals: Fifty adult albino male mice weighing 20-25 g were obtained from the Animal House Colony of the Department of Biology, King Abdulaziz University, Jeddah, Saudi Arabia. The animals were kept individually in wire bottomed cages at room temperature (25 ± 2°C) under 12 h dark-light cycles. They were maintained on 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). All animals received human care in compliance with the guidelines of the Ethical Committee of Medical Research, King Abdulaziz University, Jeddah, Saudi Arabia.

Experimental design: The doses of benzo[a]pyrene and *Ficus glomerata* extract and the route of administration were selected based on their citation in the literature. After an acclimatization period of one week, the animals 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; group (3) animals were orally treated with *Ficus glomerata* extract (250 mg kg-1 b.w) for 4 weeks; group (4) animals orally treated with *Ficus glomerata* extract (500 mg kg-1 b.w) for 4 weeks; group (5) Animal treated with 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 anesthesia. Blood samples were received in EDTA containing tubes for comet assay. Afterwards, the other 5 animals were killed and the liver samples were removed and stored at -80 ºC for gene expression analysis.

Expression of liver cancer related genes

I. Isolation of total RNA: Total RNA was isolated from liver tissues of male mice by the standard TRIzol® Reagent extraction method (Invitrogen, Germany). Briefly, tissue samples were homogenized in 1 ml of TRIzol® Reagent per 50 mg of the tissue. Afterwards, the homogenized sample was incubated for 15 minutes at room temperature. A volume of 0.2 ml of chloroform per 1 ml of TRIzol® Reagent was added. Then the samples were vortexed vigorously for 15 seconds and incubated at room temperature for 3 minutes. The samples were centrifuged for no more than 12,000 x g for 15 minutes at 4 ºC. Following centrifugation, the mixture was separated into lower red, phenol-chloroform phase, an interphase, and a

colorless upper aqueous phase. RNA was remained exclusively in the aqueous phase. Therefore, the upper aqueous phase was carefully transferred without disturbing the interphase into a fresh tube. The RNA was precipitated from the aqueous phase by mixing with isopropyl alcohol. A volume of 0.5 ml of isopropyl alcohol was added per 1 ml of TRIzol® Reagent used for the initial homogenization. Afterwards, the samples were incubated at 15 to 30 ºC for 10 minutes and centrifuged at not more than 12,000 x g for 10 minutes at 4 ºC. The RNA was precipitated which was often invisible before centrifugation, formed a gel-like pellet on the side and bottom of the tube. The supernatant was removed completely. The RNA pellet was washed once with 1 ml of 75% ethanol. The samples were mixed by vortexing and centrifuged at no more than 7,500 x g for 5 minutes at 4 ºC. The supernatant was removed and RNA pellet was air-dried for 10 minutes. RNA was dissolved in diethylpyrocarbonate (DEPC)-treated water by passing solution a few times through a pipette tip. The integrity of RNA 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).

II. Reverse transcription reaction: The complete Poly(A)$^+$ RNA isolated from male mice tissues 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. Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR): 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. First step was at 95.0°C for 3 min. Second step consisted of 40 cycles in which each cycle divided to 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.

Calculation of gene expression: First the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formulae [29]:

$Ef = 10^{-1/slope}$

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 [29]:

$Ratio_{(reference/\ target\ gene)} = Ef^{CT(reference) - CT(target)}$

Comet assay: Peripheral blood lympocytes from male mice 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 [30]. 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 [30].

Statistical analysis: All data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System [31] 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

Expression analysis of CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53 genes: The effect of several concentrations of the *Ficus glomerata* extract (250 and 500 mg/kg b.w.) and benzo[a]pyrene on the expression of liver cancer related genes (CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53) in male mice is summarized in Figures 1-7 and Table 2.

The 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 and Table 2).

On the other hand, treatment of male mice with low dose of the *Ficus glomerata* extract (250 mg/kg b.w.) increased slightly the expression of CYP1A1, CYP1A2 and CYP3A4 genes compared with the control group (Figures 1-3 and Table 2). 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 and Table 2).

