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Protective effects of curcumin during benzo(*a*)pyrene induced liver toxicity and carcinogenicity in rats

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Abstract: Phytochemicals are chemicals produced by plants through primary or secondary metabolism. It is one such family of bioactive agents that is being researched extensively the world over for its effectiveness against several cancer pathways. Curcumin belong to the family of phytochemicals and have antioxidative and anticarcinogenic properties. In the present study, chemopreventive efficacy of curcumin was investigated against Benzo[*a*]pyrene (BP), induced liver toxicity and carcinogenicity. The BP treatment resulted in a significant increase ($p \leq 0.05$) in liver functions enzymatic activity and lipid peroxidation (malonaldialdehyde, MDA) levels. The activities of antioxidant enzymes (superoxide dismutase, GSH-Px; superoxide dismutase, SOD and catalase, CAT) and glutathione (GSH) fractions were found to be significantly decreased ($p \leq 0.01$) following BP treatment. Further, BP treatment brought a significant increase ($p \leq 0.05$) in the activities of drug metabolizing enzymes (cytochrome P450, Cyt P450). Supplementation of the rat diets with curcumin (0.1 to 4.0 g/100g w/w) was able to decrease significantly ($p \leq 0.05$) the levels of MDA and increase significantly ($p \leq 0.05$) activities of antioxidant enzymes. Also, the activity of drug metabolizing enzyme (cytochrome p450) was markedly decreased by the feeding of curcumin. The results of this study suggest that treatment with curcumin proved beneficial on antioxidant status and drug metabolizing enzymes during experimentally induced liver toxicity and carcinogenicity in rats. Therefore, we recommended curcumin by a concentration of about 0.4% to be included in our daily diets, drinks and food products.

Keywords: Curcumin, lipid peroxidation, antioxidant enzymes, drug metabolizing enzymes, cytochrome P450 enzyme.

Introduction

The latest statistics reveal that cancer is now not only a leading cause of death worldwide, but that liver cancer is one of the deadliest forms. Indeed, liver cancer is the second most common cause of death from cancer worldwide, accounting for 746,000 deaths globally in 2012 (Ferlay *et al.,* 2012). Statistics on liver cancer show that 83 per cent of liver cancer cases occur in less developed countries, with the highest incidence rates in Asia and Africa. Because liver cancer is an umbrella term for many types of cancer, the signs and symptoms depend on what type of cancer is present. Hepatocellular carcinoma (HCC) is a common malignancy and now the second commonest global cause of cancer death. HCC is associated with abdominal mass, abdominal pain, emesis, anemia, back pain, jaundice, itching, weight loss and fever (Jin *et al.,* 2016).

Primary liver cancer (HCC) tends to occur in livers damaged by birth defects, alcohol abuse, or chronic infection with diseases such as hepatitis B and C, hemochromatosis (a hereditary disease associated with too much iron in the liver), and cirrhosis. More than half of all people diagnosed with primary liver cancer have cirrhosis, a scarring condition of the liver commonly caused by alcohol abuse. Hepatitis B and C and hemochromatosis can cause permanent damage and liver failure. Liver cancer may also be linked to obesity and fatty liver disease (NCI , 2009 and WCRFI, 2015). On the other side, many studies indicated that exposure to different classes of toxic chemicals such polycyclic aromatic hydrocarbons (PAH) is associated with the development of liver cancer in all vertebrata including human (Harvey,v1985; Plakunov *et al.,* 1987 and Hawkins *et al.*, 1990).

In the present study, benzo(a)pyrene (BP), the prototype and one of the most extensively studied toxic and carcinogenic PAH, was used to instigate liver carcinogenesis in rats. BP is a major carcinogenic pollutant5 and has been shown to be toxic, mutagenic and/or carcinogenic by extensive experiments *in vivo* (Harvey, 1985; Hawkins *et al.*, 1990 and *Karle et al., 2004*) and *in vitro* (Elhassaneen, 1996; and Elhassaneen, 2002) systems. BP was first isolated and characterized by Cook and his colleagues in the year 1933 and is formed when gasoline, garbage or any plant or animal materials burn incompletely (Cook *et al., 1933).* Afterthat,

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).

