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The potential preventive effects of turmeric (*Curcuma longa*) rhizomes against hepatotoxicity in rats induced by benzo[*a*]pyrene

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Abstract: Benzo[*a*]pyrene, B[*a*]P, is considered as a ubiquitous environmental and food contaminants and a top risk factor in the development of several diseases including hepatotoxicity. Several strategies to fight hepatotoxicity and its complications have been proposed, because early discovering, prevention and treatment play a pivotal role in reducing the population burden of hepatotoxicity. Therefore, the present study was designed to determine the potential preventive effects of turmeric (*Curcuma longa*) rhizome powder (TRP) against hepatotoxicity in rats induced by benzo[*a*]pyrene. Treatment of animals with B[*a*]P caused a significant increased ($p \leq 0.05$) in AST (105.27%), ALT (59.82%) and ALP (141.34%) compared to normal controls. Supplement Supplementation of the rat diets with TRP (0.25 to 1.0 g/100g w/w) prevented the rise of mean serum AST, ALT and ALP activities. The same behavior was recorded for TBARS level in serum, 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 serum. These results supported our hypothesis that such functional plant foods powder contains several classes of bioactive compounds with other compounds that are able to prevent or inhibit B[*a*]P hepatotoxicity through liver serum enzymes-lowering activity, decreasing rate on the formation of serum TBARS. Therefore, we recommended TRP by a concentration of 1% to be included in our daily diets, drinks and food products.

Keywords: Turmeric rhizome powder, liver functions, glutathione fractions, albumin, immunological parameters, TBARS.

Introduction

It has been known that cooking can produce toxic compounds in foods, if the appropriate precursors are present (Gray and Morton, 1981; Elhassaneen and Tawfik, 1998, and Elhassaneen and El-Badawy, 2013). Amongst of these compounds, polycyclic aromatic hydrocarbons (PAH) from incomplete combustion occur in several foods such as charcoal broiled and smoked goods (Emerole *et al.*, 1982; Larsson *et al.*, 1983; Van Maanen *et al.*, 1994 and Bassiouny, 1999). B[a]P is a member of the family, polycyclic aromatic hydrocarbon (PAH) that is a by-product of incomplete combustion or burning of organic (carbon-containing) items, e.g., cigarettes, gasoline, and wood. B(a)P is commonly found with other PAHs in cigarette smoke, in grilled and broiled foods, and as a by-product of many industrial processes (Elhassaneen, 2004 and Elhassaneen and El-Badawy, 2013). BaP is also found in ambient (outdoor) air, indoor air, and in some water sources (U.S. Environmental Protection Agency, 2005). 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; Plakunov *et al.*, 1987; Hawkins *et al.*, 1990) 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 toxicity and carcinogenicity in mammals, rodent and fish (Harvey, 1985 and Hawkins *et al.*, 1988 and 1990; Elhassaneen, 1996 and Elhassaneen, 2002).

Many of authorities and academic centers of research pay more attention towards the area of cancer chemoprevention compounds. One of the most impressive findings in the field of chemoprevention is the very large number of compounds that have been demonstrated to prevent the occurrence of cancer. Many of these classes are lies in an enlarged group of compounds called phytochemicals (*phyto* is Greek for plant). Phytochemicals are the bioactive compounds of plants that do not deliver energy and are not yet classified as essential nutrients but possess healthful properties beyond their use as macronutrients or micronutrients. Plants usually produce such low-molecular-weight ingredients for their protection against pests and diseases, for the regulation of their growth, or as pigments, essence, or odor (Perez-Vizcaino *et al.*, 2006). Scientists have identified thousands of phytochemicals, including flavonoids, glucosinolates (isothiocyanates

and indoles), phenolic acids, phytates, and phytoestrogens (isoflavones and lignans), in vegetables, fruits, grains, legumes, and other plant sources. A vast variety of phytochemicals that are present in the daily human diet have been found to possess substantial antimutagenic and anticarcinogenic properties (Surh, 2002). The chemopreventive effects of the majority of edible phytochemicals are often attributed to their antioxidative or anti-inflammatory activities. Besides the edible chemopreventives in vegetables, fruits, herbs, and spices, some phytochemicals in diverse plants also have other beneficial health effects such as anti-obesity, lipid-lowering, and/or antidiabetic properties (Surh *et al.*, 2001).

Turmeric (*Curcuma longa*) belongs to the *Zingiberaceae* family along with the other noteworthy members like 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) (Reviewed in Fayez, 2016).

Turmeric contains a wide variety of phytochemicals, including but not limited to curcumin, demethoxycurcumin, bisdemethoxy-curcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydro-curcumin, triethylcurcumin, turmerin, turmerones, and turmeronols (Chattopadhyay *et al.*, 2004) .Turmeric contains 2-8% curcumin. (Payton *et al.*, 2007). Traditionally many medicinal properties are attributed to this spice. Since the time of 1900 BC numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders (Aggarwal *et al.*, 2007 and Agrawal, 2003). According to our knowledge, the studies regarding the potential effects of turmeric on liver disease/cancer are so limited. Therefore, in this study, we examined the potential preventive effects of turmeric rhizomes powder against hepatotoxicity in rats induced by B[a]P.

Materials and Methods

Materials

Turmeric (*Curcuma longa*) rhizomes powder (TRP) was purchased from the local market, Bab ElKhalk, Cairo, Egypt. Benzo(a)pyrene was 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 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; a reversed phase water Adsorbosil C18 (5 µmol/L, 100 mm x4.6–mm internal diameter) for vitamin C; and normal Ultrasphere Si (5 µmol/L, 250 mm x4.6–mm internal diameter) for analysis of vitamins A and E, and curcumin.

