Journal of the College of Specific Education for Educational and Specific Studies **Issue (16) May P (2) 2021**

Bioactive compounds content in turmeric and its effects on liver disorders induced by benzo (a) pyrene in rats

محتوى المركبات النشطة حيويا في الكركم وتأثيراته على اضطرابات الكبد التي يسببها البنزوبيرين في الفئران

by
Ahmed M. Ajami [1]
Supervised by
Yousif A. Elhassaneen* Emam , O.A.**
El-bassyoni ,Ghada,M **

Abstract:

The current work was designed to study the effects of turmeric rhizome powder on liver disorders induced by Benzo(a)pyrene [B(a)P] in rats. Treatment of animals with B(a)P caused a significant increased ($p \leq 0.05$) in AST (72.71%), ALT (116.67%) and ALP (238.41%) compared to normal controls. Supplementation of the rat diets with turmeric powder (0.50, 1.00, 1.50, and 2.00 g/100g) prevented the rise of mean serum AST, ALT and ALP activities. The same behavior was recorded for MDA, the biomarker of oxidative stress in cells, levels in serum and xenobiotics metabolizing enzyme i.e. cytochrome p450 in hepatocytes. The opposite direction was recorded for the glutathione fractions (biological

[1] Researcher at Benha university.
* Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt
** Department of Home Economics, Faculty of Specific Education Benha University, Benha, Egypt

macromolecules antioxidant) in serum. These results supported our hypothesis that turmeric powder contains several categories of bioactive compounds that are able to prevent/inhibited the B(a)P induced liver disorders through liver serum enzymes-lowering activity, decreasing rate on the formation of serum MDA, decreasing the activity of xenobiotics metabolizing enzyme i.e. cytochrome p450 and increasing the levels of glutathione fractions in serum. Therefore, the present study recommended turmeric by a concentration up to 2% to be included in our daily diets, drinks and food products.

Keywords: Turmeric powder, liver functions, glutathione fractions, glycogen, MDA, cytochrome p450.

الملخص العربي :

تم تصميم العمل الحالي لدراسة تأثير مسحوق جذور الكركم على اضطرابات الكبد التي يسببها مركب البنزوبيرين في الفئران. حيث أدت معاملة الفئران بمركب البنزوبيرين الى زيادة معنوية (($p\leq0.05$في الانزيمات المعبرة عن وظائف الكبد AST (72.71٪)، ALT (116.67٪)و ALP (238.41٪)مقارنة بالمجموعة الضابطة (الطبيعية). حالت تدعيم حمية الفئران بمسحوق الكركم (٠,٥٠ و ١,٠٠ و ١,٥٠ و ٢,٠٠ جم / ١٠٠ جم) دون ارتفاع متوسط أنشطة الانزيمات السابقة AST و ALT و .ALPفى السيرم وتم تسجيل نفس السلوك لمركب المالونالدهيد ، الدليل الحيوي المعبر عن الإجهاد التأكسدي في الخلايا ، وكذلك المستويات في إنزيم استقلاب المواد الغريبة الداخة للجسم (السيتوكروم ب٤٥٠) في خلايا الكبد. كما تم تسجيل الاتجاه المعاكس لأجزاء الجلوتاثيون (الجزيئات البيولوجية المضادة للأكسدة) في سيرم الدم. دعمت هذه النتائج فرضيتنا القائلة بأن مسحوق الكركم يحتوي على عدة كجكوعات من المركبات النشطة بيولوجيًا القادرة على منع / تثبيط اضطرابات الكبد التي يسببها مركب البنزوبيرين من خلال نشاط خفض إنزيمات الكبد فى السيرم ، وتقليل معدل تكوين مركب المالونالدهيد ، وتقليل النشاط من أنزيم الأيض أي السيتوكروم ب٤٥٠، وزيادة مستويات أجزاء الجلوتاثيون في

سيرم الدم. لذلك أوصت الدراسة الحالية بتضمين الكركم بنسبة تركيز تصل إلى ٢٪ في وجباتنا الغذائية والمشروبات ومنتجاتنا الغذائية اليومية
الكلمات المفتاحية: مسحوق الكركم ، وظائف الكبد ، أجزاء الجلوتاثيون ، الجليكوجين ، المالونالدهيد، السيتوكروم ب٤٥٠.

Introduction :

Liver is a vital and principal organ present in all vertebrates (from fish to human). Such as mentioned by Crawford, (1999), it has a wide range of functions, including detoxification, protein synthesis, and production of biochemical necessary for digestion. Also, liver plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification processes. Furthermore, liver produces bile juice, 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 (reviewed in Elhassaneen, 1996; Kebamo et al., 2015).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.

The burden of liver diseases has been increasing in Egypt with a doubling of the incidence rate in the past twenty years. This has been attributed to several biological (e.g. virus infection) and environmental/dietary factors (e.g. Aflatoxin, polycyclic aromatic hydrocarbons) (Elhassaneen, 1996, 2004 and

Elhassaneen et al., 2016). Other factors such as cigarette smoking, occupational exposure to chemicals such as pesticides and heavy metals, and endemic infections in the community, as schistosomiasis, may have additional roles in the etiology or progression of the disease (Anwar et al., 2008). The World Health Organization (WHO, 2010) reported that Egypt has one of the highest incidences of hepatitis C, one of the main causes of liver cancer, in the world. The number of deaths resulting from liver cancer in Egypt had risen from 4% in 1993 to 11% in 2009. Cancer begins when cells in a part of the body start to grow out of control. There are many kinds of cancer, but they all start because of out-of-control growth of abnormal cells. Cancer cell growth is different from normal cell growth. Instead of dying, cancer cells continue to grow and form new, abnormal cells. Cancer cells can also invade (grow into) other tissues, something that normal cells cannot do. Growing out of control and invading other tissues are what makes a cell a cancer cell. In most cases the cancer cells form a tumor like liver cancer (Howlader et al., 2012). Hepatocellular cancer (HCC) can have different growth patterns like: Some begin as a single tumor that grows larger. Only late in the disease does it spread to other parts of the liver. The second type seems to start as many small cancer nodules throughout the liver, not just a single tumor. This is seen most often in people with cirrhosis (chronic liver damage) (American Cancer Society, 2013). Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world, representing the third leading cause of death from cancer and the fifth most

prevalent malignancy worldwide (Bosch et al., 1999; Farazi et al., 2006; El-Serag et al., 2007)). Owing to advances in diagnostics and therapeutics, HCC can be creatively treated when detected at an early stage by applying therapies including radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE) and surgical resection. However, curative treat¬ments are often hampered by frequent recurrence of HCC (Okuda, 2007) because the remaining liver retains the potential for de novo carcinogenesis (Kumada et al.,1997). Although systemic chemotherapy has also been challenged to patients with advanced stages of HCC, it is mostly ineffective (Thomas, 2008).