For early-stage of the disease, surgical resection and radiation ablation is currently the mainstay of liver cancer therapy. Though, chemotherapy for treating cancerous cells is often successful in killing the malignant cells but it often causes intense side effects in the body (Muhammad *et al.,* 2014). Researchers all over the world are busy in investigating several substances which have the potential to inhibit the molecular events leading to the occurrence of cancer and are called chemopreventive agents. The term of chemoprevention was coined by Sporn *et al.,* (1970) and is expressed as the use of chemicals or dietary components to block, inhibit, or reverse the development of cancer in normal or preneoplastic tissue. In the present study we will use curcumin as a phytochemical.

Curcumin, a polyphenol compound, is responsible for the yellow color of turmeric and is thought to be the most active pharmacological agent. Natural curcumin, isolated from *Curcuma longa*, 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 (Aggarwal, 2003). It 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). 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 (Reviewed in Fayez, 2016). 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 and vascular endothelial growth factor (Kavirayani, 2014)). According to our knowledge, the studies regarding the potential effects of curcumin on liver disease/cancer are so limited. Therefore, in this study, the

potential protective effects of curcumin during benzo(*a*)pyrene induced liver toxicity and carcinogenicity in rats was investigated.

Materials and Methods

Materials

Curcumin and BP were purchased from Sigma Chemical Co. (St. Louis, MO, Company agent, Cairo, Egypt).). Casein was obtained from Morgan Chemical Co., Cairo, Egypt. All organic solvents, buffers and other chemicals of analytical grade were purchased from El-Ghomhorya Company for Trading in Drug, 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.

Biological Experiments

Animals

Animals used in this study, adult male albino rats (160±9.35 g per each) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Basal Diet

The basic diet prepared according to the following formula as mentioned by (AIN, 1993) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral 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, (1963) while the salts mixture used was formulated according to Hegsted, (1941).

Experimental design

All biological experiments performed a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 1996). Rats (n=36 rats) were housed individually in wire cages in a room maintained at 25 ± 2 ^{0}C, relative humidity (55±5%), a 12-hr lighting cycle and kept under normal

healthy conditions. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, rats were segregated into six treatment groups. Animals in Group (1) served as normal controls and were administered with corn oil intraperitoneally (IP), which was used as a vehicle for the treatment of animals in BP group.Animals in Group (2) were given a single injection (IP) of BP at a dose level of 100 mg/kg body weight dissolved in corn oil according to Gunning et al., (2003). Groups (3-6) rats were given curcumin feeding in diet at a dose level of 0.1, 0.2, 0.3 and 0.4%, w/w. The treatment with curcumin to the animal belonging to groups (3) to (6) was started 14 days prior to BP injection. All the rats had free access to the diet and water and the treatments continued for a total duration of 8 weeks.

Blood collection of and preparation of liver homogenate

At the end of the study, the animals were anaesthetized using mild ether anesthesia and the blood samples were drawn from the animals belonging to all the groups by puncturing the ocular vein (retro-orbital plexus), using fine sterilized capillaries. Thereafter, serum was separated by centrifugation and stored at -40°C until analyses for enzyme activities of AST, ALT and ALP. The rats were then sacrificed; livers were removed immediately and washed with ice-chilled saline. 10% lung homogenates were prepared in ice cold Tris buffer (pH 7.4) (Sigma, ST. Louis, MO) by using mechanically driven Teflon fitted Potter-Elve- jhem type homogenizer for a few minutes till the total disruption of cells. Homogenates were centrifuged at 1.000 g for 10 minutes at 4°C. Pellets were discarded and the supernatants were used for the estimation of lipid peroxidation and reduced glutathione levels. A portion of the above supernatants were again centrifuged at 10.000 g for 20 minutes to obtain post mitochondrial fractionvwhich were utilized for the rest of biochemical estimations (Stroev and Makarova, 1989 and Liu *et al., 2015*).

Hematological analysis

Liver functions

Serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT), and serum alkaline phosphatase (ALP) activities were measured in serum using the modified kinetic method of Tietz *et al.*, (1976) and Vassault *et al.*, (1999), respectively.