In contrary, treatment of male mice with high dose of the *Ficus glomerata* extract (500 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 and Table 2), however, these level were significantly lower compared with male mice treated with benzo[a]pyrene (Figures 1-7 and Table 2). 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 mg/kg b.w.) were relatively similar to those in control and DMSO groups and lower than those in benzo[a]pyrene treated mice.

DNA Damage detected by Comet assay: The 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 3, Figure 8).

The rate 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 3). 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 3).

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 dose of the *Ficus glomerata* extract. Whereas, the rate of DNA damage was 5.2% in male mice treated with high dose of the *Ficus glomerata* extract compared with 7.4% in mice treated with low dose of the *Ficus glomerata* extract (Table 3).

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 3).

DISCUSSION

Cancer diseases are common worldwide and are characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma [32, 33]. BaP is the most potent carcinogen; it is embryo toxic and teratogenic in animals [1]. The level of BaP may be a good marker for carcinogenic potent contamination in an environmental sample [2]. In agreement with these literatures, the present study revealed that work BaP was able to induce alteration in the gene expression of several liver cancer related genes (CYP1A1, CYP1A2, CYP3A4, CYP2B6, CD59, hTRET and P53) and increased the rate of DNA damage in male mice.

The reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide, and hydroxyl radical has been implicated in the pathophysiology of various clinical disorders, including ischemia, reperfusion injury, diabetes mellitus, and cancer [34]. They play an important role in inflammation process after intoxication by ethanol and CCl4 [35]. The potential mechanism of oxidative damage is the nitration of tyrosine residue of proteins, peroxidation of lipids, degradation of DNA, and also oligonucleosomal fragments [34]. Antioxidant reactions involve multiple steps initiation, propagation, branching, and termination. Antioxidants fall into two mechanistic groups: those that inhibit or retard the formation of free radicals from their unstable precursors (initiation) and those that interrupt the radical chain reaction (propagation and branching). The former are called as preventive antioxidant and the latter as chain-breaking antioxidants [36].

Loguercio and Federico [32] and Vitaglione et al. [33] reported that there are increasing evidences that free radicals and reactive oxygen species play a crucial role in the various steps that initiate and regulate the progression of many diseases like liver diseases which is independently of the agent in its origin. In case of bioactivation, our results showed that the liver tissues and red blood cells of male mice were exposed to the damaging effects of the BaP formed toxic substance. Therefore, protective mechanisms relevant to these organs are of particular interest and one of the main objectives in present investigation.

Possible application of plant antimutagens is in dietary prevention of cancer and other mutation related diseases [37], which makes the study of plant antimutagens an important research field. The present study found that *Ficus glomerata* extract has the ability to reduce the oxidative damage induced by BaP, where *Ficus glomerata* extract reduced the expression alterations in the liver cancer related genes and declined the rate of DNA damage. In agreement with our findings, Irfan et al. [38] studied on hepatoprotective activity of *Ficus glomerata* extract on paracetamol and CCl_4 treated albino rats. Treatment with *Ficus glomerata* extract had shown significant hepatoprotecive effect.

In explanation of the protective of *Ficus glomerata* extract, reducing oxygen and hydroxyl radicals power is associated with its anti-oxidant activity and may serve as a significant reflection of the anti-oxidant activity [39]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary anti-oxidants [38]. Anti-oxidant effect of polyphenols (flavonoids) on lipid peroxidation is the result of scavenging of hydroxyl radicals at the stage of initiation and termination of peroxyl radicals [38]. Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell [38].

From the present study, it was found that the inhibition on gene expression alterations of liver related genes and DNA damage by *Ficus glomerata* extract decreased after a certain concentration which may be due to the degradation or peroxidation of the source. Plant breeders and food producers are increasingly identifying specific genotypes and varieties of fruits and vegetables rich in functional ingredients comprising of nutritive and non-nutritive antioxidants. The findings of this study support this view that some medicinal plants are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases. The providing data can just enrich the existing comprehensive data of antioxidant activity of plant materials.

In conclusion, the methanol extract of *Ficus glomerata* demonstrated dose dependent reduction in reducing power activity, superoxide anion scavenging activity and hydroxyl radical scavenging activity.

