



Antioxidant Activity of Methanol Extracts From Various Plant Parts and Their Potential Roles in Protecting the Liver Disorders Induced by Benzo(a)pyrene

Yousif Elhassaneen*, Sherif Ragab, Riham Badawy

Nutrition and Food Science Department, Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt

Email address:

yousif12@hotmail.com (Y. Elhassaneen)

*Corresponding author

To cite this article:

Yousif Elhassaneen, Sherif Ragab, Riham Badawy. Antioxidant Activity of Methanol Extracts From Various Plant Parts and Their Potential Roles in Protecting the Liver Disorders Induced by Benzo(a)pyrene. *World Journal of Public Health*. Vol. 2, No. 1, 2017, pp. 38-50. doi: 10.11648/j.wjph.20170201.15

Received: November 4, 2016; **Accepted:** November 24, 2016; **Published:** January 7, 2017

Abstract: In this study, we evaluate the antioxidant effect and total phenolics content of four selected plant parts [prickly pear peel (PPP), turmeric rhizomes (TR), red onion skin (ROS) and eggplant peel (EP)] methanolic extracts and their potential role in improving the liver disorders induced by an ubiquitous food pollutant i.e. benzo(a)pyrene (BP). The selected plant parts methanolic extracts showed considerable differences in antioxidant activity (AA=57.51 to 90.73 %) and total phenolics (11.29 to 211.45 mg GAE. g⁻¹). When all selected plant parts methanolic extracts were included in the statistical analysis, there was a positive and highly significant ($r^2=0.597-0.919$, $p \leq 0.05$) relationship between total phenolics and antioxidant activity. Also, the antioxidant potential of those methanolic extracts against BP was also studied *in vitro* in a model using Catfish liver cells in homogenate culture. BP induced many metabolic disorders and oxidative stress in fish liver homogenate namely a significant decrease in reduced glutathione (GSH) and albumin (Alb) content, and increase in malondialdehyde (MDA) formation after 24 hours of culture. Co-treatment of liver homogenate with BP and the tested selected plant parts extracts as well as their mixture by concentration 0.75% exhibited some protection effects through decreasing the rates of all those metabolic disorders and oxidative stress. That decreasing rates in different adverse effects was depending on the type of the plant parts applied. The highest therapeutic effect was recorded for the mixture of the selected plant parts extracts (PPP+TR+ROS+EP by equal amounts) followed by ROS, EP, TR and PPP, respectively. This study indicates that the compounds present in those selected extracts contain interesting bioactivities which improve many adverse effects i.e. metabolic disorders and oxidative stress in liver cells induced by BP.

Keywords: Cat Liver Homogenate, Total Phenolics, Metabolic Disorders, Oxidative Stress, Onion Skin, Eggplant Peel, Prickly Pear Peel

1. Introduction

The liver performs several vital functions: it processes many of the products that are released into the blood stream (e.g. glucose derived from glycogenesis, plasma proteins and urea), it secretes bile into the intestine to help absorb nutrient, it makes some of the clotting factors needed to stop bleeding from acute or injury, and it also stored several products in the parenchymal cells (e.g. glycogen, fat and fat soluble vitamins) [1-2].

In addition, the liver plays a very important part in biotransformation and removing the xenobiotics from the

body, of which alcohol and dietary toxins are particularly noteworthy [3-4]. The enormous functional reserve of the liver often masks the clinical impact of early liver damage. With progression of diffuse disease or disruption of bile flow, however, the consequences of liver damage can easily become life-threatening. Therefore, liver diseases are a major problem throughout the world [5].

Many environmental toxins cause liver injury to humans. Among these compounds, polycyclic aromatic hydrocarbons (PAHs) are groups of ubiquitous environmental contaminants which are strongly suspected in inducing cancer in human [6-7]. Human beings are exposed to PAH from a wide variety

of occupational, medicinal, environmental and dietary sources. Smoking, polluted air, drinking water and predominantly food are representing the major sources of PAH exposure in the general population [3, 8]. The World Health Organization stated that 99% of the oral intake of PAH contributed by food, 0.9% by inhalation and 0.1-0.3% by drinking water [9]. Benzo(a)pyrene (BP) occupies a central position among these compounds because it is one of the most extensively studied carcinogenic PAHs. It has been shown to be toxic, mutagenic and/or carcinogenic by extensive experiments in vivo [8, 10-11] and in vitro [3, 12-13] systems. Also, B(a)P exposure is associated with the development of liver cancer in mammals, rodent and fish [3, 10-11, 13-14]. It is known that the toxic, tumorigenic and carcinogenic effects of BP correlate with the cellular metabolism of this compounds to arene oxides, phenols, quinones, dihydrodiols, and epoxides and with their subsequent formation of reactive intermediates that interact covalently with DNA to form adducts [3,6]. 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 [15].