Glutathione fractions

GSH and GSSG were determined by HPLC according to the method of McFarris and Reed (1987). In brief, 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-carbooxymethyl 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.

Antioxidant enzymes

GSH-Px and CAT activities were measured as described (Splittgerber and Tappel, 1979, and Aebi, 1974, respectively). SOD activity was measured by Ransod kit (Randox laboratories mmited, Germany). Activities of SOD and GSH-Px enzymes were expressed in international unit per milliliter erythrocyte sediment and one unit of SOD was expressed as the enzyme protein amount causing 50% inhibition in 2-(4-iodophenyl)-3 (4-nitrophenol) 5-phenyltetrazolium chloride ($INTH_2$) reduction rate.

Malonaldialdehyde content (MDA)

Lipid peroxide levels measured as malondialdehyde in serum and liver were determined by as thiobarbituric acid reactive substances (TBARS) as described by Buege and Aust, (1978). Half milliliter of plasma were added to 1.0 ml of thiobarbituric acid reagent, consisting of 15% TCA, 0.375% thiobarbituric acid (TBA) and 0.01% butylated hydroxytoluene in 0.25 N HCl. Twenty-five microliters of 0.1 M $FeSO_4.7H_2O$ 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 malonaldialdehyde.

Drug Metabolizing Enzymes (Cytochrome P-450)

Cytochrome P-450 was measured by the carbon monoxide difference spectrophotometry of dithionite-reduced samples by using the method of Omura and Sato (1964).

Statistical Analysis

All measurements were done in triplicate and recorded as mean±SD. Statistical analysis was performed with the Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

Results and Discussion

Effects of curcumin feeding on BP-induced changes in liver functions of rats

Liver functions of rats injected BP and feeding curcumin were shown in Table (1). From such data it could be noticed that treatment of animals with BP caused a significant increased ($p \leq 0.01$) in AST (123.58%), ALT (76.26%) and ALP (105.47%) compared to normal control animals. Feeding of the rat diets with curcumin (0.1 to 0.4 g/100g w/w) prevented the rise of mean serum AST, ALT and ALP activities. The rate of preventative was increased with the increasing of the curcumin concentration. The rate of increasing in the liver enzymatic activities were recorded 80.74, 60.59, 29.44 and 19.41 % (For AST); 32.79, 22.93, 18.76 and 9.35% (for ALT) and 58.63, 44.76, 35.56 and 15.18% (for ALP) with the rat diets blended with 0.1, 0.2, 0.3 and 0.4 g/100g of curcumin, respectively.

In general, BP was commonly used as a hepatotoxin in the experimental study of liver diseases. The hepatotoxic effects of BP are largely due to the binding of its activated metabolites with the cellular macromolecules and induce peroxidative degradation of membrane lipids of cell wall membrane, mitochondria and lysosomes rich in polyunsaturated fatty acids (Elhassaneen, 1996). Such degradation of cellular membranes is one of the principle causes of hepatotoxicity of BP (Elhassaneen, 2004). This is confirmed by the elevation noticed in the serum 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 BP consumption in charcoal broiled meat for four weeks. Data of the present study with others reviewed that curcumin, a polyphenol compound, exhibit antioxidant, anti-inflammatory, antiviral,

antibacterial, antifungal, and anticancer activities and thus has a potential against various malignant diseases (Aggarwal *et al.,* 2007). 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 polyphenols compounds.

Table 1. Effects of curcumin feeding on BP-induced changes in liver functions of rats

Value	Control (-)	Control (+)	Curcumin (%, w/w)			
			0.1	0.2	0.3	0.4
Serum aspartate aminotransferase (AST,U/L)						
Mean	34.99^{c}	78.23^{a}	63.24^{b}	56.19^{b}	45.29^{c}	41.78^{c}
SD	5.32	8.29	10.01	6.04	3.67	5.82
% of Change	------	123.58	80.74	60.59	29.44	19.41
Serum alanine aminotransferase (ALT,U/L)						
Mean	60.65^{b}	106.9^{a}	80.54^{b}	74.56^{b}	72.03^{b}	66.32^{b}
SD	4.11	8.11	5.87	6.32	4.09	8.54
% of Change	------	76.26	32.79	22.93	18.76	9.35
Serum alkaline phosphatase (ALP,U/L)						
Mean	146.54^{c}	301.1^{a}	232.45^{b}	212.13^{b}	198.65^{bc}	168.78^{c}
SD	10.56	20.6	17.77	30.65	18.56	19.87
% of Change	------	105.47	58.63	44.76	35.56	15.18