Chemical analysis of turmeric rhizomes powder (TRP) samples

TRP samples were analyzed for moisture, protein (T.N. × 6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy) , fat (soxhelt miautomatic apparatus Velp company, Italy , petroleum ether solvent), ash, fiber and essential oil (using rotary evaporator apparatus, Velp company, Italy) contents were determined using the methods described in the A.O.A.C. (1995). Carbohydrates calculated by differences:

Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

Total phenolics, carotenoids and total dietary fiber in TRP samples were analyzed as follow: TRP was extracted with 80% acetone and centrifuged at 10,000g for 15 min. For biscuits samples, one gram of biscuit powder was extracted with 20 ml of 80% acetone and centrifuged at 8000g at room temperature. The supernatant obtained from both samples were used for the analysis of total phenolics, carotenoids, curcumin and antioxidant activity.

Total phenolics were determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Two hundred milligrams of sample was

extracted for 2 h with 2 mL of 80% MeOH containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000g for 15 min and the supernatant decanted into 4 mL vials. The pellets were combined and used for total phenolics assay. One hundred microliters of extract was mixed with 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min; 0.75 mL of sodium bicarbonate (60g/L) solution was added to the mixture after 90 min at 22 °C, absorbance was measured at 725 nm. Results are expressed as ferulic and equivalents. The total carotenoids in 80% acetone extract were determined by using the method reported by Litchenthaler (1987). Total dietary fiber content in the TRP was estimated according to the method described by Asp *et al.* (1983). Curcumin was determined in TRP extract according to the method of Wichitnithad *et al.*, (2009).

All vitamins (A, C, and E) were extracted according to methods previously detailed (Epler *et al.*, 1993; Moeslinger *et al.*, 1994 and Hung *et al.*, 1980) and were analyzed by HPLC techniques. For vitamins A and E, the chromatographic conditions were as follows: flow rate, 1.5 mL/min; detection, UV absorption at 265 nm, volume of injection, 20 µL; temperature, room temperature; and the mobile phase composition was an isocratic system of isopropanol:hexane (1:99). For vitamin C, the conditions were: flow rate, 1 mL/min; detection, UV absorption at 254 nm, volume of injection, 20 µL; temperature, room temperature, and mobile phase composition was an isocratic system of 100% methanol. Retention times and absorbance ratio against those of standards were used to identify the separated vitamins. Quantitative determination of each vitamin was determined from its respective peak area and corresponding response factor. The percent recoveries of vitamins were also studied by adding each vitamin to plasma after sample preparation and HPLC determination. Under such chromatographic conditions, mean values (\pm SD) of vitamins A, C and E, and curcumin recoveries were 89.96 ± 2.1 , 90.65 ± 1.84 , 86.09 ± 3.06 and 83.21 ± 4.01 %, respectively.

Antioxidant activity

Antioxidant activity of TRP extracts and standards (α -tocopherol, BHA, and BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the β -carotene bleaching method following a modification of the procedure described by Marco (1968). For a typical assay, 1 mL of β -carotene (Sigma) solution, 0.2 mg/mL in chloroform, was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid (J.T. Baker Chemical Co., Phillipsburg, NJ) and 0.2 mL of Tween 20 (BDH Chemical Co., Toronto, On). Each mixture was then dosed with 0.2 mL of 80% MeOH (as control) or corresponding plant extract or standard. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50 ml) was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autooxidation at 50 °C for 2 h. The absorbance of the solution at 470 nm was monitored on a spectrophotometer (beckman DU-50) by taking measurements at 10 min intervals, and the rate of bleaching of β -carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Various concentrations of BHT, BHA, and α -tocopherol in 80% methanol was used as the control. Antioxidant activity (AA) was all calculated as percent inhibition relative to control using the following equation (Al-Saikhan *et al.*, 1995).

$$AA = (R_{\text{control}} - R_{\text{sample}}) / R_{\text{control}} \times 100$$

Where: R_{control} and R_{sample} were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively.

Biological Experiments

Animals

Animals used in this study, adult male albino rats (150±8.7 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 °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, the rats were divided into two main groups, the first group (Group 1, 6 rats, as a negative control group) still fed on basal/standard diet and injected with the vehicle alone (5 ml/kg body weight) and the other main group (30 rats) was challenged with an ip injection of B[a]P (100 mg/5 ml/kg body weight) dissolved in 0.9% NaCl solution containing 0.1% Tween 20 to induce liver impaired rats then classified into five sub- groups as follow:

- Group (2): fed on standard diet only as a positive control
- Group (3): fed on standard diet containing 0.25 % turmeric powder
- Group (4): fed on standard diet containing 0.50 % turmeric powder
- Group (5): fed on standard diet containing 0.75 % turmeric powder
- Group (6): fed on standard diet containing 1.0 % turmeric powder

Blood sampling

At the end of experiment period, 4 weeks, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. 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 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 (GSH) fractions

GSH was determined by HPLC according to the method of McFarris and Reed (1987). In brief, 100 μ l 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 μ l aliquot of acidic extract was mixed with 100 μ l 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) – KHCO₃ (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 °C in the presence of 0.2 ml of 1% 1-fluoro-2,4-dinitrobenzene and injected onto the HPLC system.

Albumin

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

Protease activity assay

The protease activity was determined by adaptation the method of Rindernecitt *et al.*, (1968). Briefly, 100 μ l of plasma were added to 40 μ l of buffer (150 mM Tris base, 30 mM CaCl₂, 0.05% Brij 35) and 50 μ l of protease substrate (20% Hide powder azure, HPA, 20 % sucrose, 0.05% Brij). The tubes contents were incubated at 37 °C with continuous shaking for 2 hours. The reaction was stopped by the addition of 50 μ l of 10% TCA and the tubes were stored at 4 °C 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.

Thiobarbituric acid reactive substances (TBARS) content

TBARS were measured 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 FeSO₄·7H₂O was added and the mixture was heated for 20 min in boiling water. The samples were centrifuged at 1000 xg 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 malonaldehyde.

