Potential Chemoprevention of Liver Disorders by Dietary Curcumin in Rats Treated with Benzo(*a*)pyrene

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Abstract: Curcumin, which is a naturally occurring compound, is present in turmeric (*Curcuma longa* L., family *Zingiberaceae*) rhizomes, possesses both anti-inflammatory and antioxidant properties, and has been tested for its chemopreventive properties in skin, forestomach and colon carcinogenesis. The present study was designed to investigate the potential chemopreventive actions of dietary curcumin on Benzo(*a*)pyrene [P(a)P] induced liver disorders/toxicity and also the modulating effects of this agent on liver functions, oxidants and antioxidant status, immunological parameters in male albino rats, weighing 150±8.7 g per each. Groups of animals were fed the control (modified AIN-76A) diet or a diet containing 500-2000 mg/kg of curcumin. After two weeks, all animals, except those in the negative group (vehicle/normal saline treated group) were given two weekly s.c. injections of B(*a*)P at a dose 15 mg/5 ml/kg body weight). All groups were continued on their respective dietary regimen until the termination of the experiment at 12 weeks. The results indicate that treatment of animals with B[*a*]P caused a significant increased ($p\leq0.01$) in AST (110.38%), ALT (67.85%) and ALP (116.47%) compared to normal controls. Dietary administration of curcumin (500 to 1000 mg/kg w/w) significantly (($p\leq0.01$) inhibited serum AST, ALT and ALP activities by different rates. The same behavior was recorded for malondialdehyde (MDA) and nitrite (NO_2) levels in liver tissues, the biomarkers of oxidative stress in cells and some immunological parameters including albumin levels and protease activity in serum. The opposite direction was recorded for the glutathione fractions (biological macromolecules antioxidant) in liver tissues. These results supported our hypothesis that dietary curcumin is able to prevent or inhibit B[*a*]P hepatotoxicity through modulating liver serum enzymes activity, formation of liver MDA and NO_2 and serum immunological parameters . Therefore, we recommended curcumin by a concentration up to 2000 mg/kg to be included in our daily diets, drinks and food products.

Keywords: Turmeric, curcumin, liver tissues, liver functions, glutathione fractions, immunological parameters, malondialdehyde and nitrite.

Introduction

Liver is a vital organ present in all vertebrates. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemical necessary for digestion (Voet and Voet, 1990). This organ plays a major role in metabolism and has a number of functions in the body, including glycogen storage, d storage of nutrients (glycogen, minerals and vitamins), decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification of foreign substances such as drugs and environmental toxins. It produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton *et al.,* 1993 and Chattopadhyay and Bhattacharyya, 2007).The liver is necessary for survival; there is currently no way to compensate for the absence of liver function long term, although liver dialysis can be used short time.

Liver disease is a term generally use for conditions, diseases, and infections that affect the cells, tissues, structures, or functions of the liver. It is caused by a various

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conditions including viral infections, bacterial invasion, malnutrition, alcoholism and chemical or physical changes within the body. Also, there are many disease and condition which are responsible for hepatic disease or hepatic injury like hepatotoxicity tendency of an agent, usually a drug or toxic compounds. The toxic compounds induced different liver diseases includes pesticides, dioxins, polycyclic aromatic hydrocarbons (PAH) and aflatoxin (Elhassaneen, 1996). Amongst of these compounds, PAH from incomplete combustion occur in several foods such as charcoal broiled and smoked goods (Emerole *et al.*, 1982; Larsson *et al.,* 1983 and Bassiouny, 1999). Benzo(*a*)pyrene [B(*a*)P] is a member of the family, PAH that is a by-product of incomplete combustion or burning of organic (carbon-containing) items, e.g., cigarettes, gasoline, and wood. Also, BaP is found in ambient (outdoor) air, indoor air, and in some water sources (U.S. Environmental Protection Agency, 2005). Furthermore, many studies isolated BP from different Egyptian dietary sources including grilled, broiled, deep-fat fried and smoked foods (Elhassaneen and Tawfik, 1998; Elhassaneen, 2004 and Elhassaneen and El-Badawy, 2013).

Many of PAH compounds including B(*a*)P have been shown to be toxic, mutagenic and/or carcinogenic by extensive experiments *in vivo* (Harvey, 1985; Hawkins *et al.*, 1990 and Elhassaneen, 2004) and *in vitro* (Elhassaneen, 1996; Elhassaneen *et al*., 1997; and Elhassaneen, 2002) systems. Also, B(*a*)P exposure is associated with the development of liver cancer in mammals, rodent and fish (Harvey, 1985 and Hawkins *et al.*, 1988-1990; Elhassaneen, 1996 and Elhassaneen, 2002). It is known that the toxic, tumorigenic and carcinogenic effects of B[*a*]P correlate with the cellular metabolism of this compounds to arene oxides, phenols, quinones, dihydrodiols, and epoxides and with their subsequent formation of recative intermediates that interact covalently with DNA to form adducts (Harvey, 1985, Elhassaneen, 1996). While the Fixation of a biochemical changes by cell proliferation is considered the next step. The mutagenicity of BP is dependent upon metabolic activation. So, BP is considered a promutagen (Elhassaneen, 1996).

Many of authorities and academic centers of research reported that a large number of medicinal plants have been tested and found to contain active principles with curative properties against a variety of diseases including liver diseases (Reviewed in Jesika *et al.,* 2016). Liver protective plants contain a variety of chemical constituents like phenols, Coumarins, Lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes (Surh *et al.,* 2001 and Elhassaneen *et al.,* 2016-a). Therefore, a large number of plant parts have been claimed to have hepatoprotective activity so the development of plant based hepatoprotective drugs has been given importance in the global market.
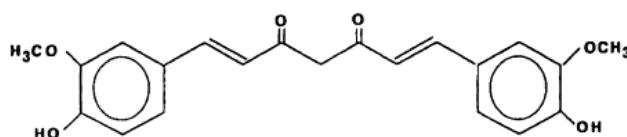

Figure 1. Structure of curcumin (diferuloylmethane)

Curcumin, a polyphenol compound (diferuloylmethane), is responsible for the yellow color of turmeric and is thought to be the most active pharmacological agent (Fayez, 2015). Natural curcumin, isolated from turmeric (*Curcuma longa* L.) rhizomes, belongs to the *Zingiberaceae* family along with the other noteworthy members like

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ginger, cardamom and galangal. It belongs to the genus *Curcuma* that consists of hundreds of species of plants that possess rhizomes and underground root like stems and is a medicinal herb of high repute all over the world particularly in South Asia, where it is also used as curry spice in foods, flavoring agent, food preservative, and color agent in mustard, margarine, soft drinks, and beverages. Such as mentioned by Aggarwal, (2003) turmeric contains curcumin I (diferuloyl methane as the major constituent), as well as curcumin II (6%) and III (0.3%). Curcumin is insoluble in water and ether, but is soluble in ethanol, dimethylsulfoxide, and other organic solvents. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer activities and thus has a potential against various malignant diseases, diabetes, allergies, arthritis, Alzheimer's disease and other chronic illnesses (Aggarwal *et al.,* 2007 and Fayez, 2015). Numerous research teams provided evidence that curcumin contributes to the inhibition of tumors formation and promotion as cancer initiation، promotion or progression of tumours is decreased or blocked by this compound (Azuine and Bhide, 1992; Chinthalapally *et al., 1995* and Elhassaneen *et al.,* 2016-a). Curcumin shows significant therapeutic potential for liver cancers because it suppresses cancer cell proliferation, induces cell cycle arrest and apoptosis via the caspase cascade, Curcumin also exerts anticarcinogenic effects by decreasing the expression of cyclooxygenase-2 (COX-2) and vascular endothelial growth factor (Chinthalapally *et al.,* 1995). According to our knowledge, the studies regarding the potential effects of curcumin on liver disease/disorders are so limited. Therefore, in this study, we examined potential chemoprevention of liver disorders by dietary curcumin in rats treated with benzo(*a*)pyrene.

Materials and Methods
Materials

Benzo(*a*)pyrene [B(a)P], γ-glutamyl glutamate and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, Company agent, Cairo, Egypt). Curcumin (>98% of diferuloylmethane) was purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). All organic solvents, buffers and other chemicals of analytical grade were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Throughout this study a SP Thermo Separation Products Liquid Chromatograph (Thermo Separation products, San Jose, CA) was used with a Consta Metvic 4100 pump, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. The columns used (Alltech, Deerfield, IL, USA) were a Spherosorb ODC-2 (5 μm, 150 x 4.6 mm I.d.) for glutathione fractions and C_{18} (5 μm, 150 x 4.6 mm I.d.) for analysis the purity and stability of curcumin on diet.