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

For example, Dawson, (1998) reported that flavonoid is known to block the hepatocellular uptake of bile acids. Also, Beattic *et al.,* (2005) reviewed that flavonoids pretreatment improved the antioxidant capacity of the liver, diminished the bilirubin concentration compared with the groups without treatment subsequently reduced the elevated levels of the following serum enzymes, AST, ALT and ALP. El-Nashar, (2007) reported that pre-treatment with flavonoids were not only able to suppress the elevation of GOT and GPT but also reduce the damage of hepatocytes *in vitro*. Furthermore, Hassan (2011) found that pre-treatment with apricot kernel extract rich in phytochemicals including polyphenols were able to reduce the damage of liver i.e. suppress the elevation of AST, ALT and ALP through the improvement of antioxidant defense system in red blood cells.

Effect of curcumin on BP-induced changes in serum glutathione fractions levels of rats

Data presented in Table (2) showed effect of feeding curcumin on serum glutathione fractions content of rats treated with BP. From such data it could be noticed that treatment of animals with BP caused a significant decreased ($p \leq 0.05$) in GSH (-38.10%) and GSSG (-19.18%) compared to normal control animals. Blending of the rat diets with curcumin (0.1 to 0.4 g/100g w/w) prevented the rise of mean serum GSH and GSSG levels. The rate of preventative was increased with the increasing of the curcumin concentration. The rate of decreasing in the serum GSH fractions were recorded -26.14, -19.98, -14.67 and -11.34% (for GSH) and -8.22, -6.85, -4.11 and -2.74 % (for GSSG) with the rat diets blending with curcumin by the ratios of 0.1, 0.2, 0.3 and 0.4 g/100g of curcumin, respectively.

Table 2. Effect of curcumin on BP-induced changes in serum glutathione fractions levels of rats

Value	Control (-)	Control (+)	Curcumin (%, w/w)			
			0.1	0.2	0.3	0.4
Reduced glutathione concentration (GSH, µmol/L)						
Mean	8.11[a]	5.02[c]	5.99[bc]	6.49[b]	6.92[ab]	7.19[ab]
SD	2.09	1.76	0.88	1.14	0.99	2.15
% of Change	------	-38.10	-26.14	-19.98	-14.67	-11.34
Oxidized glutathione concentration (GSSG, µmol/L)						
Mean	0.73[a]	0.59[b]	0.67[a]	0.68[a]	0.70[a]	0.71[a]
SD	0.12	0.11	0.18	0.12	0.10	0.18
% of Change	------	-19.18	-8.22	-6.85	-4.11	-2.74
GSH/GSSG ratio						
Mean	11.11[a]	8.51[b]	8.94[ab]	9.54[ab]	9.89[a]	10.13[a]
SD	1.69	1.10	2.23	1.15	2.10	1.20
% of Change	------	-23.41	-19.53	-14.09	-11.02	-8.85

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

These data indicated that the rate of serum GSH elevation was increased with the increasing of the curcumin blending levels. GSH is a tripeptide-thiol (γ-glutamyl cysteinyl-glycine) that has received considerable attention in terms of its biosynthesis, regulation, and various intracellular functions (Reed and Beatty, 1980; Larsson *et al*., 1983).

Among of these functions, its role in detoxifications process represent the central role through as a key conjugate of xenobiotics (such BP) electrophilic intermediates (BP oxides, hydroxides, quinines etc) and as an important antioxidant. The antioxidant functions of GSH include its role in the activities of the antioxidant enzymes system (GSH-Px and GSH-Rd). In addition, GSH can apparently serve as a nonenzymatic scavenger of oxyradicals (Halliwell and Gutteridge, 1985 and Almaadawy *et al.,* 2016). Regarding GSSG, DiGiulio (1991) mentioned that plasma 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).