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

Chemical analyses of turmeric rhizomes powder (TRP)

The proximate composition of TRP is shown in Table 1. The results showed that the moisture content was 7.55%, total protein was 7.51%, crude fat was 6.80%, crude fiber was 4.67%, ash content was 2.85% and total carbohydrate content was 70.62%. The proximate composition reported was not accordance with that observed by Kapoor, (1990) and Ruby *et al.*, (1995) but relatively accordance with that reviewed by Jaggi, (2012). These data reflected the effect of turmeric varieties on the chemical composition of rhizome. All of these components in TRP might be important from the nutrition point of view. Therefore, enrichment of different food products with TRP would enhance the nutritional quality of the product better than many food sources. Also, the total phenolics content, total carotenoids, essential oil etc in the TRP are giving such food high significant as an important functional food.

On the other side, there are the antioxidant nutrients such as vitamins C, E, β -carotene and curcumin (found in TRP) for which there are Dietary Reference Values (DRVs). However, there are thousands of

other bioactive compounds in foods that have antioxidant activity but are not classified as "nutrients." These "non-nutrient antioxidants" include phenolic compounds (found TRP) (Ajila *et al.*, 2008). Also, many studies indicated that there was a positive and significant ($p < 0.01$) relationship between all of the previous bioactive compounds and the antioxidant activity in different plant parts (Khoneem, 2009; Jaggi, 2012 and Elhassaneen *et al.*, 2013). Plant-based foods generally are considered important sources of antioxidants in the diet. Antioxidants help protect cells from the potentially damaging physiological process known as "oxidative stress" (damage to healthy cells or DNA by unpaired electrons known as free radicals). Oxidative stress is thought to be associated with the development of chronic diseases including cancer, heart disease, diabetes, rheumatoid arthritis, obesity, conditions of ageing including neurodegenerative diseases such as Parkinson's and Alzheimer's disease (Halliwell, 1991, Van Gaal *et al.*, 1998, Chaitanya *et al.*, 2010 and Elmaadawy *et al.*, 2006).

Table 1. Proximate composition of TRP

Component	Content
Water (g/100g)	7.55 ± 1.23
Total protein (g/100g)	7.51 ± 0.98
Crude fat (g/100g)	6.80 ± 1.07
Ash (g/100g)	2.85 ± 0.43
Crude fiber (g/100g)	4.67 ± 0.88
Carbohydrate (g/100g)	70.62 ± 3.11
Essential oil (g/100g)	5.82 ± 0.95
Ascorbic acid (mg/100g)	51.65 ± 12.76
Vitamin A (mg/100g)	10.71 ± 1.25
Vitamin E (mg/100g)	4.29 ± 2.05
Total carotenoids (mg.100g)	79.11 ± 10.63
Curcumin (%) - Ethanolic extract	10.23 ± 3.88
Antioxidant activity (AA, %) - Ethanolic extract	79.57 ± 6.44
Total phenolics content (mg GAE.g-1) - Ethanolic extract	19.29 ± 7.72

Each value represents the mean of three replicates ±SD.

Effects of TRP on B[a]P-induced changes in liver functions of rats

Liver functions of rats injected B[a]P and consumed TRP powder were shown in Table (2) and Figure (1). From such data it could be noticed that treatment of animals with B[a]P caused a significant increased ($p \leq 0.05$) in AST (105.27%), ALT (59.82%) and ALP (141.34%) compared to normal controls. Supplementation of the rat diets with TRP (0.25 to 1.0 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 TRP concentration. Such as shown in Figure (1), the rate of increasing in the liver enzymatic activities were recorded 67.84, 60.58, 28.64 and 22.00 % (For AST); 67.84, 60.58, 28.64 and 22.00 % (for ALT) and 91.37, 76.18, 42.23 and 37.07 (for ALP) with the rat diets supplemented by 0.25, 0.50, 0.75 and 1.0 g/100g of TRP, respectively.

Table 2. Effects of TRP on B[a]P-induced changes in liver functions of rats

Value	Control (-)	Control (+)	TRP (% , w/w)			
			0.25	0.50	0.75	1.00
Serum aspartate aminotransferase (AST,U/L)						
Mean	36.60 ^d	75.13 ^a	61.43 ^b	58.77 ^b	47.08 ^c	44.65 ^c
SD	4.27	10.21	8.79	11.18	5.12	6.35
% of Change	0.00	105.27	67.84	60.58	28.64	22.00
Serum alanine aminotransferase (ALT,U/L)						
Mean	63.29 ^d	101.15 ^a	89.03 ^b	81.27 ^b	75.44 ^{bc}	71.73 ^{bc}
SD	5.38	4.94	9.80	8.34	9.25	5.41
% of Change	0.00	59.82	40.66	28.40	19.20	13.33
Serum alkaline phosphatase (ALP,U/L)						
Mean	131.76 ^d	317.98 ^a	252.15 ^b	232.13 ^b	187.41 ^c	180.60 ^c
SD	12.65	33.23	10.77	14.71	10.87	21.77
% of Change	0.00	141.34	91.37	76.18	42.23	37.07

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

Several years ago, B[a]P was commonly used as a hepatotoxin in the experimental study of liver diseases. The hepatotoxic effects of B[a]P 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 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 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.