Animals and Diets

Adult male albino rats (150±8.7g per each, specific pathogen free) were purchased from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. All ingredients of the semi-purified diet were obtained from Al-Ghomhorya Company for Trading Drug, Chemicals pond Medical Instruments, Cairo, Egypt and were stored at 4°C prior to the preparation of diets. Rats were quarantined for 10 days and had access to modified AIN control diet (AIN, 1993). AIN control diet prepared according to the following formula: protein (10%), corn oil (10%), vitamin mixture (1%), mineral

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mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamins mixture component was that recommended by Campbell, (196 3) while the salts mixture used was formulated according to Hegsted, (1941).

Following quarantine, all the animals were randomly distributed by weight into various groups and transferred to an animal holding room. They were housed in plastic cages with filter tops (three per cage) under controlled conditions of a 12-h light/12-h dark cycle, 50% humidity, and 22 ± 2 °C temperature and kept under normal healthy conditions. The experimental diet was prepared by adding curcumin to the control diet at the expense of corn starch. The incorporation of curcumin into the control diet was done with V-blender (Moulinex Egypt, ElAraby Co., Benha, Egypt) after curcumin was premixed with a small quantity of diet in a food mixer to ensure its uniform distribution. All control and experimental diets were prepared every two weeks in our laboratory and stored in a refrigerator at 4 ^{0}C. Animals had access to food and water at all times, and food cups were replenished with fresh
diet three times per a week.

Analysis the purity and stability of curcumin on diets

The purity and stability of curcumin was analyzed on tested animal diets by HPLC. In brief, curcumin was extracted from the diet with two volumes of mixture (acetonitrile: acetic acid, 95:5). The organic layer was dried under nitrogen and re-dissolved in a mobile phase (0.1 M sodium phosphate, pH 4, and acetonitrile) and injected into HPLC. Curcumin was separated using gradient elution and was montored at a 380-nm wavelength. The results indicate that >97% of curcumin could be accounted for in feed samples stored in a cold room for three weeks.

Experimental procedure

The experiment was design to determine the efficacy of different levels (500-2000 mg.kg^{-1} curcumin) on B(a)P-induced liver disorder/toxicity. Although curcumin administered in the diet to rats at 100,000 ppm had no significant toxic effect (Ammon and Wahl, 1991), we have decided to use low dietary levels of curcumin, based on many previous studies in which 2000 ppm in the diet significantly inhibited colonic aberrant crypt foci development (Rao *et al.,* 1993) and induced chemoprevention of colon carcinogenesis.

All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into the experimental groups. The first group (Group 1, 6 rats, as a negative control group) still fed on basal/standard diet (BD) and injected with the vehicle alone (5 ml/kg body weight). Animals in group (2) was challenged two weekly s.c. injections of B[a]P at a dose rate of 15 mg/5 ml/kg body weight dissolved in 0.9% NaCI solution containing 0.1% Tween 20 to induce liver impaired rats. Groups (3-6) rats were classified and feeding as follow: group (3), fed on BD containing 500 mg.kg^{-1} curcumin; group (4), fed on BD containing 1000 mg.kg^{-1} curcumin; group (5), fed on BD containing 1500 mg.kg^{-1} curcumin and group (6), fed on BD containing 2000 mg.kg^{-1} curcumin. The treatment with curcumin to the animal belonging to groups (3 to 6) was started two weeks prior to B(a)B treated. Animals were maintained on control or experimental diets until the termination of the experiment (12 weeks). Body weights were recorded every 2 weeks. Animals were monitored daily for general health. The experiment was terminated 12 weeks after the second B(a)P treatment, at which time blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized.

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Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum according to Drury and Wallington, (1980). Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20°C until analysis.

Hematological analysis
Liver functions

Serum alanine aminotransferase (ALT) and Serum aspartate aminotransferase (AST) activities were measured in serum using the modified kinetic method of Tietz *et al.*, (1976) by using kit supplied by Biocon Company, Cairo, Egypt. Alkaline Phosphatase (ALP) activity was determined using modified kinetic method of Vassault *et al.,* (1999) by using kit supplied by Elitech Company, Cairo, Egypt.

Reduced and oxidized glutathione (GSH and GSSG)

GSH and GSSG were determined in in liver tissue homogenate extract by HPLC according to the method of McFarris and Reed (1987) as follow: 100 µl of aliquot were placed in 2 ml of 10% perchloric acid containing 1 mM bathophenanthroline disulfonic acid and homogenized. The homogenate was cold centrifuged at 10000 rpm for 5 min and the internal standard (γ-glutamyl glutamate) was added to the supernatant. A 250 µl aliquot of acidic extract was mixed with 100 µl of 100 mM iodoacetic acid in 0.2 mM cresol purple solution. The acid solution was brought to pH 8.9 by the addition of 0.4 ml of KOH (2 M) – KHCO$_3$ (2.4 M) and allowed to incubate in the dark at room temperature for 1 hr to obtain *S*-carboxymethyl derivatives. The *N*-nitrophenol derivatization of the samples were taken overnight at 4 ^{0}C in the presence of 0.2 ml of 1% 1-fluoro-2,4-dinitrobenzene and injected onto the HPLC system.

Malonaldialdehyde content (MDA)

Lipid peroxide levels measured as malondialdehyde in serum and liver samples were determined by as thiobarbituric acid reactive substances (TBARS) as described by Buege and Aust, (1978). A 500 µl of plasma or liver tissue homogenate extract were added to 1.0 ml TBA reagent, consisting of 15% TCA, 0.375% TBA and 0.01% butylated hydroxytoluene in 0.25 N HCl. Twenty-five microliters of 0.1 M FeSO$_4$.7H$_2$O was added and the mixture was heated for 20 min in boiling water. The samples were centrifuged at 1000 rpm for 10 min and the absorbance was read at 535 nm using Labo-med. Inc., spectrophotometer against a reagent blank. The absorbance of the samples was compared to a standard curve of known concentrations of malonicdialdehyde.

Nitrite (NO$_2$) determination

Nitrite was determined flourometric in serum and liver samples such as described by Misko *et al*., (1993). Ten µl of freshly prepared 2, 3-diaminonaphthalene (DAN, 0.05 mg/ml in 0.62 M HCl, protected from light) is added to 100 µl of sample and mixed immediately. Nitrate standards (> 98% pure, Sigma Chemical Company) are routinely made fresh, dissolved in DI H$_2$O, and kept on ice prior to use. After 10 min incubation at 20 ^{0}C, the reaction was terminated with 5 µl of 2.8 N NaOH. The intensity of the fluorescent signal produced by the product is maximized by the addition of base. Formation of the 2,3-diaminonaphthtriazole was measured using a Schematzu fluorescence apparatus with excitation at 365 nm rand emission read at 450 nm with a gain setting at 100%.

Albumin

Albumin was determined in plasma using kits purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt.

Protease activity (PA) assay

The PA was determined by adaptation the method of Rinderrnecitt *et al*., (1968). In brief, 100 µl of plasma were added to 40 µl of buffer (150 mM Tris base, 30 mM $CaCl_2$, 0.05% Brij 35) and 50 µl of protease substrate (20% Hide powder azure, HPA, 20 % sucrose, 0.05% Brij). The tubes contents were incubated at 37^0C with continuous shaking for 2 hours. The reaction was stopped by the addition of 50 µl of 10% TCA and the tubes were stored at 4^oC for about 15 min. After spined the tubes at 8500 rpm for 5 min, the supernatants were transferred to new tubes and the absorbencies were measured at 540 nm. Blank tubes were prepared by the same previous steps without samples addition.

Liver glycogen

Liver glycogen levels were determined after digestion of liver and precipitation of glycogen by Glycogen Assay Kit II (Colorimetric, abcam kits Co., ab169558, www.abcam.com).

Triglycerides (TG)

Enzymatic determination of TG (mg/100 ml) in serum was determined in serum using specific kits purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt.

Statistical Analysis

All measurements were done in triplicate and recorded as mean±SD. Differences in different parameters between the groups were analyzed statistically by the Student *t* test and analysis of variance using MINITAB 12 computer program (Minitab Inc., State College, PA).Differences were considered statistically significant at P ≤0.05.

Results and Discussion

Effect of curcumin on body weight changes induced by B[*a*]P in rats

The effect of curcumin on body weight (BW, g) changes induced by B[*a*]P in rats was shown in Table (1) and Figure (2). From such data it could be noticed that rats injected B[*a*]P leads to decrease the BW than the control group. At the end of the experiment (12 weeks), rats of the injected B[*a*]P group recorded 177.27% of the control (normal) group for the BW. Feeding of curcumin by different levels 500, 1000, 1500 and 2000 mg/kg induced significant decreasing on BW of the injected B[*a*]P rats which recorded 180.91, 184.55, 185.45 and 187.27% as a percent of control, respectively. The increasing rates in BW were elevated with the increasing of curcumin levels in diets. Such date are in agreement with that observed by Chinthalapally *et al.,* (1995) who reported that the chronic feeding of curcumin did not produce any gross changes in the liver, kidney, stomach, intestine, or lungs or any kind of histopathological changes in the liver or intestine attributable to toxicity.