Effects of curcumin treatments on RBCs antioxidant enzymes activities of rats subjected to BP treatment

Antioxidant defense system in rats injected BP and feeding curcumin was assessed by measuring antioxidant enzymes activities including glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) (Table 3). From such data it could be noticed that treatment of animals with BP caused a significant increased ($p \leq 0.01$) in GSH-Px (-47.06 %), CAT (-30.13%) and SOD (-40.20%) compared to normal control animals. Feeding of the rat diets with curcumin (0.1 to 0.4 g/100g w/w) prevented the rise of mean serum GSH-Px, CAT and SOD activities. The rate of preventative was increased with the increasing of the curcumin concentration. The rate of increasing in the enzymatic antioxidant activities were -33.19, -28.90, -16.62 and -12.38% (For GSH-Px); -24.96, -21.71, -12.60 and -10.12% (for CAT) and -20.35, -18.36, -10.42 and -7.94 (for SOD) with the rat diets blended with 0.1, 0.2, 0.3 and 0.4 g/100g of curcumin, respectively.

To prevent free radical damages (oxidative stress activities), the organism has developed antioxidant defenses largely based on antioxidant enzymes able to scavenge ROS. GSH-Px, SOD, CAT, and the other enzymes involved in glutathione redox cycle are the primary intracellular antioxidant enzymes that catalyze the conversion of different toxic oxygen intermediates (free radical chain reaction) to harmless products. As a group, these enzymes serve as a defence system to guard against the toxic effects of reactive oxygen intermediates (Heffiner and Repine,1989) . The superoxide radical is eliminated by SOD, which

catalyzes its conversion to hydrogen peroxide plus oxygen; and hydrogen peroxide is removed by CAT, which converts it to water plus oxygen, and by GSH-Px, which reduces it to water, using a variety of reductants available to the cell. Data of the present studies with the others indicated that BP ingestion causes depression of the antioxidant defence potential of erythrocytes and, thus, cells cannot cope with oxidant stress incurred by ingested BP (Frenkel, 1992 and Elhassaneen, 2004). It is evident that, during metabolism of BP by hepatic microsomes in the rat, substantial amounts of $.O2^-$ and catalase-inhibitable H2O2 are generated i.e. induce ROS formation. In the present study, the antioxidant properties exhibited by curcumin are important in manipulation of the BP carcinogenicity development through ROS scavenging processes in liver cells.

Table 3. Effect of curcumin treatments on RBCs antioxidant enzymes activities of rats subjected to BP treatment

Value	Control (-)	Control (+)	Curcumin (%, w/w)			
			0.1	0.2	0.3	0.4
Glutathione peroxidase (GSH-Px, U/g Hb)						
Mean	20.76 [a]	10.99 [c]	13.87 [b]	14.76 [b]	17.31 [ab]	18.19 [ab]
SD	2.07	1.55	2.21	2.41	0.95	1.42
% of Change	------	-47.06	-33.19	-28.90	-16.62	-12.38
Catalase (CAT, U/g Hb)						
Mean	184.78 [a]	129.11 [c]	138.65 [c]	144.66 [c]	161.49 [ab]	166.08 [ab]
SD	20.76	7.39	10.56	17.43	11.91	15.94
% of Change	------	-30.13	-24.96	-21.71	-12.60	-10.12
Superoxide dismutase (SOD, U/g Hb)						
Mean	4.03 [a]	2.41 [c]	3.21 [b]	3.29 [b]	3.61 [a]	3.71 [a]
SD	1.03	0.55	0.71	0.72	1.02	0.98
% of Change	------	-40.20	-20.35	-18.36	-10.42	-7.94

* Means in the same row with different litters are significantly different at $p \leq 0.01$

Effects of curcumin treatments on lipid peroxidation (LPO) levels in serum of rats subjected to BP treatment

Effects of curcumin treatments on lipid peroxidation (LPO) levels in serum of rats subjected to BP treatment are shown in Table (4). From such data it could be noticed that the serum lipid peroxide (MDA, nmol/mL) level was increased 87.74% by BP, and this increase was significantly reduced by curcumin treatment. The reducing rate in MDA

level was increased with the increasing of curcumin level. The rate of increasing in MDA was recorded 0.261, 0.217, 0.199 and 0.176% with the rat diets blended by 0.1, 0.2, 0.3 and 0.4 g/100g of curcumin, respectively.