Several studies reported that cooking can produce toxic compounds in foods, if the appropriate precursors are present. Cooking processes have been found to be a major source of toxic compounds in foods such PAHs. Although, PAHs can also be formed in curing and processing of raw food prior to cooking (Gray and Morton, 1981; Elhassaneen, 1996-1999; Elhassaneen and Tawfik, 1998; Elhassaneen et al., 1999; Huosein, 2011; Adeyeye, 2020). 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; Maanen et al., 1994; Bassiouny, 1999; Elhassaneen, 1999-2004; Hassan, 2005). Benzo(a)pyrene [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, 1996; 2004; Elhassaneen and El-Badawy, 2013; Adeyeye, 2020). B(a)P is also found in outdoor and indoor air, and in some water sources (USEPA, 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; Mehram and Sayed-Ahmed, 2020) and in vitro (Sims and Grover, 1974; 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; Elhassaneen et al., 2016; Anita, 2018). 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 (Weinstein, 1978; Philips and Sims, 1979; Harvey, 1982, 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 (MacKenzie and Angevine 1981; Elhassaneen 1996; Anita, 2018).

The modern pharmacological therapy is costly and associated with multiple side effects resulting in patient non-compliance.

Thus there is a need to explore alternative therapies particularly from herbal/plant sources as these are cost effective and possess minimal side effects. Also, it was reported that some plants exercise various bioactivities, including antioxidant, anti-inflammatory,antidiabetic, anticarcinogenic, antimutagenic etc (El-Safty, 2012 and Elhassaneen and Sayed, 2015; Elhassaneen et al., 2016-b; Elmaadawy, 2016; Elhassaneen et al., 2021).

One of the still less studied plants is the turmeric (Curcuma longa L.) belongs to the Zingiberaceae family along with the other noteworthy members like ginger, cardamom and galangal. The plant is a perennial, rhizomatous, herbaceous plant native to the Indian subcontinent and Southeast Asia, that requires temperatures between 20 and 30 °C and a considerable amount of annual rainfall to thrive. Plants are gathered each year for their rhizomes, some for propagation in the following season and some for consumption (Peter, 2008). Turmeric 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) (Rathaur et al., 2012). Turmeric contains a wide variety of bioactive compounds including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones,

and turmeronols (Chattopadhyay et al., 2004; Sello and Eldemery, 2018). Also, it 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, and inhibition of tumors formation and promotion as cancer initiation (Aggarwal et al., 2007; Nafisi et al., 2009; Agrawal and Mishra, 2010; Sello and Eldemery, 2018). According to our knowledge, the studies regarding the potential effects of turmeric on liver diseases are so limited. Therefore, the aims of the present study were determine the effects of turmeric on liver disorders induced by Benzo(a)pyrene in rats. Also, the determination of chemical composition and bioactive compounds in turmeric rhizomes powder will be in the scope of this study.

Materials and Methods

Materials

Turmeric (Curcuma longa) powder was purchased from the local markets of Benha City, Benha, Egypt. Casein, as main source of protein for rat diets preparation purchased from Morgan Company for Chemicals. Cairo, Egypt. Vitamins and salts mixtures, all organic solvents, buffers and other chemicals were of analytical grade were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical Requirements, 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.

Methods

Proximate chemical analysis of turmeric rhizomes powder samples Turmeric rhizomes powder 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).

Bioactive compounds analysis of turmeric rhizomes powder samples 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). 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. Under such chromatographic conditions, mean values (±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 of turmeric rhizomes powder samples

Antioxidant activity of turmeric extracts and standards (a-tocopherol, BHA, and BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the b-carotene bleaching method following a modification of the procedure described by Marco (1968).

Biological Experiments

Animals

Animals used in this study, adult male albino rats (130-150 g per each) were obtained from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

Basal Diet (BD)

The BD 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 vitamin and salt mixture component were formulated according to AIN, (1993).

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=50 rats), 130-150g per each, were housed individually in wire cages in a room maintained at 25 ± 2 0C, 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, 5 rats, as a negative control group) still fed on basal diet and injected with the vehicle alone (5 ml/kg body weight) and the other main group (45 rats) was were challenged with an ip injection of B(a)P (100 mg/5 ml/kg body weight) dissolved in 0.9% NaCI solution containing 0.1% Tween 20 to induce liver impaired rats then classified into eight sub- groups as follow:

- Group (2): fed on standard diet only as a positive control
- Group (3): fed on standard diet containing 0.50 % turmeric powder
- Group (4): fed on standard diet containing 1.00 % turmeric powder
- Group (5): fed on standard diet containing 1.50 % turmeric powder

Group (6): fed on standard diet containing 2.00 % 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 -20oC until analysis.

Hematological analysis

Liver functions

Serum glutamic pyruvic transaminase (SGPT/ALT), glutamic oxaloacetic transaminase (SGOT/AST) and alkaline Phosphatase (ALP) were measured in serum using the method of Tietz et al., (1976), Tietz et al., (1976) and Vassault et al., (1999), respectively.

Glutathione (GSH) determination

GSH was determined by HPLC according to the method of McFarris and Reed (1987).

Liver glycogen determination

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)

Malonaldialdehyde (MDA) content determination

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).

Drug Metabolizing Enzymes (Cytochrome P-450) determination 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± standard deviations (SD). Data were arranged with Excel Sheet (Excel Program, USA) and statistical analysis was performed with the Student t-test and MINITAB 12 computer program (Minitab Inc., State College, PA, USA).

Results and Discussion

Proximate chemical composition of turmeric powder

The proximate composition of turmeric powder is shown in Table 1. The results showed that the moisture content was 9.78%, total protein was 6.92%, crude fat was 4.44%, crude fiber was 5.11%, ash content was 2.66% and total carbohydrate content was 71.09%. The proximate composition reported was partially accordance with the recorded by Jaggi, (2012) and Sello and Eldemery (2017) but not accordance with that observed by Kapoor, (1990) and Ruby et al., (1995). Such variation in data reflected the effect of different parameters including varieties, seasonality, geographic conditions, soil, agriculture treatments etc., on chemical composition of different plant parts which among them turmeric rhizomes.

Table 1. Proximate chemical composition of turmeric powder

Component	Content (g/l100g)
Water	9.78 ±1.43
Total protein	6.92 ± 1.11
Crude fat	4.44± 0.74
Ash	2.66 ± 0.56
Crude fiber	5.11 ± 0.91
Carbohydrate (By diference)	71.09 ± 3.25

Each value represents the mean of three replicates ±SD.