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The positive effects of curcumin regarding the control of the injected B[*a*]P could be attributed to such bioactive compound and its conversion products have been shown to induce/participate in several mechanisms which contribute to their action control of adipocyte function, adiposity subsequently weight gain (Bonet *et al.,* 2015). Amongst of these mechanisms, they could be interacted with several transcription factors of the nuclear receptor superfamily, interfered with the activity of other transcription factors, modulated signaling pathways which are associated with inflammatory and oxidative stress responses; and scavenged of reactive species such ROS and RNS (Aggarwal *et al.,* 2007 and Le Lay *et al.,* 2014) .

Table 1. Effect of curcumin on body weight changes induced by B[*a*]P in rats

| Groups	Body weight (g)/week						
	0	2	4	6	8	10	12
Gp1: Control (-), Basal diet (BD)	110	169	203	245	268	285	319
Gp2: Control (+), BD + B(*a*)P	110	156	185	233	256	274	305
Gp3: 500 mg/kg curcumin treated group	110	159	189	237	258	277	309
Gp3: 1000 mg/kg curcumin treated group	110	161	191	238	260	278	313
Gp3: 1500 mg/kg curcumin treated group	110	162	195	239	261	278	314
Gp6: 2000 mg/kg curcumin treated group	110	164	198	242	263	281	316
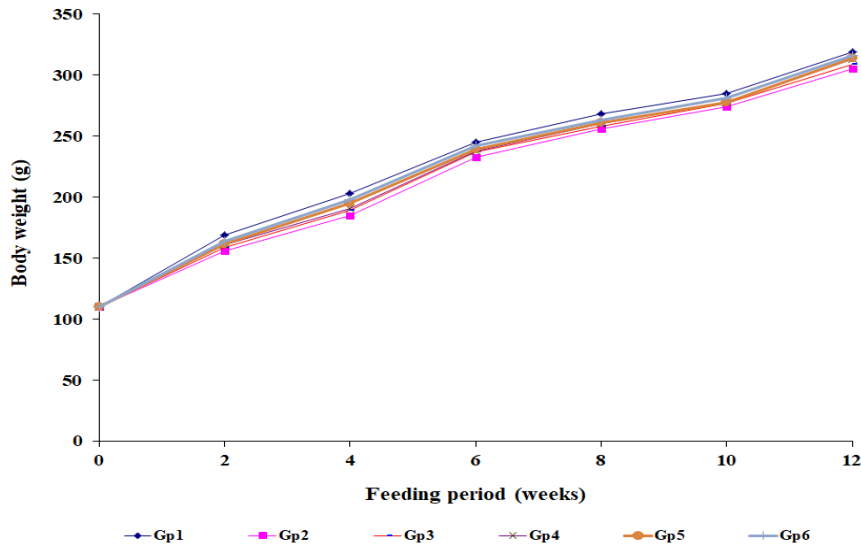
Figure 2. Effect of curcumin on body weight changes induced by B[*a*]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.

Effect of curcumin on liver functions changes induced by B[*a*]P in rats

Liver functions of rats injected B[*a*]P and consumed curcumin powder were shown in Table (2) and Figures (2-3). From such data it could be noticed that treatment of animals with B[*a*]P caused a significant increased ($p \leq 0.05$) in ALT (67.85%), AST (110.38%) and ALP (116.47%) compared to the normal controls group. Supplementation

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of the rat diets with curcumin (500 to 2000 mg/kg w/w) prevented the rise of mean serum ALT, AST and ALP activities. The prevention rate was increased with the increasing of the curcumin level feeding. The rates of increasing in the liver enzymatic activities were recorded 47.56, 29.01, 15.40 and 9.02% (For ALT); 64.55, 47.14, 26.86 and 12.58% (for ALT) and 73.23, 42.41, 35.12 and 16.24% (for ALP) with the rat diets supplemented by 500, 1000, 1500 and 2000 mg/kg of curcumin, respectively.

Long time ago, B[a]P was commonly used as a hepatotoxin i.e. liver disorders induction in different experimental biological models including *in vivo* and *in vitro* studies (Elhassaneen, 1996; Harvey, 1985 and Hawkins *et al.*, 1988-1990). The hepatotoxic effects induced by B[a]P are largely due to the binding of its activated metabolites (hydroxy- dihydroxy-, polyhydroxy-, quinones, semiquinones, epoxide

Table 2. Effect of curcumin on liver functions changes induced by B[a]P in rats

Value	Gp1 Control (-) Basal diet (BD)	Gp2 Control (+) BD + B(a)P	Curcumin treatment groups [BD+ B(a)P+ curcumin]			
			Gp3 500 mg/kg	Gp4 1000 mg/kg	Gp5 1500 mg/kg	Gp6 2000 mg/kg
Serum alanine aminotransferase (ALT,U/L)						
Mean ± SD	71.23± 5.49 [e]	119.56± 9.04 [a]	105.11± 9.99 [b]	91.89± 8.50 [c]	82.20± 9.44 [d]	77.65± 8.52 [e]
% of Change	0.00	67.85	47.56	29.01	15.40	9.02
Serum aspartate aminotransferase (AST,U/L)						
Mean ± SD	40.16± 4.37 [e]	84.48± 8.45 [a]	66.08± 9.00 [b]	59.09± 9.45 [c]	50.94± 5.24 [d]	45.21± 7.49 [e]
% of Change	0.00	110.38	64.55	47.14	26.86	12.58
Serum alkaline phosphatase (ALP,U/L)						
Mean ± SD	141.16± 12.47 [e]	305.57± 20.75 [a]	244.54± 13.61 [b]	201.03± 14.50 [c]	190.74± 12.72 [c]	164.08± 14.45 [d]
% of Change	0.00	116.47	73.23	42.41	35.12	16.24

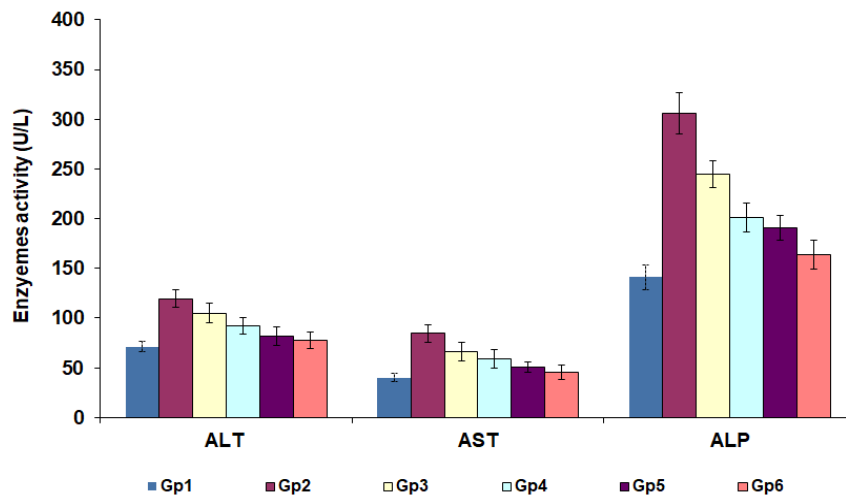*Means in the same row with different letters are significantly different at p≤0.05

Figure 2. Effect of curcumin on liver functions (U/L) changes induced by B[a]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.

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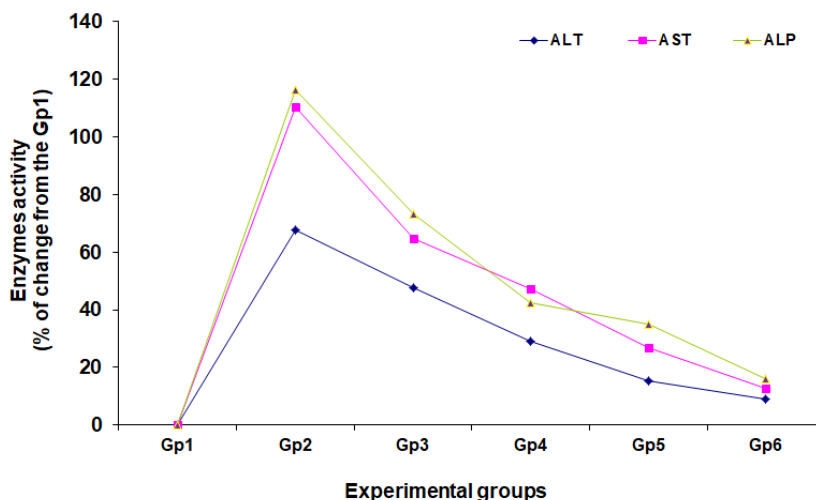**Yousif Elhassaneen, Heba Ghamry and Lamiaa Lotfy**

Figure 3. Effect of curcumin on liver functions (% of control from the Gp1) changes induced by B[*a*]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.

etc) with the cellular macromolecules such proteins and nucleic acids (Elhassaneen, 1996). Other hepatotoxic effects of B[*a*]P are due to the ability of its metabolites to induce peroxidative degradation of membrane lipids in different cell organelles including cell wall, mitochondria and lysosomes rich in polyunsaturated fatty acids (Elhassaneen, 1996 and Elhassaneen *et al.,*1997). Such degradation of cellular membranes is one of the principle causes of hepatotoxicity of B[*a*]P (Elhassaneen, 2004). This is confirmed by the elevation noticed in the serum liver marker enzymes namely AST, ALT and ALP. In related study, Elhassaneen and Al-Badawy, (2013) reported that elevations in liver functions enzymatic activities including AST, ALT and ALP in human as the result of B[*a*]P consumption in charcoal broiled meat.