Despite the increasing need for agents to protect the liver from toxins including BP damage, modern medicine is costly and associated with multiple side effects resulting in patient non-compliance, subsequently lacks a reliable liver protective drug [16]. So, there has been considerable interest in the role of complementary and alternative medicines for the treatment of liver disorders/diseases. 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. They 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.

Scientists around the world have identified thousands of phytochemicals, including flavonoids, glucosinolates (isothiocyanates and indoles), phenolic acids, phytates, and phytoestrogens (isoflavones and lignans), in different herbs and spices around the world [17]. Among the herbs, turmeric (*Curcuma longa* L.) belongs to the *Zingiberaceae* family and 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) [18]. Turmeric contains a wide variety of phytochemicals, including but not limited to curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols [19-20]. 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 [21].

On the other side, processing of fruits and vegetables may results in high amounts of waste materials/by-products such as peels, seeds and stones. Disposal of these materials usually represents a problem that is further aggravated by legal restrictions. Many studies indicated that such plant parts are considered a good source of bioactive and functional compounds [22-23]. Some major source of food wastes are prickly pear (*Opuntia ficus-indica*), onion (*Allium cepa* L.) and eggplant (*Solanum melongena*), some of the most popular vegetables and fruits. The major by-products resulting from industrial peeling of onion bulbs are brown skin, the outer two fleshy leaves and the top and bottom bulbs [22]. The outer dry layers of onion bulbs, which are not edible and removed before processing, have been shown to contain a wide spectrum of polyphenolic components [24]. Eggplant, one of the most widespread vegetable consumed around the world. The most widely cultivated varieties are elongated ovoid or slender type in a dark purple skin. Eggplant is ranked as one of the top ten vegetables in terms of oxygen radical scavenging capacity due to the fruit's phenolic constituents [25]. Anthocyanins, an important group of naturally occurring pigments of red and/or purple colored fruits, are the main phenolic compounds in eggplant peel which provide a myriad of health benefits [26-27]. Prickly pear, commonly known as prickly pear, belongs to the family *Cactaceae*. The prickly cactus pear is widely distributed in Latin America, South Africa and the Mediterranean area including Egypt, [28]. Many studies indicated that the fruits pulp and peel of prickly pears contained phenolics and other antioxidants such as biothiols and concluded that they had a positive effect in the Redox balance of humans mainly due to reduced LDL hydroperoxides levels [29-30]. So, *opuntia* fruits and young stems have been traditionally used in folk medicine to treat diabetes, hypertension, asthma, burns, edema, and indigestion [31-32].

Since high antioxidant capacity is a desired feature for the above mentioned plant parts, the aim of this study was to determine their *in vitro* potential roles in protecting the liver disorders induced by BP.

2. Materials and Methods

2.1. Materials

Selected plant parts, prickly pear (*Opuntia ficus indica*) peel (PPP) turmeric (*Curcuma longa*) rhizome (TR), red onion "*Allium cepa* L." skin (ROS) and eggplant (*Solanum melongena*) peel (EP) samples were obtained from a local supermarkets, Cairo city, Egypt. Benzo(a)pyrene (BP), Dimethyl sulfoxide (DMSO) and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All organic solvents and other chemicals were of analytical grade were purchased from El-Ghomhorya

Company for Drugs, Chemicals and Medical instruments Trading, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of the Plant Parts Extracts

After arriving of the selected plant parts samples, they were prepared for drying process by manual sorting, washing, chopped separately into small pieces and drying in under vacuum oven (Across International, Livingston, NJ) at 55 °C until arriving by the moisture in the final product to about 8%. The dried selected plant parts were homogenized in electric blender (Toshiba ElAraby, Benha, Egypt), sieved (60 mesh.inch⁻¹), packed in polyethylene bags and kept in-20°C. Powders of the selected plant parts were used for their different types extracts according to the method of Amin *et al.*, [33] with some modifications. In aqueous extraction, 20 g from dried plant+180 ml deionized water were homogenized and transferred to a beaker and stirred at 200 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of aqueous extract was removed under reduced pressure at 55°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). In organic solvents extraction, the same previous extraction procedure was carried out by using different organic solvent separately including 80% (v/v) methanol, 80% (v/v) ethanol and 100% hexane as an extraction medium. The yield of the extracts were weighted and evaluated to use the high yield one.

2.2.2. Antioxidant Activity

Antioxidant activity of the selected plant parts 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 [34]. 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 was expressed as antioxidant activity (AA) and calculated as

percent inhibition relative to control using the Al-Saikhanet *al.*, [35] equation $[AA = (R_{\text{control}} - R_{\text{sample}}) / R_{\text{control}} \times 100]$, where R_{control} and R_{sample} were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively].