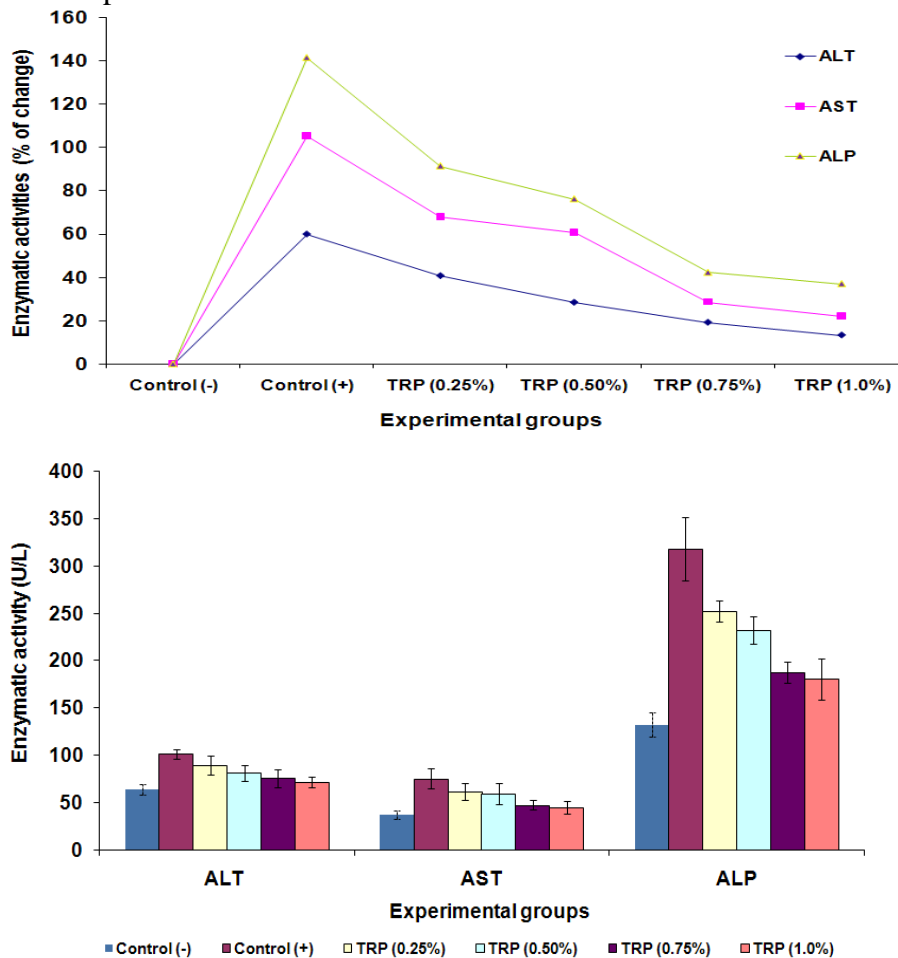


Figure 1. Effects of TRP on B[*a*]P-induced changes in liver functions of rats

Data of the present study with others reviewed that TRP is a rich source of different classes of phytochemicals such as alkaloids, carotenoids, phytosterols, phenols and organosulfurs as well as vitamins (Kapoor, 1990; Ruby *et al.*, 1995 and Jaggi, 2012). 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 (El-Nashar, 2007; Hassan, 201; El-Sayed *et al.*, 2012 and Sayed Ahmed, 2016). The possible mode of action of liver serum enzymes-lowering activity of the TRP 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 (Beattie *et al.*, 2005) and 3) improve the of antioxidant defense system in red blood cells.

Effect of TRP on B[a]P-induced changes in serum glutathione fractions levels of rats

Data presented in Table (3) and Figure (2) showed effect of feeding TFP on liver glutathione content of rats 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 GSH (-34.45%) and GSSG (-10.31%) compared to normal controls. Supplementation of the rat diets with TRP (0.25 to 1.0 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 TRP concentration. Such as shown in Figure (2), the rate of decreasing in the serum GSH fractions were recorded -28.44, -24.39, -16.44 and -13.07% (for GSH) and -7.41, -7.22, -5.98 and -3.62% (for GSSG) with the rat diets supplemented by 0.25, 0.50, 0.75 and 1.0 g/100g of TRP, respectively.

These data indicated that the rate of serum GSH elevation was increased with the increasing of the TRP concentration. 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 electrophilic intermediates and as an important antioxidant. The antioxidant functions of GSH includes 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).

Table 3. Effect of TRP on B[a]P-induced changes in serum glutathione fractions levels of rats

Value	Control (-)	Control (+)	TRP (% w/w)			
			0.25	0.50	0.75	1.00
Reduced glutathione concentration (GSH, $\mu\text{mol/L}$)						
Mean	7.42 ^a	4.86 ^{bc}	5.31 ^{bc}	5.61 ^b	6.20 ^{ab}	6.45 ^a
SD	1.22	2.03	1.22	0.79	1.03	0.78
% of Change	0.00	-34.45 ^a	-28.44	-24.39	-16.44	-13.07
Oxidized glutathione concentration (GSSG, $\mu\text{mol/L}$)						
Mean	0.69 ^a	0.61 ^{ab}	0.63 ^{ab}	0.64 ^{ab}	0.64 ^{ab}	0.66 ^a
SD	0.17	0.33	0.22	0.09	0.13	0.12
% of Change	0.00	-10.31	-7.41	-7.22	-5.98	-3.62
GSH/GSSG ratio						
Mean	10.83 ^a	7.92 ^c	8.37 ^{ab}	8.83 ^{ab}	9.63 ^a	9.77 ^a
SD	1.07	1.03	1.02	0.99	1.23	1.29
% of Change	0.00	-26.91	-22.71	-18.51	-11.13	-9.81

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

A fall in GSH observed generally accompanied by a concomitant increased in the liver lipid peroxidation (MDA content). Several reports have documented the potent antioxidant capacity of curcumin where by mitigation of lipid peroxidation and oxidative stress in several tissues were demonstrated (Nabavi *et al.*, 2012b). Such data with the others suggested that secretion of GSH from liver to blood might be blocked because of intracellular structural failure and elevation of the lipid peroxide content (Hasegawa *et al.*, 1995). TRP was described by its higher content of different classes of phytochemicals including curcumin which exhibited high antioxidative activities (Shishodia *et al.*, 2006 and) and anticarcinogenic effects (Srinivasan, 2005 and Jurenka, 2009 and Nabavi *et al.*, 2012). Additionally, DiGiulio (1991) mentioned that plasma GSSG concentration to provide a sensitive index