Decreasing in serum liver marker enzymes induced by curcumin, a polyphenol compound could be attributed to its antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer activities which have a potential against various malignant diseases (Aggarwal *et al.,* 2007 and Fayez, 2015). Similar studies reported that the effect of many plant parts on decreasing the serum liver function enzymes activity could be attributed to their high level content of that phytochemicals including curcumin (El-Nashar, 2007; El-Sayed *et al.,* 2012; Fayez, 2015 and Sayed Ahmed, 2016). The possible mode of action of liver serum enzymes-lowering activity of the curcumin could be explained by one or more of the following process: 1) block the hepatocellular uptake of bile acids (Dawson, 1998), 2) improve the antioxidant capacity of the liver (Beattic *et al.,* 2005) and 3) improve the of antioxidant defense system in red blood cells (Fayez, 2015).

Effect of curcumin on biological antioxidants (liver tissue glutathione fractions levels) changes induced by B[*a*]P in rats

Data presented in Table (3) and Figures (4-5) showed the effects of curcumin on biological antioxidants (liver tissue glutathione fractions levels) changes induced by B[*a*]P in rats. From such data it could be noticed that treatment of animals with B[*a*]P

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caused a significant decreased ($p \leq 0.05$) in GSH (-38.35%) and GSSG (-19.30%) compared to normal controls. Supplementation of the rat diets with curcumin (500 to 2000 mg/kg w/w) prevented the rise of mean serum GSH and GSSG levels. The rate of preventative effects was increased with the increasing of the curcumin concentration. The rate of decreasing in the serum GSH fractions were recorded and -31.09, -26.00, -18.91 and -11.32% (for GSH) and -21.51, -19.17, -13.84 and -8.79 % (for GSSG) with the rat diets supplemented by 500, 1000, 1500 and 2000 mg/kg of curcumin, respectively.

Table 3. Effect of curcumin on liver tissue glutathione fractions levels changes induced by B[a]P in rats

Value	Gp1 Control (-) Basal diet (BD)	Gp2 Control (+) BD + B(a)P	Curcumin treatment groups [BD+ B(a)P+ curcumin]			
			Gp3 500 mg/kg	Gp4 1000 mg/kg	Gp5 1500 mg/kg	Gp6 2000 mg/kg
Reduced glutathione level (GSH, μmol/mg tissue protein)						
Mean ± SD	9.89 ± 1.17 [a]	6.10 ± 1.04 [cd]	6.81 ± 1.16 [c]	7.32 ± 0.82 [b]	8.02 ± 1.07 [ab]	8.77 ± 1.81 [a]
% of Change	0.00	-38.35	-31.09	-26.00	-18.91	-11.32
Oxidized glutathione level (GSSG, μmol/mg tissue protein)						
Mean ± SD	0.91 ± 0.23 [a]	0.73 ± 0.23 [cd]	0.71 ± 0.32 [cd]	0.74 ± 0.19 [c]	0.78 ± 0.14 [c]	0.83 ± 0.20 [b]
% of Change	0.00	-19.30	-21.51	-19.17	-13.84	-8.79
GSH/GSSG ratio						
Mean ± SD	10.87 ± 1.21 [a]	8.30 ± 1.21 [c]	9.54 ± 0.98 [ab]	9.95 ± 1.77 [ab]	10.23 ± 1.11 [a]	10.57 ± 1.07 [a]
% of Change	0.00	-23.61	-12.21	-8.45	-5.88	-2.77

[*] Means in the same row with different letters are significantly different at $p \leq 0.05$

These data indicated that the rate of liver tissues GSH fractions elevation was increased with the increasing of the curcumin consumption quantity. In general, GSH is a tripeptide-thiol (γ-glutamyl cysteinyl-glycine) and GSSG those have received considerable attention in terms of their biosynthesis, regulation, and various intracellular functions (Reed and Beatty, 1980; Larsson *et al*., 1983). Among of these functions, their roles in detoxifications process represent the central role through as a key conjugate of xenobiotics electrophilic intermediates [such Benzo(*a*)pyrene metabolites] and as an important antioxidant. The antioxidant functions of GSH fractions include their roles in the activities of the antioxidant enzymes system including glutathione perioxidase (GSH-Px) and glutathione reductase (GSH-Rd). In addition, GSH fractions can apparently serve as a nonenzymatic scavenger of oxyradicals (Halliwell and Gutteridge, 1985; Fayez, 2015 and Almaadawy *et al.,* 2016). Additionally, Fayez (2015) mentioned that liver cells GSSG concentration to provide a sensitive index of whole body oxidative stress in the rat. Increased fluxes of oxyradicals might be decreased in the GSH/GSSG ratio, due either to direct radical scavenging or to increased peroxidase activity (Almaadawy *et al.,* 2016). A high in GSH observed generally accompanied by a concomitant fall in GSSG and increased in the ratio of GSH/GSSG. So, we think that curcumin causes improvement on the antioxidant defense potential of liver tissues.
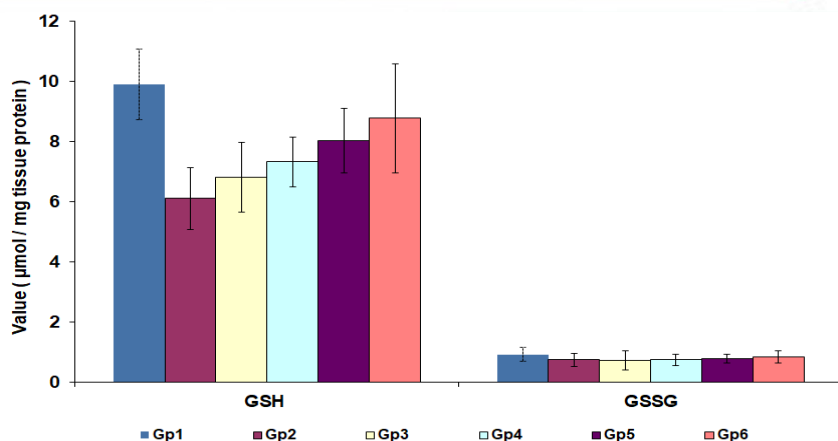
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Figure 4. Effect of curcumin on liver tissue glutathione fractions levels (µmol/mg tissue protein) changes induced by B[*a*]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.
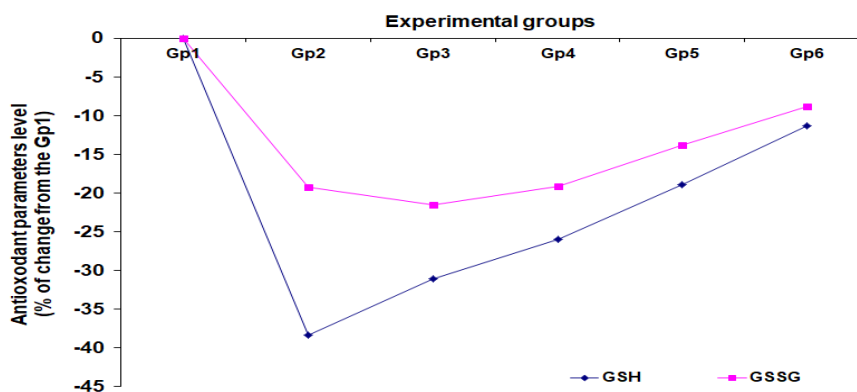

Figure 5. Effect of curcumin on liver tissue glutathione fractions levels (% of control from the Gp1) changes induced by B[*a*]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.