2.2.3. Total Phenolics

Total phenolics of the selected plant parts extracts were determined by using Folin-Ciocalteu reagent [36]. A 100 μ l of each 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⁻¹) solution was added to the mixture after 90 min at 22°C, absorbance was measured at 725 nm. Results are expressed as gallic acid and equivalents.

2.2.4. Biological Experiments

(i). Fish

Catfish (*Clarias lazera*), one year age were collected from the Nile River, Egypt by arrangement with some fisherman's and transported to the laboratory in 50-liters plastic trash cans. Fish were held outside the laboratory in 50-liter sinks including Nile River water with two aerators pushed oxygen at a flow rate of 1 L/min. Fish were fed a daily ration of 2% of their body weight/day (Basic Flake, Aquarium design, Union City, CA) and the amount adjusted every two weeks. The water temperature adjusted in the range 18-25°C by using a heating system inside the Aquarius. The photoperiod was 12-h light and 12-h dark. No diseases were observed in experimental fish.

(ii). Preparation of Catfish Liver Homogenate

Catfish liver homogenate were prepared according to the method mentioned by Elhassaneen, [3] with some few modifications. Briefly, Catfish were anesthetized in tricaine methane sulfonate (MS-222; Sigma Chemical Co., St. Louis, MO) and weight was recorded. Livers were excised to a 60 × 15 mm petri dish (Baxter Healthcare Corp., McGaw Park, IL) containing Hank's Balanced Salt Solution (HBSS; Sigma Chemical Co.). Other tissues unless livers were cut away and the HBSS were removed. The livers were minced with a sterilize scissors and resuspended in RPMI-1640 (Sigma Chemical Co.) adjusted to 330 mOs/kg and supplemented with 25 mM 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethane sulfonic acid buffer (HEPES), 2 mM L(+)-glutamine, 100 IU/ml penicillin, 100 μ g/ml streptomycin and 10% fetal Calf serum (FCS; all from Sigma Chemical Co.) to give a concentration (10 mg protein/ml).

(iii). Determination of the Ideal Concentrations of the Selected Plant Parts Extract for Applying in Co-treatment With BP

Liver homogenate of Catfish were seeded at 100 μ l homogenate (1 mg protein)/well of 96 flat tissue culture plates. 100 μ l of RPMI-1640/FCS growth medium was added to each well. Seven tenfold dilutions of BP were prepared and 100 μ l of different dilutes were added to each well (as positive control replicates). Mixture of selected plant parts methanolic extracts (prickly pear peel + turmeric rhizome + red onion skin + eggplant peel extracts by equal amounts) were prepared in

concentrations (0.25-1.00%) and 100 µl of different dilutes was added to each well (as treated replicates). All plates were incubated at 27°C for 4 h in the presence of 5% CO₂ tension. The plates were prepared for NR assay.

(iv). *Effect of Selected Plant Parts Methanolic Extracts on the Metabolic Disorders and Oxidative Stress Induced by BP in Liver Cells*

Liver homogenate of Catfish were seeded at 100 µl homogenate (1 mg protein)/well of 96 flat tissue culture plates. 100 µl of RPMI-1640/FCS growth medium was added to each well. Seven tenfold dilutions of BP were prepared and 100 µl of different dilutes were added to each well (as positive control replicates). Mixture of selected plant parts methanolic extracts were prepared in concentrations (0.25-1.00%) and 100 µl of different dilutes was added to each well (as treated replicates). All plates were incubated at 27°C for 4 h in the presence of 5% CO₂ tension. The plates were prepared for Glutathione (GSH), albumin (Alb) and malondialdehyde (MDA) measurements..

(v). *GSH Measurement*

Reduced glutathione content (GSH) was measured by the spectrophotometric recycling method of Tietze [37] in the presence of 5,5'-ditiobis (2-nitrobenzoic acid) (DTNB), NADPH and glutathione reductase (GR) in a DU70 Beckman spectrophotometer (CA).

(vi). *Alb Measurement*

Levels of albumin (Alb) were determined using a commercially available kit (Bioassay systems, Hayward, CA) based on an established method that utilizes bromocresol green which forms a coloured complex specifically with albumin that is detectable at 620 nm. Known quantities of human albumin were used to establish the standard curve. Specific levels of albumin secretion were normalized to total protein levels.

(vii). *MDA Measurements*

The extent of lipid peroxidation was measured by the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric (532 nm) product, proportional to the MDA present (Sigma-Aldrich, St. Louis, MO). The activity of lipid peroxidation was expressed as the amount of MDA normalized by the amount of cellular protein.