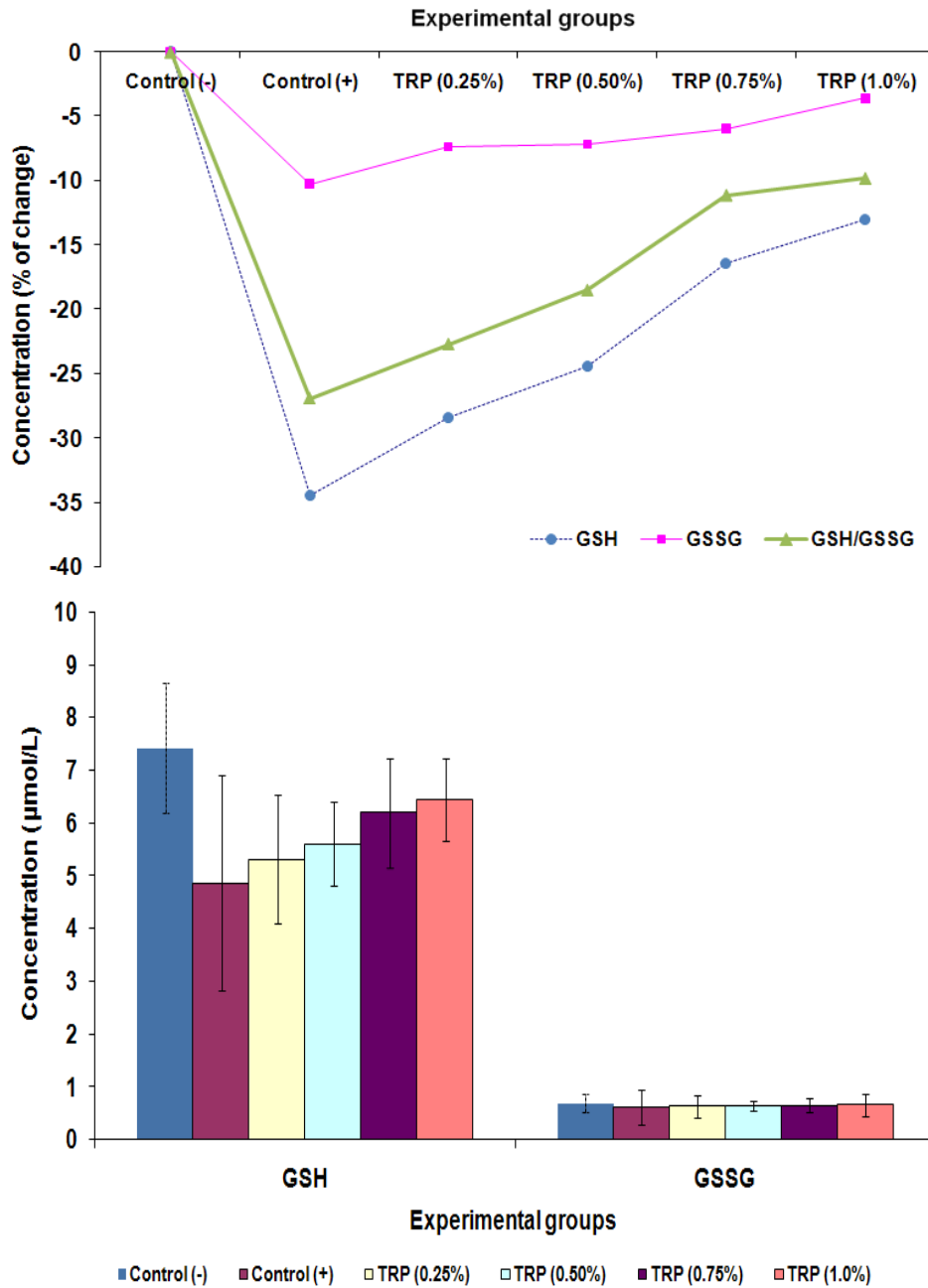


Figure 2. Effect of TRP on B[a]P-induced changes in serum glutathione fractions levels of rats

oxyradicals might be decreased in the GSH/GSSG ratio, due either to of whole body oxidative stress in the rat. Increased fluxes of direct radical scavenging or to increased peroxidase activity (Almaadawy *et al.*, 2016).

Effect of TRP on B[a]P-induced changes in serum immunological parameters levels of rats

Data presented in Table (4) and Figures (3) showed effect of feeding TFP on some immunological parameters (albumin level and protease activity) in serum of rats 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 (-22.91%) and protease activity (-28.63%) compared to normal controls. Supplementation of the rat diets with TRP (0.25 to 1.0 g/100g w/w) prevented the rise of mean serum albumin level and protease activity. The rate of preventative was increased with the increasing of the TRP concentration. Such as shown in Figure (3), the rate of increasing in the serum albumin level and protease activity were recorded -18.55, -15.10, -9.73 and -8.66% (for albumin) and -22.84, -19.41, -12.54 and -11.70% (for protease activity) with the rat diets supplemented by 0.25, 0.50, 0.75 and 1.0 g/100g of TRP, respectively.