Effect of curcumin on serum immunological parameters levels changes induced by B[*a*]P in rats

Data presented in Table (4) and Figures (6-7) showed effect of feeding curcumin on some immunological parameters (albumin level and protease activity) in rats serum treated with B[*a*]P. From such data it could be noticed that treatment of animals with B[*a*]P caused a significant decreased ($p \leq 0.05$) in albumin level (-25.83%) and protease activity (-29.24%) compared to normal controls. Supplementation of the rat diets with curcumin (500 to 2000 mg/kg w/w) prevented the rise of mean serum albumin level and protease activity. The rate of preventative was increased with the increasing of the curcumin quantity. The rate of increasing in the serum albumin level and protease activity were recorded -21.35, -18.09, -11.51 and -5.61% (for albumin) and -24.46, -18.65, -15.59 and -14.54% (for protease activity) with the rat diets supplemented by 500, 1000, 1500 and 2000 mg/kg of curcumin, respectively. The present data are in

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Table 4. Effect of curcumin on serum immunological parameters levels changes induced by B[*a*]P in rats

Value	Gp1 Control (-) Basal diet (BD)	Gp2 Control (+) BD + B(*a*)P	Curcumin treatment groups [BD+ B(*a*)P+ curcumin]			
			Gp3 500 mg/kg	Gp4 1000 mg/kg	Gp5 1500 mg/kg	Gp6 2000 mg/kg
Albumin concentration (g/dl)						
Mean ± SD	3.91± 0.21 [a]	2.90 ± 0.14 [c]	3.08 ± 0.31 [ab]	3.20 ± 0.29 [ab]	3.46 ± 0.33 [ab]	3.69 ± 0.28 [a]
% of Change	0.00	-25.83	-21.35	-18.09	-11.51	-5.61
Protease activity (PA, U/L)						
Mean ± SD	3.53 ± 0.46 [a]	2.50 ± 0.44 [abc]	2.66 ± 0.51 [ab]	2.87 ± 0.42 [ab]	2.98 ± 0.23 [ab]	3.02 ± 0.71 [ab]
% of Change	0.00	-29.24	-24.46	-18.65	-15.59	-14.54

[*] Means in the same row with different letters are significantly different at p≤0.05
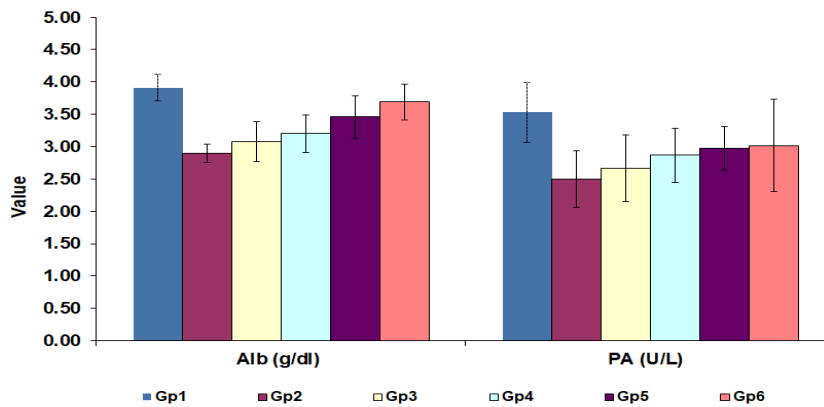

Figure 6. Effect of curcumin on serum immunological parameters [albumin concentration (Alb, g/dl) and protease activity (PA, U/L)] levels changes induced by B[*a*]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.
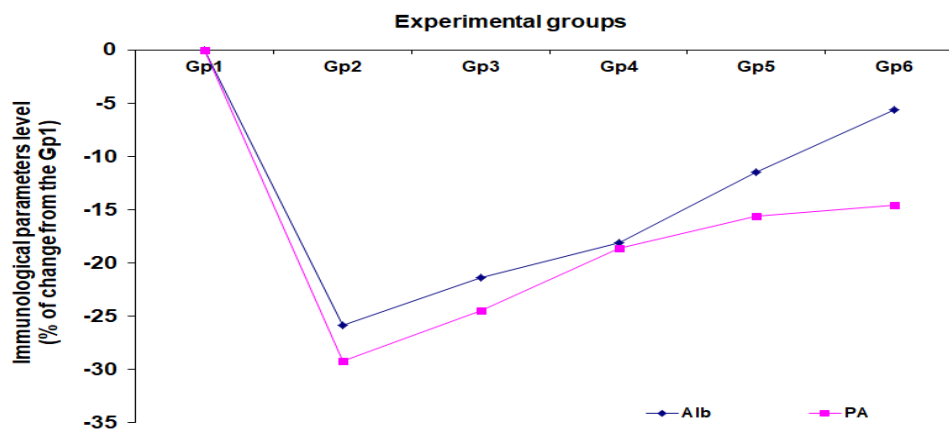

Figure 7. Effect of curcumin on serum immunological parameters [albumin concentration (Alb) and protease activity (PA)] levels (% of control from the Gp1) changes induced by B[*a*]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.

accordance with that observed by Wang *et al.*, (2007) who found that B[*a*]P induced significant decrease in the serum albumin content of experimental animals. Such as mentioned by Elhassaneen *et al.,* (2016-b) hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases. Subsequently, decline in albumin can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases.

Regarding the serum protease activity, numerous studies have reported that proteases could play an important role in immunological functions and humoral host defense (Neurath ,1989; Troll and Kennedy, 1993 and Almaadawy *et al.,* 2016). Other studies outlining the direct relationship between protease expression and protozoal virulence have implicated proteases as being involved in pathogenicity (Wilson *et al.*, 1989; Keene *et al.*, 1989). In our previous studies, protease activity was found decreased as noticed in the present study. For example, Elhassaneen *et al.*, (1997) and Elhassaneen, (2001) reported that a significant decreasing in the protease activity of fish isolated liver cells as a consequence of exposure to different categories of pollutants including paper industry effluent, pesticides and B[*a*]P. The present study proved that curcumin exhibited therapeutic effects against B[*a*]P through removal some of the immunotoxic effects such improving serum albumin levels and protease activity.

Effect of curcumin on biological oxidants changes induced by B[*a*]P in rats: serum and liver tissues malondialdehyde (MDA) and nitrite (NO$_2$) content

Data presented in Table (5) and Figures (8-9) showed effect of feeding curcumin on some biological oxidants changes in rats serum treated with B[*a*]P. From such data it could be noticed that treatment of animals with B[*a*]P caused a significant decreased ($p \leq 0.05$) in MDA concentration -39.43%) and NO$_2$ concentration (-23.55%) compared to normal controls. Supplementation of the rat diets with curcumin (500 to 2000 mg/kg w/w) increased the rise of mean serum MDA and NO$_2$ concentrations. The rate of rising was increased with the increasing of the curcumin quantity. The rate of increasing in the serum MDA and NO$_2$ were recorded -33.79, -32.25, -26.36 and -24.53% (for MDA concentration) and -20.65, -17.75, -15.22 and -9.06% (for NO$_2$ concentration) with the rat diets supplemented by 500, 1000, 1500 and 2000 mg/kg of curcumin, respectively.

The opposite direction was observed for the effect of feeding curcumin on some biological oxidants changes in rats liver tissues treated with B[*a*]P such as shown in Table (6) and Figures (10-11). From such data it could be noticed that treatment of animals with B[*a*]P caused a significant increased ($p \leq 0.05$) in MDA concentration (249.43%) and NO$_2$ concentration 186.14%) compared to normal controls. Supplementation of the rat diets with curcumin (500 to 2000 mg/kg w/w) decreased the rise of mean serum MDA and NO$_2$ concentrations. The rate of rising was decreased with the increasing of the curcumin quantity. The rate of decreasing in the liver tissue MDA and NO$_2$ were recorded 201.53, 102.40, 86.00 and 47.43% (for MDA concentration) and 111.39, 67.57, 50.00 and 41.83% (for NO$_2$ concentration) with the rat diets supplemented by 500, 1000, 1500 and 2000 mg/kg of curcumin, respectively.

In similar studies, lab evidences for B[*a*]P-associated oxidative stress have been provided by measurement of either biomarkers or end-products of free radical-mediated oxidative processes (Hasegawa *et al.,* 1995 and Fayez, 2015). For instance, lipid peroxidation markers such as malondialdehyde (MDA), major products of the oxidation of polyunsaturated fatty acids, lipid hydroperoxides and conjugated dienes

Table 5. Effect of curcumin on serum malondialdehyde (MDA) and nitrite (NO$_2$) contents induced by B[a]P in rats

Value	Gp1 Control (-) Basal diet (BD)	Gp2 Control (+) BD + B(a)P	Curcumin treatment groups [BD+ B(a)P+ curcumin]			
			Gp3 500 mg/kg	Gp4 1000 mg/kg	Gp5 1500 mg/kg	Gp6 2000 mg/kg
MDA concentration (nmol/ml)						
Mean ± SD	0.168 ± 0.02 [a]	0.102 ± 0.01 [c]	0.111 ± 0.054 [b]	0.114 ± 0.060 [b]	0.124 ± 0.043 [b]	0.127 ± 0.045 [b]
% of Change	0.00	-39.43	-33.79	-32.25	-26.36	-24.53
NO$_2$ concentration (nmol/L)						
Mean ± SD	2.76 ± 0.54 [a]	2.11± 0.32 [abc]	2.19 ± 0.434 [ab]	2.27 ± 0.220 [ab]	2.34 ± 0.493 [ab]	2.51 ± 0.625 [a]
% of Change	0.00	-23.55	-20.65	-17.75	-15.22	-9.06