Table 4. Effects of curcumin treatments on MDA concentration (nmol/mL) in serum of rats subjected to BP treatment

Value	Control (-)	Control (+)	Curcumin (%, w/w)			
			0.1	0.2	0.3	0.4
Mean	0.155^{c}	0.291^{a}	0.261^{a}	0.217^{b}	0.199^{b}	0.176^{c}
SD	0.011	0.062	0.033	0.048	0.032	0.025
% of Change	------	87.74	68.39	40.00	28.39	13.55

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

Accompanied by a concomitant reduce in biological macromolecules antioxidants (GSH fractions), high concentrations of oxidant i.e. MDA as established in the present study in rats treatment with curcumin. In our opinion, if there were no change in the antioxidant defense system of rats feeding ingested curcumin, it would be difficult to observe high concentrations of MDA. BP metabolism results in the production of ROS that in turn cause mutations to the DNA which if not repaired helps in promotion of carcinogenesis (Samarth *et al.,* 2006). ROS initiates lipid peroxidation (LPO) i.e. formation of MDA directly by reacting with the lipids of membranes or by acting as second messengers for the primary free radicals (Rajendran *et al.,* 2008). In the present study, we observed a significant rise in the LPO levels as well as ROS levels upon BP treatment. In similar study, as the result of charcoal broiled meat consumption i.e. containing PAH, high levels of MDA in the plasma of human were associated with rather low levels of biological antoxidants including enzymatic and non-enzymatic systems (Elhassaneen, 2004). Several reports have documented the potent antioxidant capacity of curcumin where by mitigation of lipid peroxidation and oxidative stress in several tissues were demonstrated (Bohm *et al.,* 1997 and Nabavi *et al*., 2012).

Effects of curcumin treatments on the activity of cytochrome P450 in liver of rats subjected to BP treatment

Effects of curcumin treatments on the activity of cytochrome P450 in liver of rats subjected to BP treatment was shown in Table (5). From such data it could be noticed that treatment of animals with BP caused a significant increased ($p \leq 0.01$) in cytochrome P450 (39.74%) compared to

normal control animals. Feeding of the rat diets with curcumin (0.1 to 0.4 g/100g w/w) prevented the rise of mean serum cytochrome P450 activity. The rate of preventative was increased with the increasing of the curcumin concentration. The rate of increasing in the cytochrome P450 activities were 12.58, 9.93, 7.28 and 2.65 with the rat diets blended with 0.1, 0.2, 0.3 and 0.4 g/100g of curcumin, respectively. In similar study, Liu *et al.,* (2015) reported that BP treatment brought about a significant increase in the activities of drug metabolizing enzymes (cytochrome P450 and b5) in lungs of mice and the activities of these enzymes were markedly decreased by the administration of phytochemicals including curcumin and quercetin.

Table 5. Effects of curcumin treatments on the activity of cytochrome P450 (nanomoles/mg protein) in liver of rats subjected to BP treatment

Value	Control (-)	Control (+)	Curcumin (%, w/w)			
			0.1	0.2	0.3	0.4
Mean	1.51 [b]	2.11[a]	1.7 [b]	1.66 [b]	1.62 [b]	1.55[b]
SD	0.28	0.37	0.39	0.22	0.09	0.18
% of Change	-----	39.74	12.58	9.93	7.28	2.65