Table 4. Effect of TRP on B[a]P-induced changes in serum immunological parameters levels of rats

Value	Control (-)	Control (+)	TRP (% w/w)			
			0.25	0.50	0.75	1.00
Serum albumin concentration (Alb, g/dl)						
Mean	3.80 ^a	2.93 ^{ab}	3.10 ^{ab}	3.23 ^a	3.43 ^a	3.47 ^a
SD	0.20	0.10	0.40	0.28	0.29	0.27
% of Change	0.00	-22.91	-18.55	-15.10	-9.73	-8.66
Serum protease activity (PA, U/L)						
Mean	3.11 ^a	2.22 ^{abc}	2.40 ^{ab}	2.51 ^{ab}	2.72 ^{ab}	2.75 ^a
SD	0.16	0.23	0.10	0.19	0.12	0.43
% of Change	0.00	-28.63	-22.84	-19.41	-12.54	-11.70

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

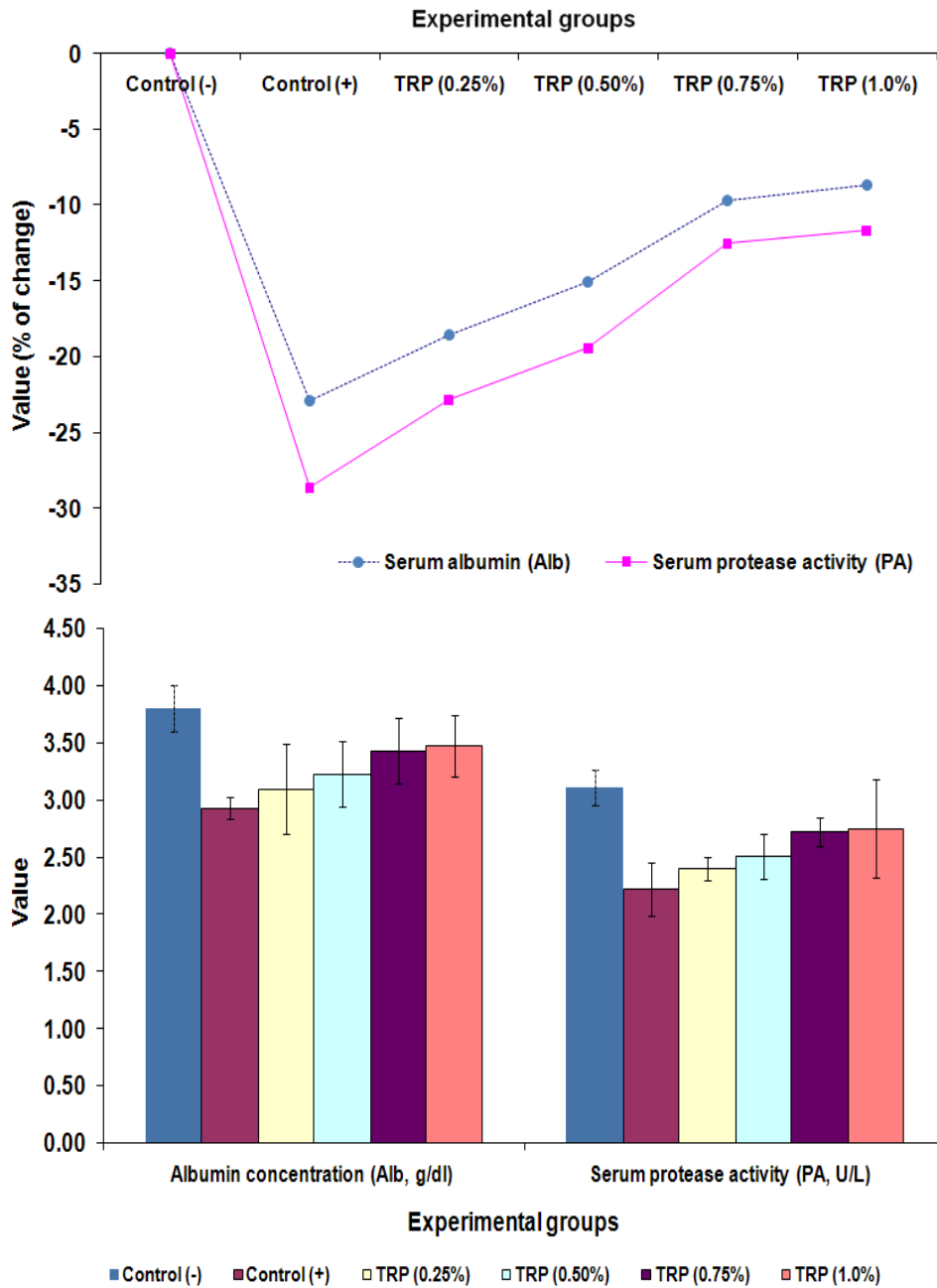


Figure 3. Effect of TRP on B[a]P-induced changes in serum immunological parameters levels of rats

In according with the study of Wang *et al.* (2007) , the present study showed that B[a]P induced significant decrease in the serum albumin content. It was reported that hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases, hence decline in total protein and albumin can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. In similar study, side treatment with artichoke significantly increased the reduced levels of serum total protein and albumin which was in agreement with previous study (Abd El-Aleem *et al.*, 2009).

On the other side, numerous studies have revealed that proteases could play an important role in immunological functions and humoral host defense (reviewed in Neurath,1989). 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 several studies, protease activity was found decreased as found 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 paper industry effluent, pesticides, heavy metals, polycyclic aromatic hydrocarbons etc. Co-treatment of rats with B[a]P and TRE exhibited therapeutic effects through decreasing the immunotoxic effects i.e. albumin levels and protease activity.

Effect of TRP on B[a]P-induced changes in serum TBARS concentration of rats

Serum lipid peroxidation of rats injected with B[a]P and consumed TRP was shown in Table (5) and Figures (4). From such data it could be noticed that the serum lipid peroxide level was increased 68.86% by B[a]P, and this increase was significantly reduced in the B[a]P+TRP. The rate of TBARS (malonaldehyde level, MDA) reducing was increased with the increasing of the TRP concentration. Such as shown in Figure (4), the rate of increasing in TBARS was recorded 54.56, 44.22, 24.95 and 20.98% with the rat diets supplemented by 0.25, 0.50, 0.75 and 1.0 g/100g of TRP, respectively.