[*] Means in the same row with different letters are significantly different at p≤0.05
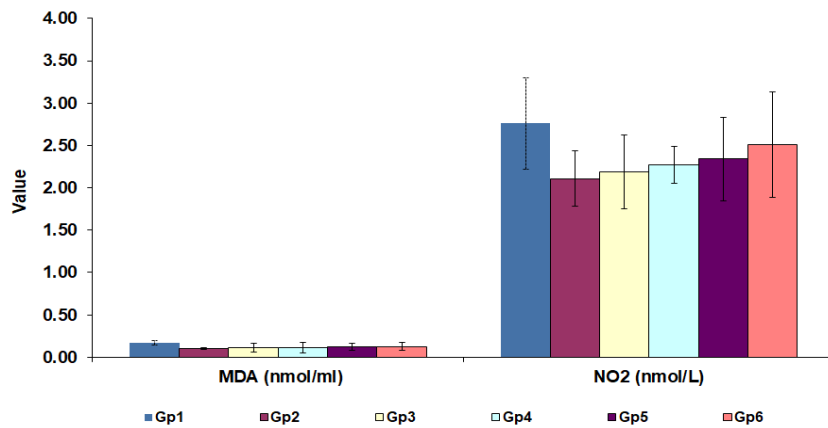

Figure 8. Effect of curcumin on serum malondialdehyde (MDA, nmol/ml) and nitrite (NO$_2$, nmol/L) contents changes induced by B[a]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.
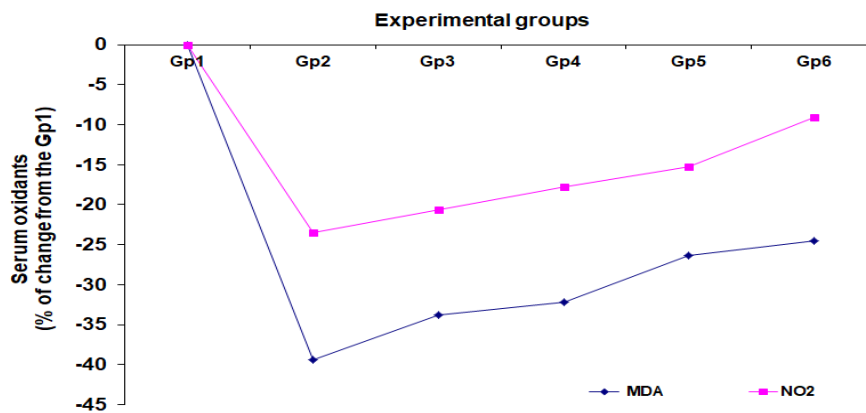

Figure 9. Effect of curcumin on serum malondialdehyde (MDA) and nitrite (NO$_2$) contents [% of control from the Gp1] changes induced by B[a]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.

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Table 6. Effect of curcumin on liver tissue malondialdehyde (MDA) and nitrite (NO$_2$) contents changes induced by B[a]P in rats

Value	Gp1 Control (-) Basal diet (BD)	Gp2 Control (+) BD + B(a)P	Curcumin treatment groups [BD+ B(a)P+ curcumin]			
			Gp3 500 mg/kg	Gp4 1000 mg/kg	Gp5 1500 mg/kg	Gp6 2000 mg/kg
MDA concentration (nmol/mg tissue protein)						
Mean ± SD	3.55 ± 0.32 [d]	12.42 ± 1.13 [a]	10.72 ± 1.05 [a]	7.19 ± 0.97 [b]	6.61± 0.85 [bc]	5.24 ± 0.95 [c]
% of Change	0.00	249.43	201.53	102.40	86.00	47.43
NO$_2$ (nmol/g tissue protein)						
Mean ± SD	4.04 ± 0.63 [d]	11.56 ± 2.11 [a]	8.54 ± 1.45 [b]	6.77 ± 1.29 [c]	6.06 ± 0.99 [c]	5.73 ± 1.18 [c]
% of Change	0.00	186.14	111.39	67.57	50.00	41.83

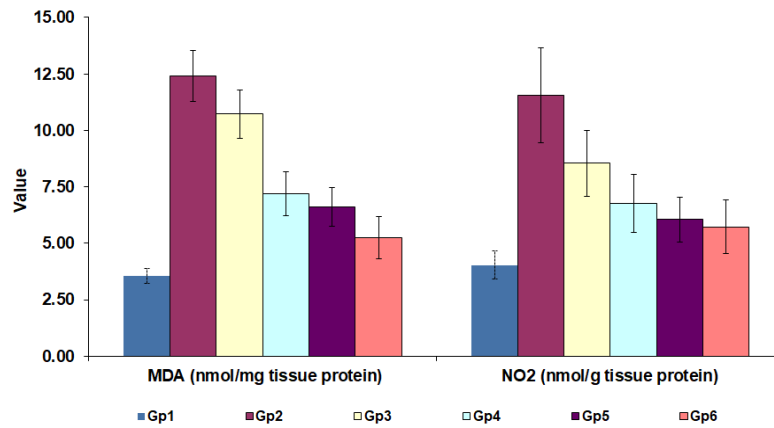[*] Means in the same row with different letters are significantly different at p≤0.05

Figure 10. Effect of curcumin on liver tissue malondialdehyde (MDA, nmol/mg tissue protein) and nitrite (NO$_2$, nmol/g tissue protein) contents changes induced by B[a]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.
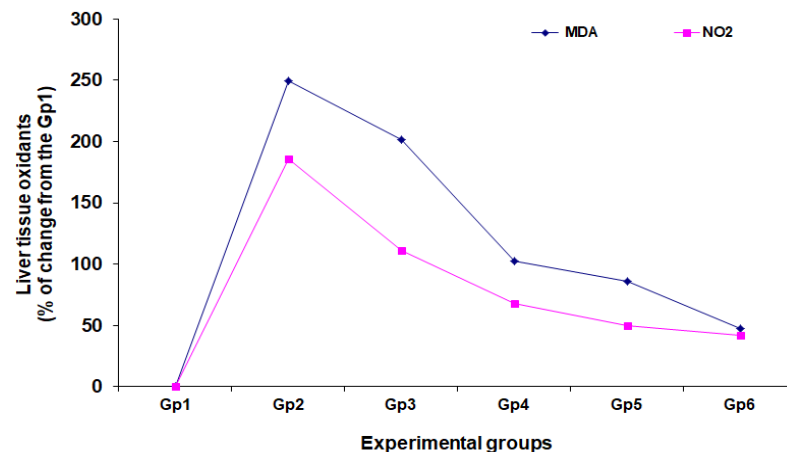

Figure 11. Effect of curcumin on liver tissue malondialdehyde (MDA) and nitrite (NO$_2$) contents [% of control from the Gp1] changes induced by B[a]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.

are found to be increased in plasma from rats treated with B[a]P (Fayez, 2015). Also, the same behavior was recorded for reactive nitrogen substances (RNS) including nitric oxides such NO_2. Systemic metabolic alterations associated with B[a]P contribute to the increase in oxidative stress have been reported by Elhassaneen (1996).

Several decades ago, interest in the possible significance of MDA on human health has been stimulated by reports that are mutagenic and carcinogenic compound (Shamberger *et al*., 1974 and Mukai and Goldstein, 1976)). Nitric oxide synthase catalyzes the conversion of L-arginine to citrulline and highly reactive free radical species, nitric oxide (NO) (Manahan, 1989). Nitric oxide, in turn, can react with molecular oxygen and water to form nitrite and nitrate; with hemoglobin to form iron-nitrosyl adducts and/or nitrate in blood, with superoxide anion to make nitrate, and with the amino and thiol groups of protein to produce nitrosylated species (Manahan, 1989; Misko *et al*., 1993). The excess production of nitric oxides has been implicated in the pathogenesis and tissue destruction of a growing number of immunological and inflammatory diseases including septic shock, arthritis, graft rejection and diabetes (Jacob *et al*., 1992).

The present data proved that consumption of curcumin clearly protected against the changes in liver lipid peroxide level. On the other hand, serum lipid peroxide levels dropped in all B[a]P-treated animals. Such data with the others (Hasegawa *et al.,* 1995) suggested that secretion of lipoprotein from liver to blood might be blocked because of intracellular structural failure and/or because of the energy depletion suggested by the marked decrease in glycogen content. The increases of serum enzyme activities were clearly inhibited by curcumin at this time. A marked fall in serum triglyceride levels was noted in all B[a]P treated animals (See Tables 2-5 and Figures 5-8), probably being related to the low levels of serum lipid peroxide in all treated groups.

Regarding the RNS, Endothelial NO synthase- (eNOS-) and inducible NO synthase- (iNOS-) dependent NO are abundant in adipocytes. iNOS expression has been shown to be increased in white adipose tissue (WAT) derived from diet-induced or genetic models of obesity (Perreault and Marette, 2001). Similarly, both eNOS and iNOS are expressed at higher levels in WAT from obese patients compared to lean controls (Elizalde *et al.,* 2000 and Engeli *et al.,* 2004).