ACKNOWLEDGMENT

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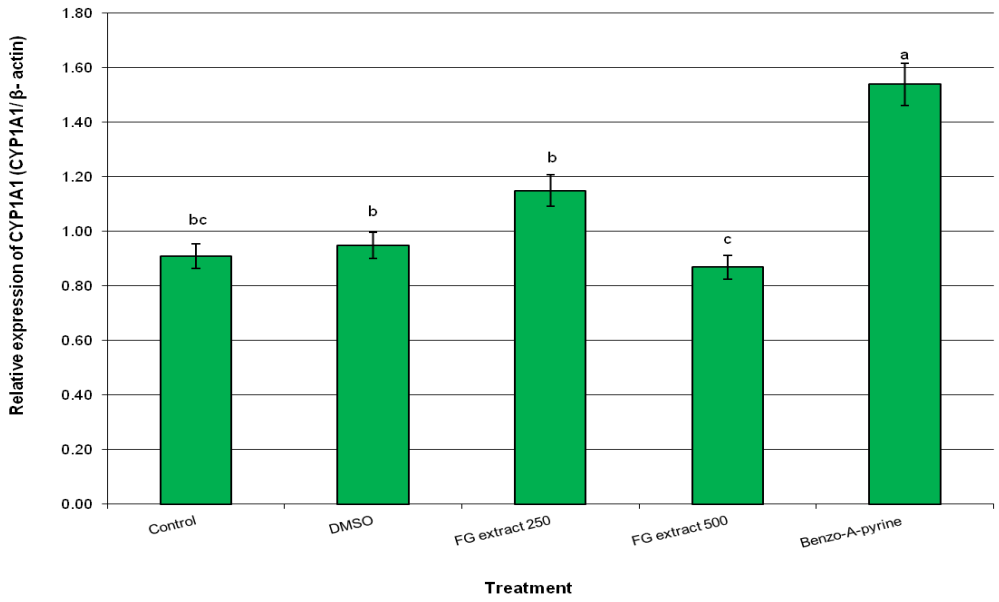

Figure 1: The relative expression of CYP1A1 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficus glomerata* extract. Mean values in the same column with different superscript differ significantly ($P< 0.05$).

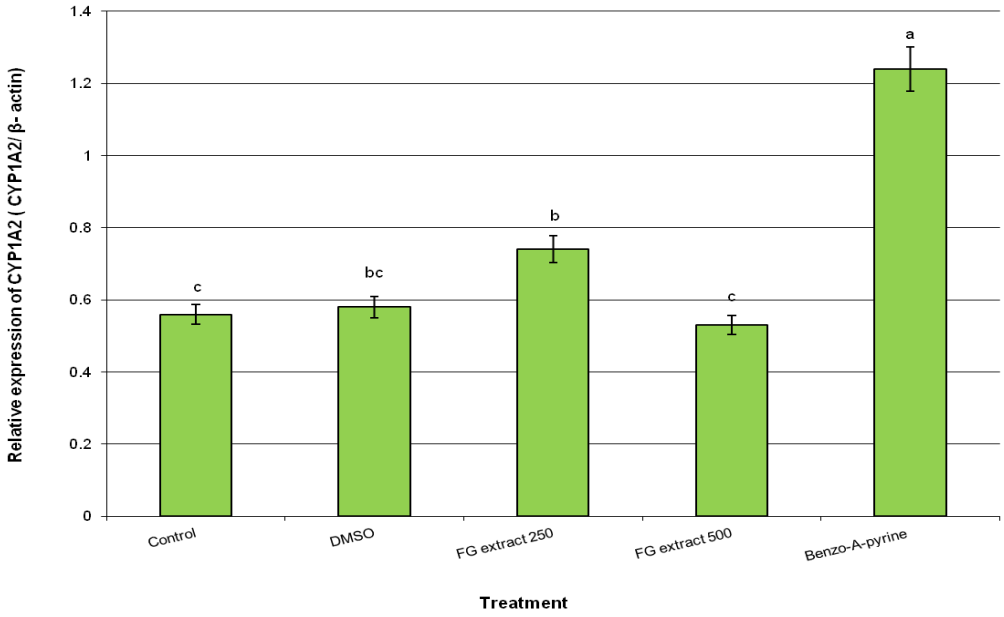

Figure 2: The relative expression of CYP1A2 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficus glomerata* extract. Mean values in the same column with different superscript differ significantly ($P< 0.05$).