Accompanied by a concomitant reduce in biological macromolecules antioxidants (GSH fractions), high concentrations of

Table 5. Effect of TRP on B[a]P-induced changes in serum TBARS concentration (nmol/mL) of rats

Value	Control (-)	Control (+)	TRP (% , w/w)			
			0.25	0.50	0.75	1.00
Mean	0.168 ^c	0.284 ^a	0.260 ^a	0.243 ^a	0.210 ^b	0.204 ^b
SD	0.024	0.123	0.052	0.063	0.044	0.044
% of Change	0.00	68.86	54.56	44.22	24.95	20.98

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

oxidant i.e. TBARS as established in the present study in rats feeding of TRP. In our opinion, if there were no change in the antioxidant defense system of rats feeding ingested TRP, it would be difficult to observe high concentrations of TBARS. High levels of malonaldehyde (MDA), one of the most important compounds in TBARS, in the plasma of patients were associated with rather low levels of biological antioxidants (Elhassaneen, 2004). Several reports have documented the potent antioxidant capacity of curcumin i.e. the main bioactive component in TRP where by mitigation of lipid peroxidation and oxidative stress in several tissues were demonstrated (Boham *et al.*, 1997 and Nabavi *et al.*, 2012b).

Correlation studies

In the correlation analysis, important differences were found between oxidative and antioxidant defense system in B[a]P-induced changes in rats and the same rats feeding TRP (Figure 5). From such data it could be noticed that there was a strong negative significant ($p \leq 0.05$) relationship between GSH and TBARS concentrations in plasma ($r^2 = -0.870$). This correlation confirm that if there were no change in the antioxidant defense system of B[a]P treated rats, it would be difficult to observe high concentrations of TBARS. 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, in some model systems, a combination of bioactive compounds including α -tocopherol and β -carotene i.e. such as found in TRP interact synergistically to inhibit lipid peroxidation subsequently increased TBARS (Bohm *et al.*, 1997).

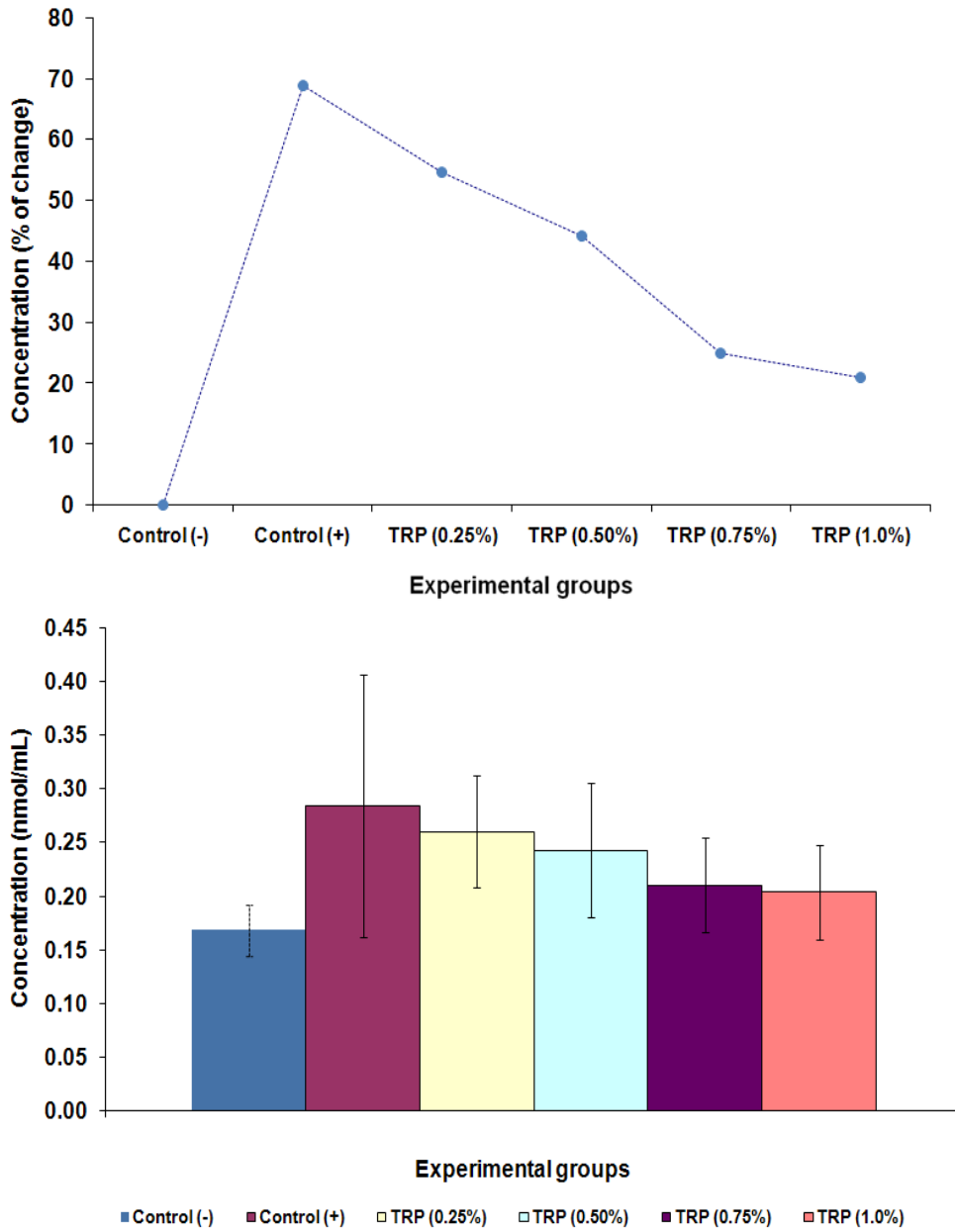


Figure 4. Effect of TRP on B[a]P-induced changes in serum TBARS concentration (nmol/mL) of rats