Bioactive compounds and antioxidant activity of turmeric powder

Data in Table (2) showed the bioactive compounds and antioxidant activity of turmeric powder. From such data it could be noticed that there are the antioxidant vitamins such as vitamins A *(β*-carotene), C, and E, found in turmeric powder for which there are Dietary Reference Values (DRVs). Such data was partially accordance with the recorded by Sello and Eldemery (2017). Vitamins are important in maintenance of the body equilibrium thereby reducing the risk associated with nutrient deficiency in humans. Vitamin C functions as a cofactor in many enzymatic reactions in animals (including humans) that mediate a variety of essential biological

functions, including wound healing and collagen synthesis. Another biochemical role of vitamin C is to act as an antioxidant (a reducing agent) by donating electrons to various enzymatic and non-enzymatic reactions (DRI, 2000; Ang et al., 2018). Also, vitamin C has an effect on cancer. Within the normal range of dietary intake without additional dietary supplementation, are people who consume more vitamin C at lower risk for developing cancer (*Stratton and Godwin, 2011; Cortés-Jofré et al., 2020)* Also, for people diagnosed with cancer, will large amounts of ascorbic acid administered intravenously treat the cancer (*Papaioannou et al., 2011; Xu* er al., 2013). Vitamin A plays a role in a variety of functions throughout the body, such as vision, gene transcription, immune function, embryonic development and reproduction, bone metabolism, haematopoiesis, skin and cellular health, teeth and mucous membrane (2020). Vitamin E has the following functions: it is an antioxidant, it helps keep the immune system strong against viruses and bacteria, it helps form red blood cells and widen blood vessels to keep blood from clotting inside them, it helps the body use vitamin K and cells also use vitamin E to interact with each other (Traber and Bruno, 2020). Also, many biological functions have been postulated, including a role as a fat-soluble antioxidant. In this role, vitamin E acts as a radical scavenger, delivering a hydrogen (H) atom to free radicals (*DRI, 2019).* A further

revision of *USFD* , (2012) allowed that vitamin E may reduce risk of renal, bladder and colorectal cancers.

Table 2. Bioactive compounds and antioxidant activity of turmeric powder

Component	Content
Vitamins:	
Vitamin A (β-carotene, mg/100g)	20.34± 3.22
Ascorbic acid (mg/100g)	64.63± 4.56
Vitamin E (mg/100g)	8.98 ± 0.98
Bioactive compounds:	
Essential oil (g/100g)	4.01 ± 0.54
Total carotenoids (mg.100g)	91.32 ± 8.65
Curcumin (%) - Ethanolic extract	13.72 ± 2.65
Total phenolics content (mg GAE.g-1) - Ethanolic extract	21.65 ± 10.0.4
Antioxidant activity (AA)	
AA, % (Ethanolic extract)	81.48 ± 4.05

Each value represents the mean of three replicates ±SD.

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 turmeric). Such as shown in table (4) Turmeric powder is rich in different classes of bioactive compounds including phenolics (21.65 ± 10.0.4 mg GAE.g^{-1} extract), essential oils (4.01 ± 0.54 g/100g), total carotenoids (91.32 ± 8.65, mg.100g) and Curcumin (13.72 ± 2.65 % in ethanolic extract). 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; Elhassaneen *et al.,* 2013; Sello; Eldemery, 2017 ; Elhassaneen et al., 2019; Mehram et al., 2021). 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, anemia, liver diseases, 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.,* 2016; Elhassaneen *et al.,* 2016 $_{a-b}$, 2019 and 2020; Mehram et al., 2021).

Finally, all of these macro and micro nutrients found in turmeric powder might be important from the nutrition point of view. Therefore, enrichment of different food products with turmeric powder would enhance the nutritional quality of the product better than many food sources. Also, bioactive compounds including phenolics, carotenoids, essential oil etc in the turmeric powder are giving such food high significant as an important functional food.

The effect of turmeric powder on liver functions of rats injected with B(*a*)P

Liver functions of rats injected B(*a*)P and consumed turmeric powder were shown in Table (3). From such data it could be noticed that treatment of animals with B(*a*)P caused a significant($p \leq 0.05$) increased in AST (72.71%), ALT (116.67%) and ALP (238.41%) compared to normal controls. Supplementation of the rat diets with turmeric powders (0.50, 1.00, 1.50, and 2.00 g/100g) prevented the rise of mean serum AST, ALT and ALP activities. A does-response exhibited between the turmeric powder and the rate of preventative. Such as shown in Figures (1-7), the rate of increasing in the liver enzymatic activities were recorded 45.02, 36.63, 28.53 and 24.33% (For AST); 88.96, 85.91, 63.79 and 56.40% (for ALT); and 169.16, 144.60, 121.16 and 88.77% (for ALP) with the rat diets supplemented by 0.50, 1.00, 1.50, and 2.00 g/100g of turmeric powder, respectively. Such data are in accordance with that observed by Mehram and Sayed-Ahmed (2020) who found that B(*a*)P induced liver disorders in rats and protective effects of mulberry (*Morus alba* L.) leaves (contains bioactive compounds such as found in turmeric powder) were recorded.

In general, B(*a*)P is one of the most commonly used hepatotoxins, *in vitro* and *in vivo*, in the experimental study of liver diseases. The hepatotoxic effects of B(*a*)P are largely due to its active metabolites, oxides, hydroxyls, polyhydroxyls and quinones radicals (Elhassaneen, 1996). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of cell wall membrane,

mitochondria, lysosomes and endoplasmic reticulum rich in polyunsaturated fatty acids (Elhassaneen, 1996 and Elhassaneen et al., 1997; Elhassaneen et al., 2016-a). This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of B(*a*)P (Hasegawa *et al.,* 1995;Elhassaneen, 1996; Elhassaneen, 2004 and Mehram and Sayed-Ahmed, 2019). This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP and decrease in protein (Elhassaneen, 2004; Elhassaneen and Al-Badawy, 2013). In the assessment of liver damage by B(*a*)P the determination of enzyme levels such as AST, ALT and ALP is largely used (Hassan, 2011; Elhassaneen and Al-Badawy, 2013; Elhassaneen et al., 2016 $_{a-b}$; Saleh, 2016 ; Mehram and Sayed-Ahmed, 2019). Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. High levels of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury, AST catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Voet and Voet, 1990). Serum ALP, bilirubin and total protein levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure (Muriel and Garcipiana, 1992).

Table (3). Effects of turmeric powder on liver functions of rats injected with B(*a*)P

Rats no.	Control (Ve^-) Std diet	Control (Ve^+) B(*a*)P	Turmeric Powder (%, w/w)			
			0.50	1.00	1.50	2.00
Aspartate aminotransferase activity, AST, U/L						
Mean	62.33 d	107.66 a	90.40 b	85.17 b	80.1bc	77.50 bc
SD	6.75	8.53	6.52	6.32	7.76	6.94
% of Change	0.00	72.71	45.02	36.63	28.53	24.33
Alanine aminotransferase activity, ALT, U/L						
Mean	33.53 d	72.65 a	63.36 b	62.33 b	54.92 c	52.44 c
SD	6.37	11.33	14.36	12.46	11.48	9.80
% of Change	0.00	116.67	88.96	85.91	63.79	56.40
Alkaline phosphatase activity, ALP, U/L						
Mean	96.83 f	327.67 a	260.62 b	236.8 c	214.14 d	182.77 e
SD	18.60	43.29	18.80	28.25	19.90	13.75
% of Change	0.00	238.41	169.16	144.60	121.16	88.77

* Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$.