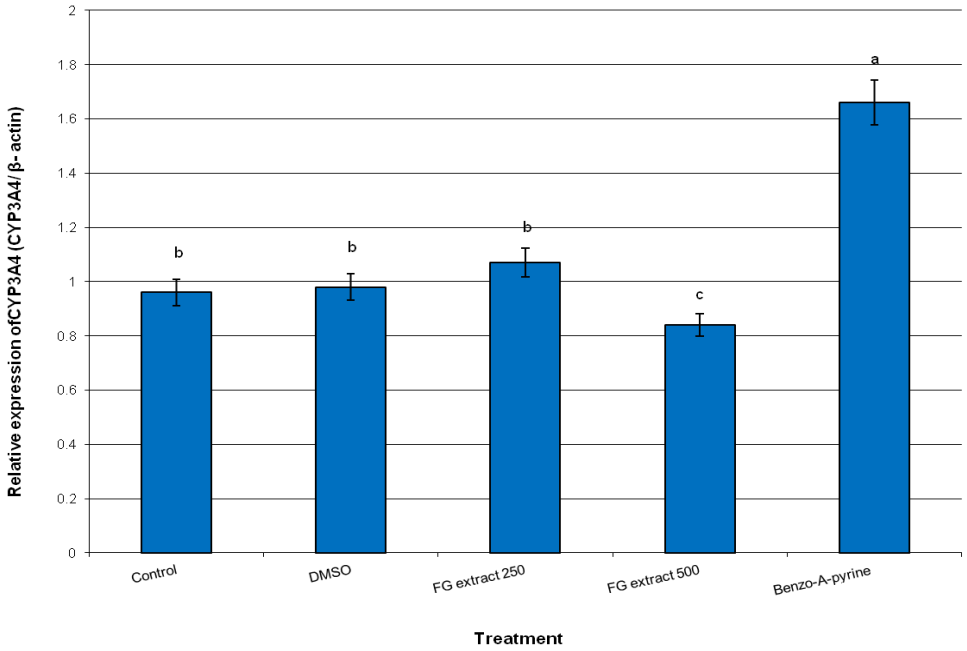

Figure 3: The relative expression of CYP3A4 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficus glomerata* extract. Mean values in the same column with different superscript differ significantly ($P< 0.05$).