Such data are in accordance with that observed by Fayez, (2015). Also, Hasegawa *et al.,* (1995) found that previous drinking of green tea (containing polyphenolic compounds such curcumin) clearly protected against the changes in liver lipid peroxide level. On the other hand, serum lipid peroxide levels dropped in all B[a]P-treated animals. Such data with the others (Hasegawa *et al.,* 1995) suggested that secretion of lipoprotein from liver to blood might be blocked because of intracellular structural failure and/or because of the energy depletion suggested by the marked decrease in glycogen content. The increases of serum enzyme activities were clearly inhibited by TRP at this time. A marked fall in serum triglyceride levels was noted in all B[a]P treated animals (See tables 2-5 and Figures 5-8), probably being related to the low levels of serum lipid peroxide in all treated groups.

Effect of curcumin on liver tissue glycogen concentration changes induced by B[a]P in rats

Liver glycogen content of rats treated with B[a]P and consumed curcumin powders were shown in Table (7) and Figures (12-13). From such data it could be noticed that the liver glycogen content was decreased -86.64% by B[a]P, and this

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Table 7. Effect of curcumin on liver tissue glycogen concentration (mg/g tissue) changes induced by B[a]P in rats

Value	Gp1 Control (-) Basal diet (BD)	Gp2 Control (+) BD + B(a)P	Curcumin treatment groups [BD+ B(a)P+ curcumin]			
			Gp3 500 mg/kg	Gp4 1000 mg/kg	Gp5 1500 mg/kg	Gp6 2000 mg/kg
Mean ± SD	13.45 ± 1.02 [a]	1.80 ± 0.33 [e]	2.73 ± 0.45 [d]	4.14 ± 0.67 [bc]	5.81 ± 1.15 [bc]	7.95 ± 1.55 [b]
% of Change	0.00	-86.64	-79.71	-69.21	-56.82	-40.86

[*] Means in the same row with different letters are significantly different at p≤0.05
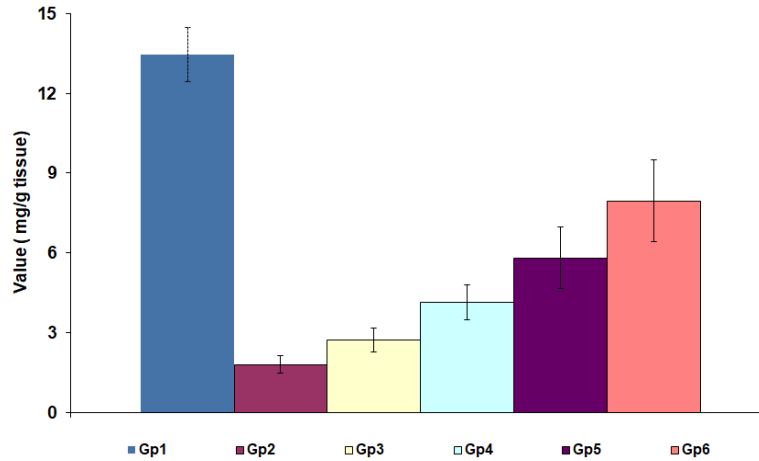

Figure 12. Effect of curcumin on liver tissue glycogen concentration (mg/g tissue) changes induced by B[a]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.
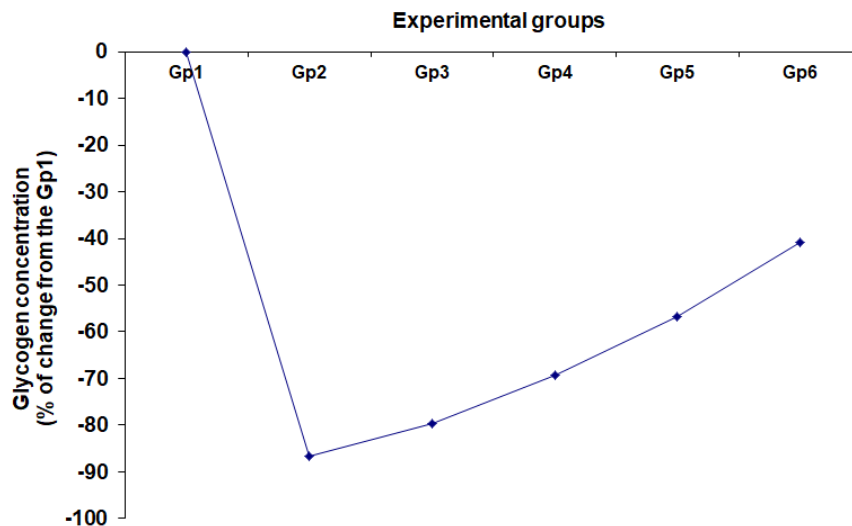

Figure 13. Effect of curcumin on liver tissue glycogen concentration (% of control from the Gp1) changes induced by B[a]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.

decrease was significantly elevated in the B[*a*]P+curcumin powders. The rate of glycogen elevation was increased with the increasing of the curcumin powders quantity. The rate of increasing in liver tissue glycogen was recorded -79.71, -69.21, -56.82 and -40.86 with the rat diets supplemented by 500, 1000, 1500 and 2000 mg/kg of curcumin powder, respectively. These data indicated that curcumin was induced an effective role in manipulation of the liver glycogen content induced by B[*a*]P in rats. In similar study carried out by Hasegawa *et al.,* (1995) found that previous drinking of green tea (containing polyphenolic compounds like curcumin) clearly protected against the changes in liver glycogen content. Such data with the others (Hasegawa *et al.,* 1995) and Fayez (2015) suggested that secretion of lipoprotein from liver to blood might be blocked because of intracellular structural failure and/or because of the energy depletion suggested by the marked decrease in glycogen content.

Effect of curcumin on serum triglyceride concentration changes induced by B[*a*]P in rats

Serum triglycerides (TG) level of rats treated with B[*a*]P and consumed curcumin powders were shown in Tables (8) and Figures (14-15). From such data it could be noticed that the TG content was decreased -79.98% by B[*a*]P, and this decrease was significantly elevated in the B[*a*]P+ curcumin powders. The rate of TG elevation was increased with the increasing of the curcumin powders quantity. The

Table 8. Effect of curcumin on serum triglyceride concentration (mg/dl) changes induced by B[a]P in rats

Value	Gp1 Control (-) Basal diet (BD)	Gp2 Control (+) BD + B(*a*)P	Curcumin treatment groups [BD+ B(*a*)P+ curcumin]			
			Gp3 500 mg/kg	Gp4 1000 mg/kg	Gp5 1500 mg/kg	Gp6 2000 mg/kg
Mean ± SD	147.91± 11.168 [a]	29.61 ± 4.788 [abc]	33.23 ± 5.579 [ab]	39.91 ± 7.972 [ab]	42.11 ± 6.132 [b]	49.91 ± 8.219 [b]
% of Change	0.00	-79.98	-77.53	-73.02	-71.53	-66.26

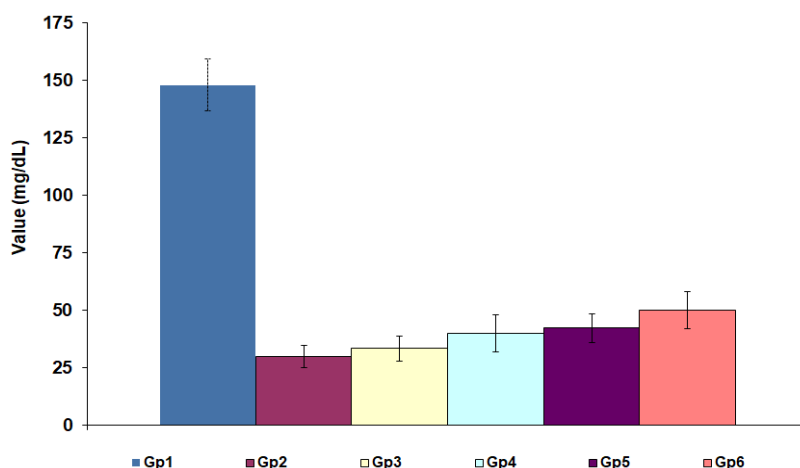[*] Means in the same row with different letters are significantly different at p≤0.05

Figure 14. Effect of curcumin on serum triglyceride concentration (mg/dl) changes induced by B[*a*]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.