It is reviewed in several studies plant parts including turmeric powder are a rich source of different classes of phytochemicals such alkaloids, carotenoids, phytosterols, phenols and organosulfurs (Harborne, 1998; Harborne and Mabry, 1982, Beattic *et al.,* 2005; Sddiqi *et al.,* 2012; Elhassaneen *et al.,*

2013; Sello and Eldemery, 2017; Elhassaneen et al., 2019; Mehram et al., 2021). Many 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 those bioactive compounds. For example, Hassan, (2011) found that different doses of apricot kernel extracts showed high-decreased in serum GOT, GPT and ALP after 12 week of feeding when compared with control group.). Also, El-Sayed *et al.*, (2012) found that the addition of tested plant parts such Henada, lemon balm leaves, hawthorn leaves, rose of jericho and corn cob silk by 5 and 10% of the diet intake in the presence of CCl_4 induced significantly improvements in all liver functions including the serum activities of AST, ALT and ALP. The potential mode of action of liver serum enzymes-lowering activity of the turmeric powder could be explained by one or more of the following process: 1) flavonoid is known to block the hepatocellular uptake of bile acids (Dawson, 1998), 2) flavonoids pretreatment improved the antioxidant capacity of the liver, diminished the bilirubin concentration compared with the groups without treatment (Beattic *et al.*,2005), 3) pre-treatment with flavonoids were not only able to suppress the elevation of AST and ALT but also reduce the damage of hepatocytes *in vitro* through exhibited strong antioxidant activity and scavengers against reactive oxygen species (ROS) (El-Nashar, 2007), 4) pre-treatment with apricot kernel extract rich in phytochemicals 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

(Hassan, 2011), and 5) pre-treatment with phenolics and carotenoids were not only able to suppress the elevation of AST, ALT and ALP but also reduce the damage of rats hepatocytes through exhibited strong antioxidant activities against oxidative stress biomarkers (Mehram and Sayed Ahmed, 2020).

Effects of turmeric powder on serum albumin of rats injected with B(*a*)P

Data presented in Table (4) showed effect of feeding turmeric powders on serum albumin of rats treated with B(*a*)P. It was noticed that the mean value of albumin for control (-) group was 4.05 g/dl while there were significantly (P≤0.05) decreased in serum albumin for control (+) group with percent of change -18.91 compared to control (-) group. Feeding with turmeric powders, lead to significantly (P ≤0.05) increased in serum albumin compared to control (+) group by the ratio of -21.13, -14.87, -11.70 and -8.79% with the rat diets supplemented by 0.50, 1.00, 1.50 and 2.00 g/100g of turmeric powder, respectively. A does-response exhibited between the turmeric powder and the rate of preventative. Such data are in accordance with that observed by Mehram and Sayed-Ahmed (2020) who found that benzo(*a*)pyrene induced liver disorders in rats and protective effects of mulberry leaves (contains bioactive compounds such as found in turmeric powder) including manipulation of albumin content was recorded. Also, In similar studies of Wang *et al*. (2007), Koneri *et al*. (2008), Hedge and Joshi (2009) and Elhassaneen et al., 2016-

a) they reported that B(*a*)P induced significant decrease in the serum albumin content. Also, it was reported that hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases (Koneri *et al*., 2008). Therefore, decline in serum albumin can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. Furthermore, side treatment with artichoke significantly increased the reduced levels of serum albumin which was in agreement with previous studies (Abd El-Aleem *et al*., 2009).

Table (4). Effects of turmeric powder on serum albumin concentration (g/dl) of rats injected with B(*a*)P

Rats no.	Control (Ve^-) Std diet	Control (Ve^+) B(*a*)P	Turmeric Powder (%, w/w)			
			0.50	1.00	1.50	2.00
Sum	20.27	16.44	15.99	17.25	17.90	18.49
Mean	4.05 [a]	3.29 [b]	3.20 [bc]	3.45 [b]	3.58 [b]	3.70 [b]
SD	0.16	0.39	0.25	0.30	0.23	0.21
% of Change	0.00	-18.91	-21.13	-14.87	-11.70	-8.79

* Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$.

Effects of turmeric powder on in liver glycogen concentration (mg/g tissue) of rats injected with B(*a*)P

Data presented in Table (5) showed effect of feeding turmeric powders on liver glycogen of rats treated with B(*a*)P. It was noticed that the mean value of liver glycogen for control (-)

group was 13.85 mg/g tissue which decreased after B(*a*)P injection by -89.80%. Such liver glycogen decrease was significantly elevated in the co-treatment with B(*a*)P plus turmeric powder. A does-response exhibited between the turmeric powder and the rate of preventative. The rate of increasing in glycogen was recorded -86.58, -79.29, -66.06 and -54.60 % (from the positive control group) with the rat diets supplemented by 0.50, 1.00, 1.50 and 2.00 g/100g of turmeric powder, respectively. Such data are in accordance with that observed by Mehram and Sayed-Ahmed (2020) who found that benzo(*a*)pyrene induced decrease in liver glycogen in rats and protective effects of mulberry leaves (contains bioactive compounds such as found in turmeric powder) was recorded. Also, Hasegawa et al., (1995) found that previous drinking of green tea clearly protected against the changes in liver glycogen content induced by B(*a*)P. Such data with the others 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 (Hasegawa *et al.,* 1995, Mehram and Sayed-Ahmed, 2019).

Table (5). Effects of turmeric powder on in liver glycogen concentration (mg/g tissue) of rats injected with B(*a*)P

Rats no.	Control (Ve^-) Std diet	Control (Ve^+) B(*a*)P	Turmeric Powder (%, w/w)			
			0.50	1.00	1.50	2.00
Mean	13.85 [a]	1.40 [d]	1.86 [d]	2.87 [c]	4.70 [c]	6.29 [b]
SD	1.65	0.15	0.23	0.34	0.76	0.74
% of Change	0.00	-89.88	-86.58	-79.29	-66.06	-54.60

* Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$.

Effects of turmeric powder on in serum triglycerides concentration of rats injected with B(*a*)P

Serum triglycerides level of rats injected with B(*a*)P and consumed turmeric powder was shown in Table (6). From such data it could be noticed that the serum triglycerides level was decreased -87.15 by B(*a*)P, and this decrease was significantly ($p \leq 0.05\%$) elevated in the co-treatment of B(*a*)P plus turmeric powder. A does-response exhibited between the turmeric powder and the rate of preventative. The rate of increasing in triglycerides was recorded -86.80, -83.91, -78.33 and -74.52% (from the positive control group) with the rat diets supplemented by 0.50, 1.00, 1.50 and 2.00 g/100g of turmeric powder, respectively. Such data are in accordance with that observed by Mehram and Sayed-Ahmed (2020) who found that benzo(*a*)pyrene induced increase in serum triglycerides in rats

and protective effects of mulberry leaves (contains bioactive compounds such as found in turmeric powder) was recorded.

Table (6). Effects of turmeric powder on in serum triglycerides concentration (mg/dl) of rats injected with B(*a*)P

Rats no.	Control (Ve^-) Std diet	Control (Ve^+) B(*a*)P	Turmeric Powder (%, w/w)			
			0.50	1.00	1.50	2.00
Sum	757.66	97.38	100.02	121.91	164.18	193.08
Mean	151.53 a	19.48 cd	20.00 c	24.38 c	32.84 b	38.62 b
SD	22.24	3.11	3.43	4.37	6.42	5.99
% of Change	0.00	-87.15	-86.80	-83.91	-78.33	-74.52

* Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$.