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

Correlation studies

In the correlation analysis, important differences were found between lipid peroxidation (LPO and antioxidant defense systems (enzymatic and non-enzymatic) in BP induced changes in rats and the same rats feeding curcumin (Figures 1). From such data it could be noticed that there was a strong negative significant ($p \leq 0.01$) relationship between MDA and GSH concentration in plasma ($r^2 = 0.808$), GSH-Px ($r^2 = 0.768$), CAT ($r^2 = 0.701$) as well as SOD ($r^2 = 0.721$) in RBC's. These correlations confirm that if there were no change in the antioxidant defense system of BP 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 BP were associated with rather low levels of different oxidative parameters including GSH. Also, in some model systems, a combination of bioactive compounds including α-tocopherol and β-carotene i.e. partially similar curcumin in mode of action interact synergistically to inhibit lipid peroxidation subsequently decreased TBARS (Bohm *et al*., 1997). Also, Liu *et al.,* (2015) reported that administration of phytochemicals (curcumin and quercetin) both separately as well as in combination for a period of 22 weeks to BP

treated mice were able to increase the antioxidant enzymatic defense system activities in correlation with the decreasing of the lipid peroxidation (MDA) concentration.

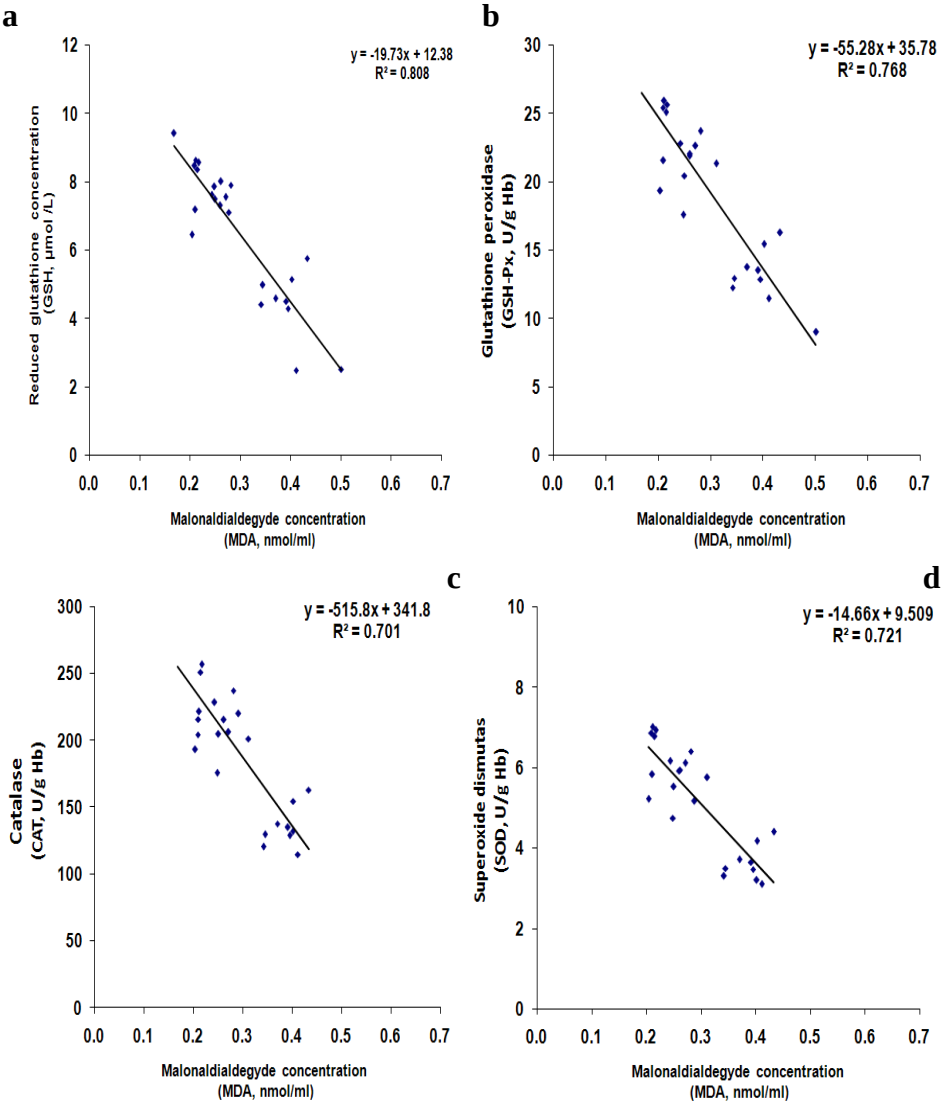

Figure 4. Correlation between lipid peroxidation (LPO) and antioxidant defense system in rats treated with PB and feeding curcumin: (a) MDA vs. GSH, (b) MDA vs. GSH-Px, (c) MDA vs. CAT, and (d) MDA vs. SOD.