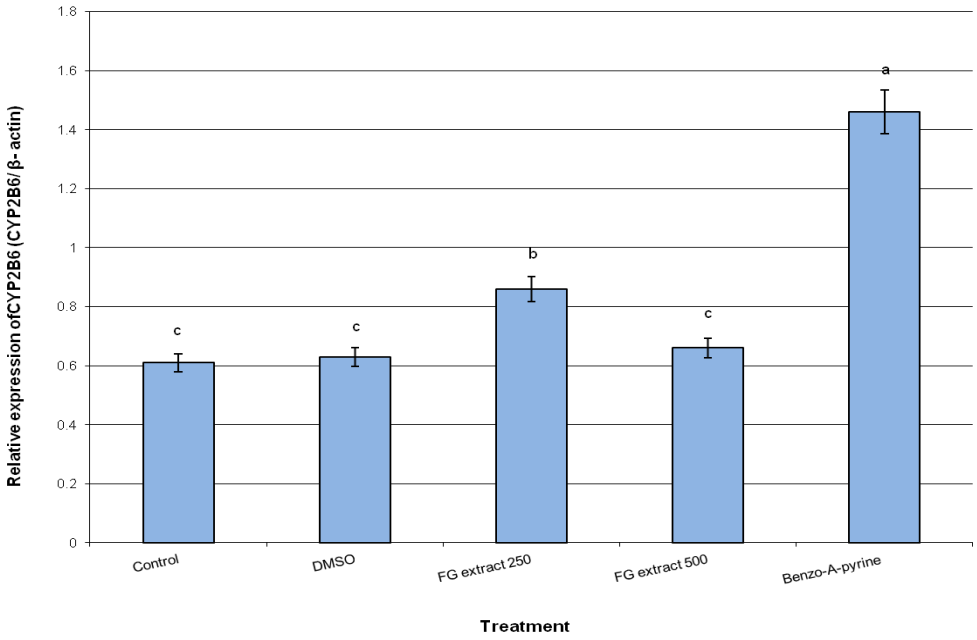

Figure 4: The relative expression of CYP2B6 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficus glomerata* extract. Mean values in the same column with different superscript differ significantly ($P< 0.05$).

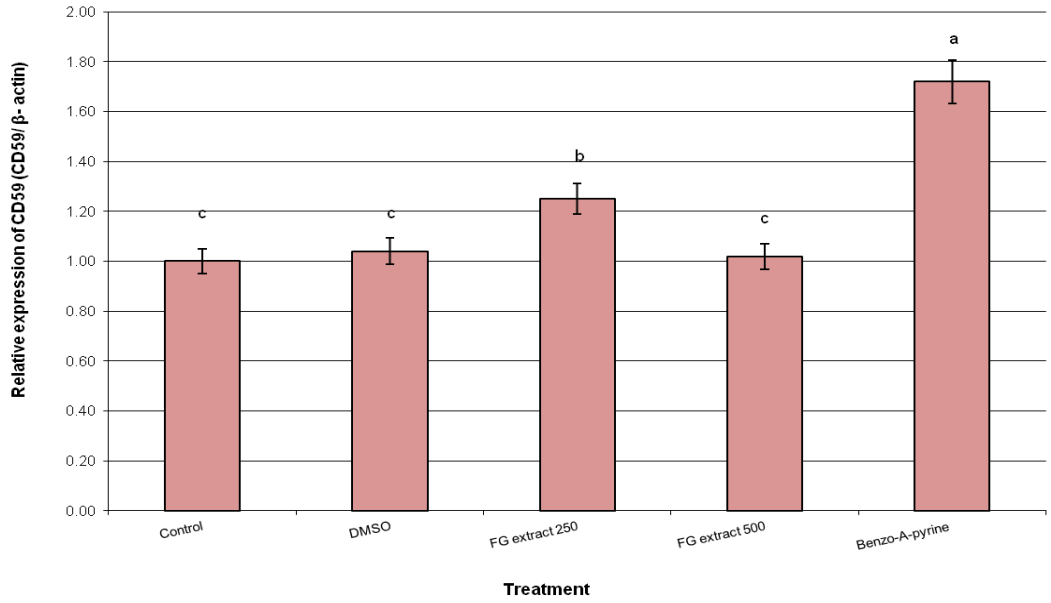

Figure 5: The relative expression of CD59 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficus glomerata* extract. Mean values in the same column with different superscript differ significantly ($P< 0.05$).