Effects of turmeric powder on liver glutathione (GSH) content of rats injected with B(*a*)P

Data presented in table (7) showed the effect of feeding turmeric powder on liver glutathione (content of rats treated with B(*a*)P. from such data it could be noticed that the mean value of GSH for control (-) group was 9.56 g/dl while there were significantly ($P \leq 0.05$) decreased in liver GSH for control (+) group with percent of change -38.63 compared to control (-) group. The feeding with turmeric powder lead to significantly ($P \leq 0.05$) increased in serum GSH compared to control (+) group by the ratio of were -36.59, -27.74, -20.07 and -14.73% for turmeric powder. Data also indicated that the rate of liver

GSH elevation was increased with the increasing of the turmeric powder concentration. Such data are in accordance with that observed by Mehram and Sayed-Ahmed (2020) who found that benzo(*a*)pyrene induced decrease in liver GSH in rats and protective effects of mulberry leaves (contains bioactive compounds such as found in turmeric powder) was recorded.

Table (7). Effects of turmeric powder on liver glutathione (GSH, µmol / mg tissue protein) content of rats injected with B(*a*)P

Rats no.	Control (Ve^-) Std diet	Control (Ve^+) B(*a*)P	Turmeric Powder (%, w/w)			
			0.50	1.00	1.50	2.00
Mean	9.56	5.87	6.06	6.91	7.64	8.15
SD	1.14	1.05	0.72	1.04	1.27	1.40
% of Change	0.00	-38.63	-36.59	-27.74	-20.07	-14.73

* Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$.

In general, GSH is a tripeptide-thiol (γ-glutamyl cysteinyl-glycine) that has received considerable attention in terms of its biosynthesis, regulation, and various intracellular functions. Among of these function do two constitute roles in detoxifications as follow: as a key conjugate of electrophilic intermediates, principally via glutathione-s-transferase activities in phase II metabolism, and as an important antioxidant (Elhassaneen, 1996 and 2004). The antioxidant

functions of GSH includes its role in the activities of GSH-Px and AS-Px . In addition, GSH can apparently serve as a nonenzymatic scavenger of oxyradicals (Halliwell and Gutteridge, 1985 and Elhassaneen, 1996). Several studies noticed that a fall in GSH observed generally accompanied by a concomitant increased in the liver lipid peroxidation (MDA content) (Elhassaneen, 2004; Mahran et al., 2018; Mehram and Sayed-Ahmed, 2020). Several reports have documented that potent antioxidant capacity of curcumin (the main bioactive compound in turmeric) where by mitigation of lipid peroxidation and oxidative stress in several tissues were demonstrated (Nabavi *et al*., 2012). The present data with the others suggested that secretion of GSH from liver to blood might be blocked because of intracellular structural failure, elevation of the lipid peroxide content and/or the energy depletion suggested by the marked decrease in glycogen content (Hasegawa *et al.*, 1995; Sello and Eldemery, 2017; Mehram and Sayed-Ahmed, 2020). Turmeric powder was described by its higher content of different categories of bioactive compounds including curcumin which exhibited high antioxidative activities and anticarcinogenic effects (Boone et al., 1992; Chauhan, 2002; Srinivasan, 2005, Shishodia *et al.*, 2006; Jurenka, 2009; Nabavi *et al.*, 2012; Zheng *et al.*, 2014) . Supplementation of the rat diets with turmeric powder leads to reduce the lipid peroxide content and/or elevate the glycogen content subsequently elevate the GSH content in liver.

Effects of turmeric powder on serum lipid peroxidation of rats injected with B(*a*)P

Lipid peroxidation in serum and liver of rats injected with B(*a*)P and feeded turmeric powder were shown in Table (8-9). From such data it could be noticed that the liver lipid peroxide level (MDA) was increased 262.07% by B(*a*)P injection. This increasing was significantly (P≤0.05) reduced in the co-treatment of B(*a*)P plus turmeric powder. A does-response exhibited between the turmeric powder and the rate of preventative. Such as data were shown, the rate of increasing in MDA was recorded 199.08, 117.79, 98.07 and 87.22 % of the control negative group with the rat diets supplemented by 0.50, 1.00, 1.50 and 2.00 g/100g of turmeric powder, respectively. The opposite direction was observed for the lipid peroxidation in serum of rats injected with B(*a*)P and fed turmeric powder were shown in Tables (5). From such data it could be noticed that the serum lipid peroxide level (MDA) was decreased -49.73% by B(*a*)P injection. This increasing was significantly (P≤0.05) elevated in the co-treatment of B(*a*)P plus turmeric powder. A does-response exhibited between the turmeric powder and the rate of preventative. Such as data were shown, the rate of decreasing in MDA was recorded -47.00, -47.32, -44.10 and -39.50 % of the control negative group with the rat diets supplemented by 0.50, 1.00, 1.50 and 2.00 g/100g of turmeric powder, respectively Such data are partially in accordance with that observed by Mehram and Sayed-Ahmed (2020) who found that benzo(*a*)pyrene induced increasing in liver MDA in rats and protective effects of mulberry leaves

(contains bioactive compounds such as found in turmeric powder) was recorded.

Table (8). Effects of turmeric powder on liver lipid peroxidation (MDA, nmol/mg tissue protein) of rats injected with B(*a*)P

Rats no.	Control (Ve^-) Std diet	Control (Ve^+) B(*a*)P	Turmeric Powder (%, w/w)			
			0.50	1.00	1.50	2.00
Mean	3.88 ^{e}	14.04 ^{a}	11.59 ^{b}	8.44 ^{c}	7.68 ^{c}	7.26 ^{cd}
SD	0.94	3.92	2.84	1.60	1.45	2.90
% of Change	0.00	262.07	199.08	117.79	98.07	87.22

* Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$.

Table (9). Effects of turmeric powder on serum lipid peroxidation (MDA, nmol/ml) of rats injected with B(*a*)P

Rats no.	Control (Ve-) Std diet	Control (Ve+) B(a)P	Turmeric Powder (%, w/w)			
			0.50	1.00	1.50	2.00
Mean	0.179 a	0.090 bc	0.095 bc	0.095 bc	0.100 b	0.109 b
SD	0.044	0.022	0.022	0.023	0.024	0.025
% of Change	0.00	-49.73	-47.00	-47.00	-44.10	-39.50

* Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$.

Many studies displayed the potent antioxidant capacities of curcumin (the main bioactive component in turmeric powder) where by mitigation of lipid peroxidation and oxidative stress in several tissues were demonstrated (Nabavi *et al*., 2012, Elhassaneen *et al.*, 2016-a). Also, Hasegawa et al., (1995) found that previous drinking of green tea 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 rats. Such data with the others 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 (Hasegawa *et al.*, 1995; Mehram and Sayed-Ahmed 2020). The increases of serum enzyme activities were clearly inhibited by turmeric powder consumption. Therefore, a marked decreasing in serum triglyceride levels was noticed in all B(*a*)P treated rats probably being related to the low levels of serum lipid peroxide in all treated groups.