Conclusion

BP is considered as a ubiquitous environmental and food contaminants as well as a top risk factor in the development of several diseases including liver toxicity and carcinogenicity. Curcumin exhibit inhibiting effects probably by modulating regulators of drug metabolizing enzymes and antioxidant defense systems and thereby adversely affecting the toxic/carcinogenic process to the benefit of the biological system. Therefore, curcumin show great prospects in dealing with the condition of liver toxicity and carcinogenicity. All the observations discussed clearly suggest that curcumin hold great potential to be used as an effective preventive measure against the occurrence of liver toxicity and cancer in a section of human population who have a family history of liver cancer as well those who are constantly exposed to toxins/ carcinogens from different sources.

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التأثيرات الوقائية للكركمين أثناء إحداث البنزوبيرين لسمية وسرطان الكبد فى الفئران

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الملخص العربى:

المواد الكيميائية النباتية (الفيتوكيماويات) هي المواد الكيميائية التي تنتجها النباتات من خلال الأيض الابتدائي أو الثانوي، كما تعد تلك المواد واحدة من العائلات ذات العوامل النشطة بيولوجيا التي يجري بحثها على نطاق واسع في جميع نحاء العالم لفعاليتها ضد العديد من مسارات السرطان. ينتمى الكركمين إلى عائلة من المواد الكيميائية النباتية والذى له خصائص مضادة للأكسدة ومضاد للسرطان .وفي الدراسة الحالية سوف يتم التحقق التحقيق من الفعالية الوقائية الكيميائية للكركمين ضد مركب البنزوبيرين الذى يمتلك القدرة على إحداث السمية والسرطانية فى الكبد. أدت المعاملة بالبنوبيرين إلى حدوث زيادة معنوية ($p\leq0.05$) في وظائف الكبد، ومستويات أكسدة الدهون (المالونالدهيد). كما حدث إنخفاض معنوى ($p\leq0.01$) للإنزيمات المضادات للأكسدة (الجلوتاثيون بيروأكسيديز، السوبر أكسيد ديسميوتيز، الكاتاليز) وأجزاء الجلوتاثيون بعد المعاملة بالبنزوبيرين. وعلاوة على ذلك، أحدثت المعاملة بالبنزوبيرين الى زيادة معنوية ($p\leq0.05$) في أنشطة انزيمات استقلاب المواد الغريبة داخل الجسم (إنزيم السيتوكروم P450). ولقد أدى تدعيم حمية الفئران مع الكركمين بتركيزات ٠,١ إلى ٤,٠ جم / ١٠٠ جم وزن/وزن إلى حدوث خفض معنوي ($p\leq0.05$) فى مستويات المالونالدهيد ، وكذلك زيادة معنوية ($p\leq0.05$) فى أنشطة الإنزيمات المضادة للأكسدة وانخفاض فى درجة نشاط انزيمات استقلاب المواد الغريبة داخل الجسم (إنزيم السيتوكروم P450) . وتثبت نتائج هذه الدراسة أن المعاملة بالكركمين لها فوائد عديدة من خلال تحسن حالة مضادات الأكسدة وإنزيمات استقلاب المواد الغريبة داخل الجسم أثناء التجارب الخاصة بحث السمية والقدرة على إحداث السرطان فى كبد الفئران. لذلك، توصى الدراسة بتضمين الكركمين بتركيز قد يصل الى ٠,٤٪ في الوجبات الغذائية اليومية والمشروبات والمنتجات الغذائية.

الكلمات المفتاحية: الكركمين، أكسدة الدهون، الانزيمات المضادة للأكسدة، إنزيمات استقلاب المواد الغريبة داخل الجسم، انزيم السيتوكروم P450.