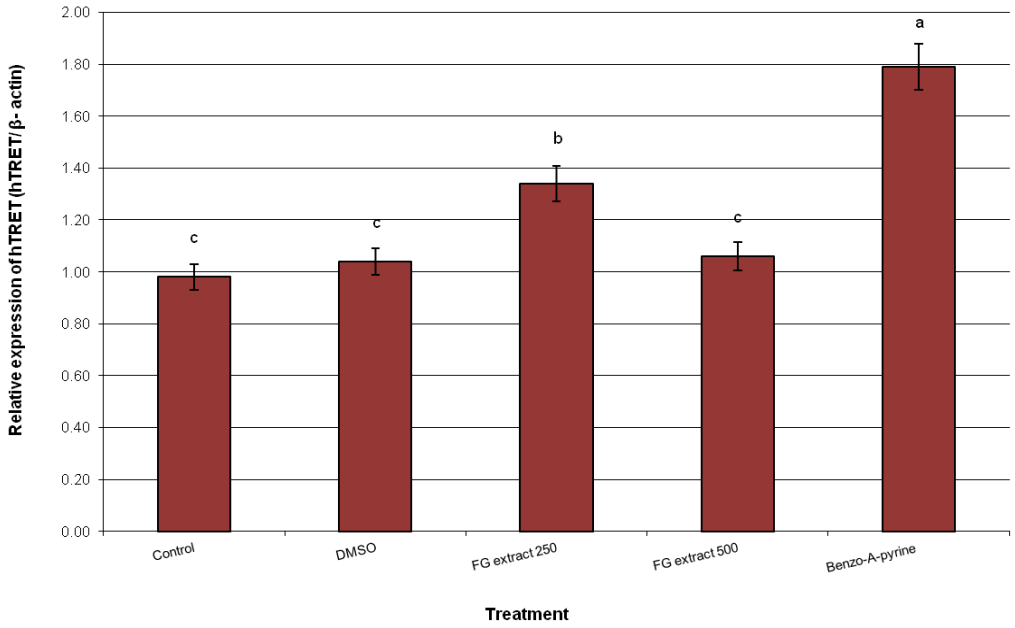

Figure 6: The relative expression of hTRET gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficus glomerata* extract. Mean values in the same column with different superscript differ significantly ($P< 0.05$).

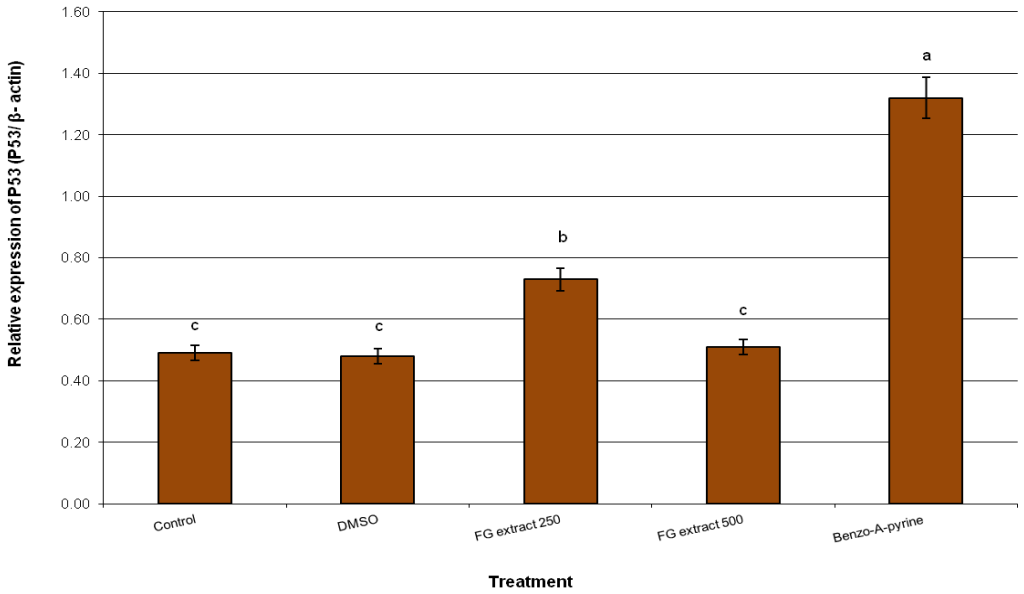

Figure 7: The relative expression of P53 gene in liver of male mice after exposure to Benzo-A-pyrine and/or *Ficus glomerata* extract. Mean values in the same column with different superscript differ significantly ($P< 0.05$).