Effects of turmeric powder on the activity of cytochrome-P450 (cyto-p450) in liver of rats injected with B(*a*)P

Effects of turmeric powder treatments on the activity of cyto - P450 in liver of rats subjected to B(*a*)P treatment was shown in Table (10). From such data it could be noticed that treatment of rats with B(*a*)P caused a significant increased ($p \leq 0.05$) in cyto-P450 by (47.46%) compared to normal/negative control animals. Feeding of the rat diets with turmeric powder by 0.50, 1.00, 1.50 and 2.00 g/100g w/w prevented the rising of mean liver cyto- P450 activity. The rate of preventative effect was

elevated with the increasing of the turmeric powder concentration. The rate of decreasing in the cyto- P450 activities were -35.03, 28.81, 16.38 and 7.34% of the negative control group with the rat diets blended with 0.50, 1.00, 1.50 and 2.00 g/100g of turmeric powder, respectively. In similar study, Mehram and Sayed-Ahmed (2020) found that benzo(*a*)pyrene induced increasing in liver cyto-p450 in rats and protective effects of mulberry leaves (contains bioactive compounds such as found in turmeric powder) was recorded. Also, Liu *et al.*, (2015) reported that B(*a*)P treatment brought about a significant increase in the activities of drug metabolizing enzymes including cyto- p450 in lungs of mice and the activities of these enzymes were markedly decreased by the administration of bioactive compounds including curcumin (the main bioactive in turmeric powder) and phenolic compounds.

Table (10). Effects of turmeric powder on the activity of cytochrome P450 in liver (nano moles/mg protein) of rats injected with B(*a*)P

Rats no.	Control (Ve^-) Std diet	Control (Ve^+) B(*a*)P	Turmeric Powder (%, w/w)			
			0.50	1.00	1.50	2.00
Mean	1.77 [f]	2.61[a]	2.39 [ab]	2.28 [b]	2.06 [c]	1.90 [d]
SD	0.041	0.023	0.022	0.023	0.024	0.025
% of Change	0.00	47.46	35.03	28.81	16.38	7.34

* Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$.

In conclusion, benzo(*a*)pyrene is considered a food contaminant and a top risk factor in the development of several diseases including liver disorders. Oxidative stress appears as a major contributor in the development of many metabolic complications associated liver disorders/diseases. Lowering oxidative stress to prevent such liver metabolic disorders and complications therefore constitutes an interesting target. Feeding of turmeric applied in foods has been proven to be essential in the treatment and/or prevention of liver disorders induced by B(*a*)P through oxidative stress reduction and xenobiotics metabolizing enzyme i.e. cytochrome p450 activity decreasing. Therefore, we recommended turmeric powder to be included in our daily live dishes and beverages.

References

- Abd El-Aleem, I.M.; El-Deeb, A.E.; Abd El-Rahman, A.A. and El Hadary, A.E.A. (2009): Antioxidant Properties of Artichoke leaves and Onion skin Carbo Tetrachloride – Induced Rat Liver Injury; 24 (11): 43-64.
- Adeyeye, S. O. (2020). Polycyclic Aromatic Hydrocarbons in Foods: A Critical Review. Current Nutrition & Food Science. 16 (6): 866 – 873.
- Aggarwal, B. B.; Sundaram, C.; Malani, N.; and Ichikawa, H. (2007): Curcumin the Indian solid gold. Adv. Exp. Med. Biol., 595: 1–75 .
- AIN. American Institute of Nutrition. (1993). Purified diet for laboratory Rodent, Final report. J. Nutrition. 123:1939-1951.

- *Ang A, Pullar JM, Currie MJ, Vissers M (2018).* "Vitamin C and immune cell function in inflammation and cancer". *Biochemical Society Transactions. 46 (5): 1147–1159*.
- Anita L. (2018). Polycyclic Aromatic Hydrocarbons: Sources, Importance and Fate in the Atmospheric Environment. Current Organic Chemistry, 22 (11): 1050 – 1069
- Anwar W, Khaled Hussein M, Amra Hassan A, El-Nezami Hani, Loffred Christopher A. (2008). Changing pattern of hepatocellular carcinoma (HCC) and its risk factors in Egypt: possibilities for prevention. Mutat Res, 659:176–84.
- Asp, N.G.; Johnson, C.G.; Hallomer, H. and Siljestrom, H (1983). Rapid enzymatic assay of insoluble dietary fiber. Journal of Agricultural and Food Chemistry, 31, 476-482.
- Bassiouny, E. (1999). The effect of grilled meat consumption on health " M. Sc. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt.
- Beattic, J.; Crozier, A. and Duthie, G. (2005): Potential Health Benefits of berries. Current Nutrition and Food Science. 1: 71-86.
- Boone, C.W.; Steele, V.E. and Kelloff, G.J. (1992). Screening of chemopreventive (anticarcinogenic) compounds in rodents. Mut Res;267:251-255.

- Buege, J.A. and Aust, S.D. (1978): Microsomal lipid peroxidation in Packer L., (ed), Methods in enzymology, New York, NY, Academic, 52: 302 - 310.
- Chaitanya, K.V.; Pathan, A.A.K.; Mazumdar, S.S.; Charavarthi, G.P.; Parine, N. and Bobbarala, V. (2010): Role of oxidative stress in human health: An overview. Journal of Pharmacy Research. 3: 1330-1333.
- Chattopadhyay, I.; Biswas, K.; Bandopadhyay, U.; and Banerjee, R. K. (2004): Turmeric and curcumin: Biological actions and medicinal applications. Curr Sci., 87: 44-53.
- Chauhan, D.P. (2002). Chemotherapeutic potential of curcumin for colorectal cancer. Current pharmaceutical Design, 8 (19): 1695 – 1706.
- Cortés-Jofré M, Rueda JR, Asenjo-Lobos C, Madrid E, Bonfill Cosp X (2020). "Drugs for preventing lung cancer in healthy people". The Cochrane Database of Systematic Reviews. 2020 (3): CD002141.
- Crawford, J. M. (1999). The Liver and the Biliary Tract., Pathologic Basis of Disease. Eds. R. S. Cotran, V. Kumar, and T. Collins. W. B. Saunders Company: Philadelphia, USA.
- Dawson, R. M.(1998). The toxicology of microcystins. Toxicon.,36:953-62.
- *DRI, (2000).* "Vitamin C". *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: The National Academies Press. 2000. pp. 95–185*

- Drury, R.A. and Wallington, E.A. (1980). Carlton's Histological Technique. 5th ed. Oxford University.
- Elhassaneen A. and Tawfik, L. (1998).(A) The presence of some carcinogens in human foods distributed in Egyptian local markets. Journal of Home Economics, 8 (3): 23 - 38.
- Elhassaneen Y. and Sayed, R. (2015). Polycyclic aromatic hydrocarbons formation in charcoal broiled meatballs are reduced by addition of onion peel extracts to ground beef. Proceeding of 2nd International Conference on Food and Biosystems Engineering (2nd I.C. FABE2015), 28-31 May, 2015), [Vol. II, PP. 95-104], Mykonos, Greece .
- Elhassaneen, Y. (2002). New and quickly biological method for detection the potential chemical toxins and/or carcinogens in foods. Proceedings of2nd scientific Conference on Foodborne Contamination and Egyptian's Health (24–24 April), Faculty of Agriculture, Mansoura University, Mansoura, Egypt, pp 371-394.
- Elhassaneen, Y. A. (1999). Toxicological and biochemical effects of polycyclic aromatic hydrocarbon compounds produced in fish by cooking and processing. Six Arabic conference on Food Science and Technology, March 16 – 18, Cairo, Egypt.
- Elhassaneen, Y. A.; Khater, O.M.; Tawfeek, L.M. and Sanad, M. (1997): In vitro biochemical studies on pollution of fish with paper industry effluents. Second Egyptian Conference of Home Economics (25-26 May),

Faculty of Home Economics, Menoufia University, Egypt.