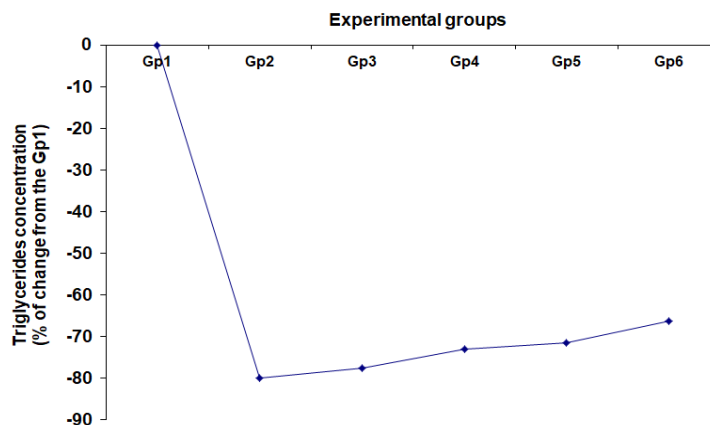**Yousif Elhassaneen, Heba Ghamry and Lamiaa Lotfy**

Figure 15. Effect of curcumin on serum triglyceride concentration (% of control from the Gp1) changes induced by B[*a*]P in rats

Gp1, control (-) group; Gp2, control (+) group; Gp3, 500 mg/kg curcumin treatment group; Gp4, 1000 500 mg/kg curcumin treatment group; Gp5, 1500 mg/kg curcumin treatment group; Gp6, 2000 mg/kg curcumin treatment group.

rate of increasing in liver tissue glycogen was recorded -77.53, -73.02, -71.53 and -66.26% with the rat diets supplemented by 500, 1000, 1500 and 2000 mg/kg of curcumin powder, respectively. These data indicated that curcumin was induced an effective role in manipulation of the serum TG content induced by B[*a*]P in rats. In similar study carried out by Hasegawa e*t al.,* (1995) found that previous drinking of green tea clearly protected against the changes in serum triglycerides level. Also Fayez (2015) found that curcumin lowers the atherogenic risks by reducing the insulin resistance, triglyceride, visceral fat and total body fat.

Correlation studies

In the correlation analysis, important differences were found between biological oxidant, biological antioxidant, immunological parameters, glycogen and triglycerides content in liver/serum of rats feeding curcumin and treated with B[*a*]P (Table 9). From such data it could be noticed that there was a strong negative significant (p≤ 0.05) relationship between GSH fractions (GSH and GSSG) and MDA (r^2 =- 0.9371 and - 0.8943, respectively) and NO$_2$ (r^2 = - 0.9064 and - 0.9064, respectively) concentrations in liver tissues. Those correlations confirm that if there were no change in the biological antioxidant defense system of B[a]P treated rats, it would be difficult to observe high concentrations of MDA. In similar study, Fayez, (2016) reported that high levels of MDA in the plasma of rats treated with B[*a*]P were associated with rather low levels of different oxidative parameters including GSH. Also, a combination of bioactive compounds in turmeric including curcumin interact synergistically to inhibit lipid peroxidation subsequently increased MDA (Sello and Eldemery, 2017). On the same time, high negative significant (p≤ 0.05) relationship between immunological parameters (Alb and PA), glycogen and TG and oxidants parameter (MDA and NO$_2$) concentrations were observed in liver tissues. Those relationships confirmed that curcumin was induced an effective roles in manipulation of the liver glycogen content and serum TG level induced by B[*a*]P in rats. Such relationships were confirmed by the study of Hasegawa

et al., (1995) who reported that drinking of green tea (containing polyphenolic compounds like curcumin) clearly protected against the changes in liver glycogen content. Also, Fayez (2015) secretion of lipoprotein from liver to blood might be blocked in rats consumed turmeric powder including curcumin because of intracellular structural failure and/or the energy depletion suggested by the marked decrease in glycogen content.

Table 9. Correlation between biological oxidant, biological antioxidant, immunological parameters, glycogen and triglycerides content in liver/serum of rats feeding curcumin and treated with B[*a*]P

Parameters	R^{2*}	Parameters	R^2
MDA (liver)/GSH (liver)	- 0.9371	NO_2 (liver)/GSH (liver)	- 0.9064
MDA (liver)/GSSG (liver)	- 0.8943	NO_2 (liver)/GSSG (liver)	- 0.8710
MDA (liver)/Glycogen (liver)	- 0.7954	NO_2 (liver)/Glycogen (liver)	- 0.7895
MDA (liver)/TG (serum)	- 0.8554	NO_2 (liver)/TG (serum)	- 0.8399
MDA (liver)/Alb (serum)	- 0.7182	NO_2 (liver)/Alb (serum)	- 0.7349
MDA (liver)/PA (serum)	- 0.7693	NO_2 (liver)/PA (serum)	- 0.7852

* P ≤ 0.05

Conclusion

B[*a*]P is considered as a ubiquitous environmental and food contaminants and a top risk factor in the development of several diseases including liver disorders/toxicity. Oxidative stress appears as a major contributor in the development of many metabolic complications associated hepatotoxicity. Lowering oxidative stress to prevent such metabolic disorders and complications therefore constitutes an interesting target. Dietary curcumin has been proven to be essential in the treatment and/or prevention of liver disorders/toxicity through modulating liver serum enzymes activity, formation of liver MDA and NO_2 and serum immunological parameters . So, we recommended curcumin by a concentration up to 2000 mg/kg to be included in our daily diets, drinks and food products.

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Yousif Elhassaneen, Heba Ghamry and Lamiaa Lotfy

الوقاية الكيميائية المحتملة من اضطرابات الكبد بواسطة الكركمين الغذائي فى الفئران المعاملة بالبنزوبيرين

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الملخص

يعد الكركم من المركبات الطبيعية التت توجد بتركيزات عالية فى ريزومات نبات الكركم (Curcuma longa L.) والذى ينتمى الى العائلة النباتية الزنجبيلية (Zingiberaceae) ويتميز بإمتلاكه للخصائص المضادة للالتهابات والأكسدة، كما تم اختباره للخصائص المضادة للالتهاب الكيميائي في الجلد ، وسرطانات المعدة والاثنى عشر والقولون. لذلك تم تصميم الدراسة الحالية للتحقيق في الوقاية الكيميائية المحتملة من اضطرابات الكبد عن طريق الكركمين الغذائي فى الفئران المعاملة بالبنزوبيرين، وذلك من خلال التأثيرات المعدلة التى يحدثها الكركمين على وظائف الكبد، وحالة المؤكسدات ومضادات الأكسدة ، والمقاييس المناعية في ذكور الفئران البيضاء (وزن 150 ± 8.7 جرام/فأر). لذلك تم تغذية مجموعات من الحيوانات على العلائق الضابطة (القياسية) أو علائق غذائية قياسية تحتوى على 500–2000 ملليجرام/كيلوجرام من الكركمين. وبعد إنقضاء أسبوعين من التغذية تم حقن جميع الحيوانات وباستثناء تلك الموجودة في المجموعة الضابطة السالبة (المجموعة التى تم معاملتها بالمادة الحاملة للمادة المحدثة للسمية الكبدية– المحلول الملحي العادي)، بالبنزوبيرين مرتين فى الأسبوع بجرعة 15 مجم / 5 مل / كجم من وزن الجسم لإحداث الإضطرابات/السمية الكبدية، ثم استمرت جميع المجموعات على النظام الغذائي الخاص بكل منها حتى نهاية التجربة التى استمرت 12 أسبوعا. ولقد أوضحت النتائج أن معاملة الفئران بالبنزوبيرين قد تسببت فى زيادة معنوية (p≤0.01) فى نشاط انزيمات الكبد ALT, AST, ALP وذلك بنسب 67,85 ، 110,38 ، 116,47 مقارنة بالمجموعة الضابطة الطبيعية. كما أدى خلط وجبات الفئران بالكركمين بتركيزات 500، 1000، 1500، 2000 ملليجرام/كيلوجرام قد أحدث إنخفاضا معنويا (p≤0.05) فى درجة نشاط تلك الإنزيمات وبمعدلات مختلفة تزايدت مع زيادة درجة الكركمين. ولقد سجل نفس السلوك لمستويات مركب المالونالدهيد وأكسيد النيتروز فى أنسجة الكبد والتى تعد مؤشرات حيوية على الإجهاد التأكسدى والإلتهابات فى أنسجة الجسم المختلفة وكذلك بعض المؤشرات المناعية الهامة مثّل مستوى الألبيومين ونشاط انزيم البروتيز فى السيرم. كما سجل سلوكا معاكسا فيما يتعلق بجزيئات الجلوتاثيون (الجزيئات الحيوية المضادة للأكسدة) فى الكبد. ولعل نتائج الدراسة الحالية تؤيد الفرضية أن الكركمين الغذائى له القدرة على منع أو تثبيت السمية الكبدية للبنزوبيرين وذلم من خلال تعديل الخطوط المتعلقة بنشاط إنزيمات الكبد، تكوين مركبات المالونالدهيد وأكسيد النيتروز، مستوى بعض المؤشرات المناعية الهامة فى السيرم. لذلك توصى الدراسة الحالية بإستخدام الكركم بتركيزات حتى 2000 ملليجرام/كجم فى وجباتنا ومشروباتنا اليومية وكذلك كإضافات طبيعية لبعض المنتجات الغذائية.

الكلمات المفتاحية: الكركم ، الكركمين، أنسجة الكبد، وظائف الكبد ، الجلوتاثيون، المؤشرات المناعية، المالونالدهيد، أكسيد النتريت.

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