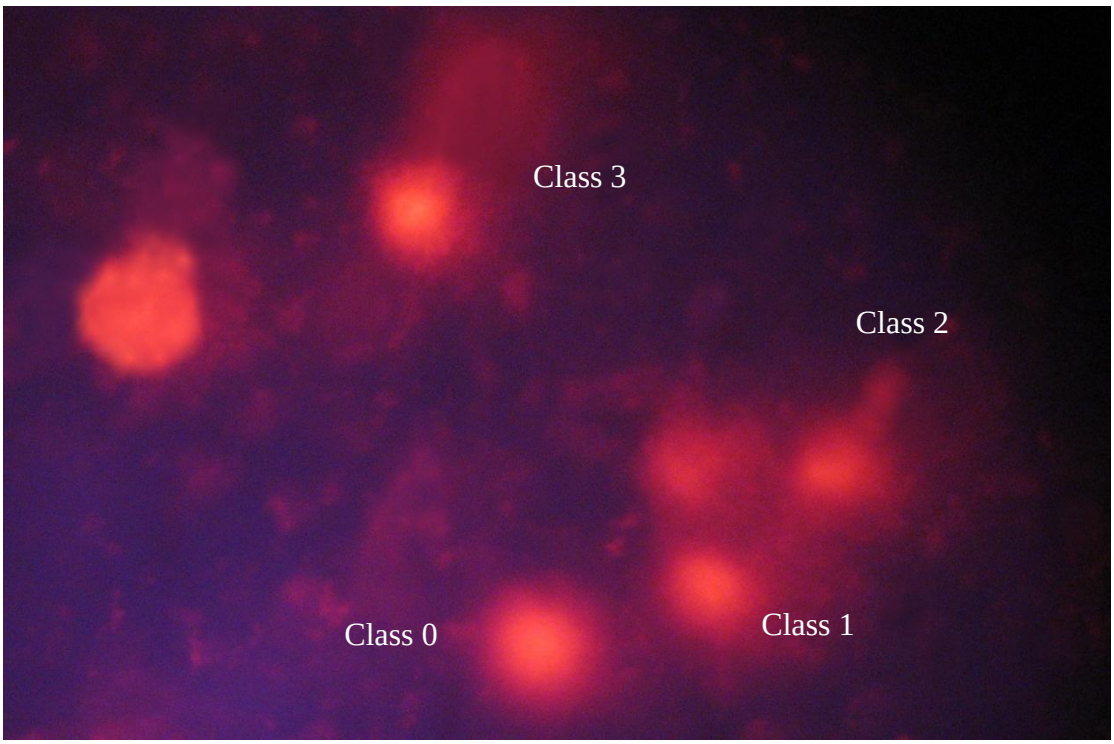

Figure 8: DNA damage in male mice treated with Benzo-A-pyrine and/or *Ficus glomerata* extract**.** Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.

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

Table 2: Quantitative values of the expression of liver related genes in mice treated with Benzo-A-pyrine and/or *Ficus glomerata* extract (mg/kg bw.).

Treatment	Quantitative values of RT-PCR (Mean±SEM)						
	CYP1A1	CYP1A2	CYP3A4	CYP2B6	CD59	hTRET	P53
Control	0.91 ± 0.02^{bc}	0.56 ± 0.01^{c}	0.96 ± 0.02^{b}	0.61 ± 0.01^{c}	1.00 ± 0.03^{c}	0.98 ± 0.03^{c}	0.49 ± 0.01^{c}
DMSO	0.95 ± 0.02^{b}	0.58 ± 0.01^{bc}	0.98 ± 0.02^{b}	0.63 ± 0.01^{c}	1.04 ± 0.03^{c}	1.04 ± 0.03^{c}	0.48 ± 0.01^{c}
FG extract 250	1.15 ± 0.02^{b}	0.74 ± 0.01^{b}	1.1 ± 0.02^{b}	0.86 ± 0.01^{b}	1.25 ± 0.03^{b}	1.34 ± 0.03^{b}	0.73 ± 0.02^{b}
FG extract 500	0.87 ± 0.02^{c}	0.53 ± 0.01^{c}	0.84 ± 0.01^{c}	0.66 ± 0.01^{c}	1.02 ± 0.01^{c}	1.06 ± 0.02^{c}	0.51 ± 0.01^{c}
Benzo-A-pyrine	1.54 ± 0.03^{a}	1.24 ± 0.02^{a}	1.66 ± 0.03^{a}	1.46 ± 0.03^{a}	1.72 ± 0.04^{a}	1.79 ± 0.04^{a}	1.32 ± 0.02^{a}

Table 3: 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 250	5	500	37	463	21	9	7	7.4
FG extract 500	5	500	26	474	14	6	5	5.2
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.

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