- Elhassaneen, Y. and El-Badawy, A. (2013). Influence of Charcoal Broiled Meat Consumption on the Liver Functions and Non-Enzymatic Antioxidants in Human Blood. Food and Nutrition Sciences, 4 (1): 90 – 99.
- Elhassaneen, Y.; ElKhamisy, A.; Sayed-Ahmed, R. and Marzouk, E. (2019). Antioxidant activities and total phenolics content in some Egyptian herbs. Port Saied Specific Research Journal (PSSRJ), 11 (1): 211-228.
- Elhassaneen, Y.; Gorge, S.; Elkhamisy, A. and Negm, S. (2016). Removal Techniques of Aflatoxin from Contaminated Commodities: Review Study. 4th International-18 th Arab Conference of Home Economics "Home Economics and Development Issues" 5-6 April, 2016, Faculty of Home Economics. Minoufiya University, Egypt. Journal of Home Economics (Special issue), 26 (2): 23-41..
- Elhassaneen, Y.A. (1996). Biochemical and technological studies on pollution of fish with pesticides and polycyclic aromatic hydrocarbons. Ph.D. Thesis., Faculty of Agriculture, Mansoura University, Egypt.
- Elhassaneen, Y.A. (2004). The effect of charcoal broiled meat consumption on antioxidant defence system of erythrocytes and antioxidant vitamins in plasma. Nutrition Research, 24 (6): 435 - 446.
- Elmaadawy, A.; Arafa, R. and Elhassaneen, Y. (2016). Oxidative Stress and antioxidant defense systems status

in obese rats feeding some selected food processing by-products applied in bread. Journal of Home Economics, 26 (1): 1 -37.

- El-Nashar, N. G. (2007). Development of primary liver cell culture from fish as a valuable tool in nutrition and biotechnology research. Ph.D. Thesis, Faculty of Home Economics, Minoufiya University, Egypt.
- El-Safty, A. (2012). Production of some important nutritional and functional compounds from the by-products of food processing companies. Ph.D. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt.
- El-Sayed, M.; El- Sherif. F., Elhassaneen, Y. and Abd El-Rahman, A. (2012). Potential Therapeutic Effects of some Egyptian Plant Parts on Hepatic Toxicity Induced by Carbon Tetrachloride in Rats. Life Sci. J., 9(4): 3747-3755.
- Eman B Mehram; Sara A. Ahmed; Yousif A Elhassaneen (2021). Role of Oxidant/Antioxidant Status in the Pathogenesis of Iron-Deficiency Anemia: Case Study on Children of Qalyubiyya and Minoufiya Governorates, Egypt. Bulletin of the National Nutrition Institute of the Arab Republic of Egypt 55(1): 39-72.
- Emerole, G. O.; Uwaifo, A. O.; and Bababunmi, E.A. (1982): The presence of aflatoxine and some polycyclic aromatic hydrocarbons in human foods. Cancer Lett. , 15: 123-129.

- Epler, K.S.; Zeigler, R.G. and Craft, N.E. (1993). Liquid chromatographic method for the determination of carotenoids, retinoids and tocopherols in human serum and in food. J Chromatog, 619:37–48.
- Gray, J.I.; and Morton, D.I. (1981): Some toxic compounds produced in food by cooking and processing: A review. J. Human Nutr., 35: 5–23.
- Halliwell, B. (1991): Reactive oxygen species in living systems: Source, biochemistry and role in human disease. American Journal of Medicine, 91: 14s-21s.
- Halliwell, B. and Gutteridge, J.M. (1985): Free radicals in biology and medicine. Clarendon Press. Oxford. UK.
- Harborne, J.B. (1998). phytochemical methods: A guige to modern techniques of plant analysis. The 3 rd Ed., Chapman & Hall, London.
- Harborne, J.B. and Mabry, T.J. (1982). The Flavonoids: Advances in Research. Chapman and Hall, London.
- Harvey, R. G. (1985). Polycyclic hydrocarbons and carcinogenesis. ACS Symp. Ser. 283, American Chemical Society, Washington, D.C., USA.
- Hasegawa, R.; Chujo, T.; Sai-Kato, K.; Umemura, T.; Tanimura, A.; and Kurokawa, Y. (1995): Preventive effects of green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with 2-nitropropane. Food Chem Toxicol., 33(11): 961-970.
- Hassan, A.A. (2011). The effect of phytochemicals on prevention and/or treatment of liver cancer induced by some food pollutants. Ph.D. Thesis, Faculty of Home

Economics, Minoufiya University, Shebin El-Kom, Egypt.

- Hassan, A.A., (2005), The effect of antioxidants on the formation of toxic and carcinogenic substances in some smoked food. M. Sc. Thesis, Fac. Of. Home Economics, Minufyia Univ. Egypt.
- Hawkins, E. W.; Walker, W. W.; Overstreet, R. M.; Lytle, T. F.; and Lytle, J. S. (1988). Dose-related carcinogenic effect of water- borne benzo(a) pyrene on livers of two small fish species. Ecotoxicology and environmental safety, 16: 219-231.
- Hawkins, E. W.; Walker, W. W.; Overstreet, R. M.; Lytle, T. F.; and Lytle, J. S. (1990). Carcinogenic effects of some polycyclic aromatic hydrocarbons on the Japanese medaka and Guppy in waterborne exposures. The Science of the Total Environment, 94: 155-167.
- Hung, S.S.; Cho, Y.C. and Slinger, S.J. (1980): Hight performance liquid chromatographic determination of alpha-tocopherol in fish liver, J. Assoc. Off. Anal. Chem., 63 (4): 889 - 893.
- Huosein, M. (2011). The effect of phytochemicals on toxic and/or carcinogenic substances formed during cooking and processing of meat " Ph.D. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt.
- Jaggi, L. (2012). Turmeric, Curcumin and Our Life: A Review. Bull. Environ. Pharmacol. Life Sci.; 1 (7): 11 – 17

- Jurenka, J.S. (2009): Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. Altern Med Rev., 14(2): 141-53.
- Kapoor, L.D. (1990). Viola odorata L. Handbook of Ayurvedic Medicinal Plants Boca Raton: CRC Press; pp. 335.
- Kebamo, S.; Shibiru T. and Bekesho G. (2015). The Role of Biotransformation in Drug Discovery and Development, J Drug MetabToxicol:5: 1-13.
- Khoneem, A. (2009).Antioxidant activity of some vegetables, spices and herbs distributed in Egyptian local markets " M.Sc. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt.
- Koneri, R.; Balaraman, R. and Firdous V.K.M. (2008). Hepatoprotective effects of Momordica cymbalaria Fenzl. Against carbon tetrachloride induced hepatic injury in rats. Pharmacologyonline, 1: 365-374.
- Larsson.B.K..Sahllerg.G.P.Eriksson,A.T.andBusk,L.A.(1983): Polycyclic aromatic hsdrocarbons in grilled food. J. Agric. Fd Chem., 31:867-873.
- Litchenthaler, H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148, 350-383.
- Liu, Y., Wu, Y. and Zhang, Y. (2015). Protective effects of curcumin and quercetin during benzo(a)pyrene induced lung carcinogenesis in mice. European Review

for Medical and Pharmacological Sciences, 19: 1736-1743.