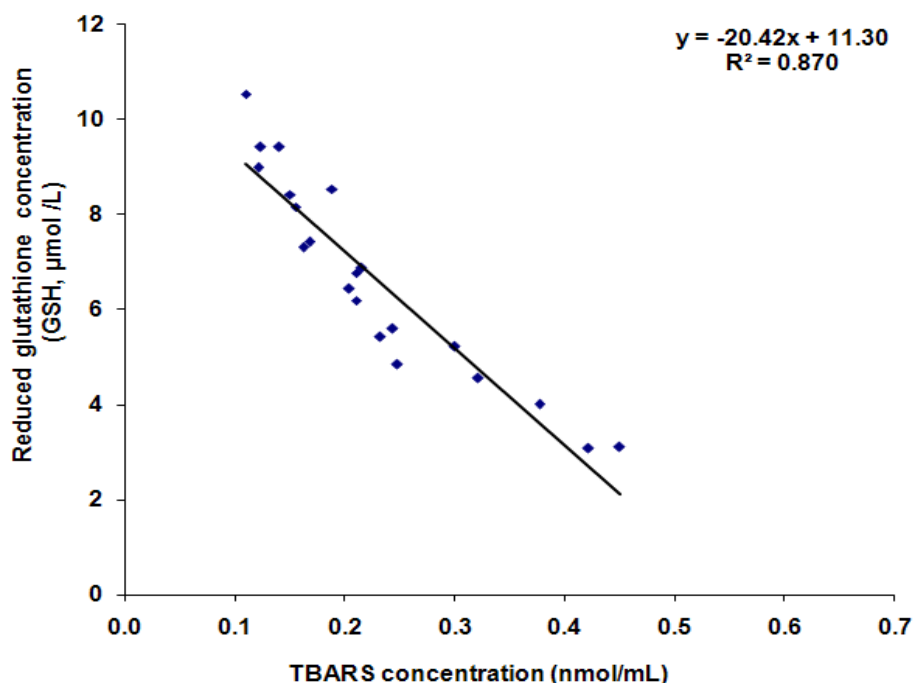


Figure 5. Correlation between oxidative stress (TBARS) and antioxidant defense system (GSH) in rats treated with B[a]P and feeding TRP.

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 hepatotoxicity. 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. Feeding of some selected medicals plants parts applied in foods has been proven to be essential in the treatment and/or prevention of hepatotoxicity but also beneficial for oxidative stress reduction. Overall, the present study supports the benefits of dietary modification, including bioactive/ antioxidant compounds supplementation, in alleviating oxidative stress associated hepatotoxicity.

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التأثيرات الوقائية المحتملة لريزومات الكركم تجاه السمية الكبدية في الفئران المستحثة بالبنزوبيرين

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بعد البنزوبيرين من أكثر الملوثات البيئية والغذائية إنتشارا ، كما أنه من أكثر العوامل خطورة في نمو وتطور العديد من الأمراض والتي منها التسمم الكبدى. لذلك وضعت العديد من الإستراتيجيات لمحاربة التسمم الكبدى ومضاعفاته، حيث أن الإكتشاف والمعالجات المبكرة والوقاية تلعب دورا حيويا في إختزال عدد السكان المصابين بهذا المرض. لذلك أجريت الدراسة الحالية بهدف إستبيان التأثيرات الوقائية المحتملة لريزومات الكركم تجاه السمية الكبدية في الفئران المستحثة بالبنزوبيرين. ولقد أوضحت النتائج أن معاملة الفئران بالبنزوبيرين قد تسببت في زيادة معنوية ($p \leq 0.05$) في نشاط انزيمات الكبد ALT, AST, ALP وذلك بنسب 105,27، 59,82، 141,34 % مقارنة بالمجموعة الضابطة الطبيعية. كما أدى خلط وجبات الفئران بمسحوق ريزومات الكركم بتركيزات 0,25-0,10 % (وزن/وزن) قد أحدث إنخفاضاً معنوياً ($p \leq 0.05$) في درجة نشاط تلك الإنزيمات وبمعدلات مختلفة تزايدت مع زيادة درجة التركيز. ولقد سجل نفس السلوك لمستوى مواد حامض الثيوباربتيكورك الفعالة والذي يعد مؤشرا حيويا على الجهد التأكسدى والإلتهابات في أنسجة الجسم المختلفة وكذلك بعض المؤشرات المناعية الهامة مثل مستوى الألبومين ونشاط انزيم البروتينز في السيرم. كما سجل سلوكا معاكسا فيما يتعلق بجزئيات الجلوتاثيون (الجزئيات الحيوية المضادة للأكسدة) في السيرم. ولعل نتائج الدراسة الحالية تؤيد الفرضية أن مساحيق النباتات الوظيفية تحتوى على العديد من مجموعات المركبات النشطة حيويا بجانب مركبات أخرى يكون لها القدرة على منع أو تثبيث السمية الكبدية للبنزوبيرين والتي تشمل خفض النشاط لإنزيمات الكبد، ونقص تكوين مواد حامض الثيوباربتيكورك الفعالة وزيادة مستوى بعض المؤشرات المناعية الهامة في السيرم. لذلك توصى الدراسة الحالية بخلط مسحوق ريزومات الكركم بتركيز قد يصل الى 0,1% في الأطباق والمشروبات اليومية وكذلك كإضافات لبعض المنتجات الغذائية.

الكلمات المفتاحية: مسحوق ريزومات الكركم ، وظائف الكبد ، جزئيات الجلوتاثيون، ألبومين، المؤشرات المناعية، مواد حامض الثيوباربتيكورك الفعالة.