- Maanen van, J.M.S.; Moonen, E.J.C.; Maas, L.M., Kleinjans, J.C.S. and Schooten van, F.j. (1994). Formation of aromatic DNA adducts in white blood cells in relation to urinary excretion of 1-hydroxypyrene during consumption of grilled meat. Carcinogenesis, 15: 2263-2268.
- Mahran, M. Z.; Abd Elsabor, R. G. and Elhassaneen, Y. A. (2018). Effect of feeding some selected food processing by-products on blood oxidant and antioxidant status of obese rats. Proceeding of the 1st Scientific International Conference of the Faculty of Specific Education, Minia University, "Specific Education, innovation and labor market" 16-17 Juli, 2018, Minia, Egypt.
- Marco, G. J. (1968). A rapid method for evaluation of antioxidants. J. Am. Oil Chem. Soc., 45: 594-598.
- McFarris, M.W. and Reed, D.J. (1987): HPLC of thiols and disulfides: dinitrophenol derivatives. Methods Enzymol. 143: 101 – 109.
- Moeslinger, T.; Brunner, M. and Spieckermann, G. (1994): Spectrophotometric determination of dehydroascorbic acid in biological samples. Analytical Biochemistry. 221: 290 - 296.
- Muriel, P.; Garciapina, T.; Perez-Alvarez,V. and Mourelle, M. (1992). Silymarin protects against

paracetamol-induced lipid peroxidation and liver damage. J. Applied Toxicology; 12:439-442.

- Nabavi, S.F.; Nabavi, S.M.; Abolhasani, F.; Moghaddam, A.H.; and Eslami, S. (2012): Cytoprotective effects of curcumin on sodium fluoride-induced intoxication in rat erythrocytes. B Environ Contam Toxicol, 88: 486–90.
- Omura, T. and Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes. II. Solubilisation, purification and properties. J Biol Chem., 239: 2379-2385.
- Papaioannou D, Cooper KL, Carroll C, Hind D, Squires H, Tappenden P, Logan RF (October 2011). "Antioxidants in the chemoprevention of colorectal cancer and colorectal adenomas in the general population: a systematic review and meta-analysis". Colorectal Disease. 13 (10): 1085–99.
- Payton, F.; Sandusky, P.; and Alworth, W.L. (2007): NMR study of the solution structure of curcumin. J. Nat.Prod., pp: 143-146.
- Peter, K. V. (2008). Underutilized and Underexploited Horticultural Crops, Volume 2. New India Publishing. India, pp. 341.
- Plakunov, I.; Smolarek, T.A.; Fischer, L.D.; Wiley, J.C. and Baird, W.M. (1987): Separation by ion-pair high-performance liquid chromatography of the glucuronid, sulfate and glutathione conjugates formed

from benzo(a)pyrene in cell cultures rodents, fish and humans. Carcinogenesis, 8: 59-66.

- Ruby, A. J., Kuttan, G., Dinesh Babu, K., Rajasekharan, K. N. and Kuttan, R., Antitumor and antioxidant activity of natural curcuminoids. Cancer Lett., 1995, 94, 79–83.
- Saleh, A. (2016). Study the effect of nutritional antioxidant on liver injuries induced by carbon tetrachloride in vitro". Ph.D. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt.
- Sayed-Ahmed, S.; Shehata, N.; Elhassaneen, Y.; (2020). Potential Protective Effects of Ganoderma lucidum Powder against Carbon Tetrachloride Induced Liver Disorders in rats: Biological, Biochemical and Immunological Studies, Egypt. Bulletin of the National Nutrition Institute of the Arab Republic of Egypt 56(2): 99-132.
- Shishodia, S.; Sethi, G.; and Aggarwal, B.B. (2006): Curcumin: getting back to the roots. Annu New York Acad Sci., 1056(1): 206 – 217.
- Singleton, V. L. and Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 16: 144-158.
- Srinivasan, K. (2005): Plant foods in the management of diabetes mellitus: Spices as beneficial antidiabetic food adjuncts. International Journal of Food Sciences and Nutrition, 56(6): 399 – 414.

- Stratton J, Godwin M (June 2011). "The effect of supplemental vitamins and minerals on the development of prostate cancer: a systematic review and meta-analysis". Family Practice. 28 (3): 243–52.
- Tietz, N. W. (1976). Fundamentals of Clinical Chemistry. Philadelphia, W. B.Saunders.p243.
- *Traber MG, Bruno RS (2020). "Vitamin E". In BP Marriott, DF Birt, VA Stallings, AA Yates (eds.). Present Knowledge in Nutrition, Eleventh Edition. London, United Kingdom: Academic Press (Elsevier). pp. 115–36.*
- *USFD , (2012).* "Alliance for Natural Health v. Sebelius, Case No. 09-1546 (D.D.C.)". *US Food & Drug Administration. Washington, DC.*
- Van Gaal, L.F.; Vertommen, J. and De Leeuw, I.H. (1998): The in vitro oxidizability of lipoprotein particles in obese and non-obese subjects. Atherosclerosis. 137: S39-S44.
- Vassault, A.; Grafmeyer, D.; Graeve, J.; Cohen, R.; Beaudonnet, A.; and Bienvenu, J. (1999): Quality specifications and allowable standards for validation of methods used in clinical biochemistry. Ann Biol Clin (Paris)., 57(6): 685-95.
- Voet, D. and Voet, G. (1990). Biochemistry, 1st Edition, John Wiley and Sons, USA
- Wang, FR.; Ai, H.; Chen, XM. and Lei, CL. (2007): Hepatoprotective effect of a protein-enriched fraction from the maggots (Musca domestica) against CCl4-

induced hepatic damage in rats. Biotechnol. Lett., 29(6):853-858.

- WHO (2010). Global Surveillance, Control of Hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Viral Hepat, 6:35–47.
- Wichitnithad, W.; Jongaroonngamsang, N.; Pummangura, S. and Rojsitthisak, P. (2009). A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts. Phytochem Anal. 20(4):314-319.
- Xu X, Yu E, Liu L, Zhang W, Wei X, Gao X, et al. (November 2013). "Dietary intake of vitamins A, C, and E and the risk of colorectal adenoma: a meta-analysis of observational studies". European Journal of Cancer Prevention. 22 (6): 529–39.
- Zheng, A.; Li, H.; Wang, X.; Feng, Z.; Xu, J. and Cao, K. (2014).Anticancer effect of a curcumin derivative B63: ROS production and mitochondrial dysfunction. Curr Cancer Drug Targets. 14(2):156-66.