(viii) *Measurement of Protein Concentration*

Protein concentration was measured by a BCA kit (Pierce Biotechnology, Rockford, IL) following the manufacturer's protocol.

2.2.5. Statistical Analyses

All of the biological experiments were performed at least three times, using four wells for each concentration of tested agent. Data for the dose-response toxicity and metabolic disorders curves were presented as the arithmetic mean. Analysis of variance was performed on data using the General Linear Models Procedure (GLM) of the Statistical Analysis System [38].

3. Results and Discussion

3.1. Extractive Value of Selected Plant Parts Using Different Organic Solvents

The extractive values in different organic solvents are based on the quantity, which are soluble in them. It makes a valuable test to check the quality of drug/additive/food supplement etc and any variation in the chemical constituents may cause a change in the extractive values. Thus, it helps in the determination of the adulteration and is an index of the purity of the material. The extractive value of selected plant parts therefore, were determined by successive extraction in different solvents using a Soxhlet's apparatus. The results are shown in Table (1). The extractive value for selected plant parts in water and hexane was low (1.54-3.09%) while relatively high in methanol and ethanol (2.49-8.51%). Similar data was recorded by Saleh [39] for other plant parts including orange peel, sweet violet blossoms and marjoram leaves. All of those data confirmed that selected plant parts components were found in both lipohylic and hydrophilic phases i.e. accordance with the known rule "like dissolve like". The variation in the extractive values may be possible due to the presence of specific compound according to the solubility, soil condition, atmospheric condition and water content of the sample [39-40].

Table 1. Extractive value of selected plant parts using different organic solvents.

Selected plant parts	Mean extract, yield (%) ±SD			
	Water	Methanol	Ethanol	Hexane
Prickly pear peel (PPP)	2.09±0.74 ^a	3.01±1.01 ^c	2.54±1.14 ^c	1.54±0.66 ^b
Turmeric rhizome (TR)	2.03±0.65 ^a	3.99±0.99 ^c	2.49±0.99 ^c	2.21±0.59 ^{ab}
Red onion skin (ROS)	2.91±1.10 ^a	8.51±1.76 ^a	6.01±1.03 ^a	3.09±1.02 ^a
Eggplant peel (EP)	2.41±1.02 ^a	5.79±1.28 ^b	3.51±1.38 ^b	2.39±0.87 ^a

* Each value represents the mean of three replicates ±SD. Mean values with the different letters in the same column mean significantly different at level p≤0.05.

3.2. Antioxidant Activity and Total Phenolics of Selected Plant Parts Methanolic Extracts

3.2.1. Antioxidant Activities

The antioxidant activities and total phenolics of four selected plant parts methanolic extracts are shown in Table (2). From such data it could be noticed that the selected plant parts

methanolic extracts showed considerable differences in antioxidant activity (AA=57.51 to 90.73 %). Red onion skin (ROS) and eggplant peel extracts showed strong activity because of its high phenolics content (211.45 and 63.20mg GAE. g⁻¹, respectively) while prickly pear peel (PPP) showed relatively low content in both antioxidant activity and the total phenolics (57.51 % and 11.29 mg GAE. g⁻¹, respectively).

The decrease in absorbance of β -carotene in the presence of different selected plant parts methanolic extracts (and well-known antioxidants used as standards) with the oxidation of β -carotene and linoleic acid is shown in Figure (1). Such data indicated that ROS extract recorded the lowest decreasing followed by EG, TR and PPP extracts, respectively. The values of ROS extract absorbance through 120 min are coming well i.e. closing the line of 50 mg.L⁻¹ of α -tocopherol followed by the rest of the parts extracts. These data proved the very high stability of the ROS extract relatively high stability of the rest tested plant parts extracts when comparing with that more common standard α -tocopherol.

In a similar studies, Elhassaneen *et al.*, [23], Saleh [39] and Ahmed, [41] found that many food by-products/plant parts extracts including potato peel powder (PPP), red onion skin

powder (ROSP), prickly pear peel (PPP) and mango peel powder (MPP) recorded highly antioxidant activity and phenolic content. Almost of those plant parts extracts exhibited high antioxidant stability when comparing with the α -tocopherol as the standard antioxidant. In comparing with the other related study, Velioglu *et al.*, [42] determined the antioxidant activities and total phenolics of 28 plant products and by-products, including sunflower seeds, flaxseeds, wheat germ, buckwheat, several fruits, vegetables, and medicinal plants and found that the total phenolics content varied from 169 to 10548 mg/100 g of dry product. Antioxidant activity of methanolic extract evaluated according to the β -carotene bleaching method expressed as AOX, AA, ORR, and AAC ranged from 0.05, 53.7, 0.009 and 51.7 to 0.26, 99.1, 0.46 and 969.3, respectively.

Table 2. Antioxidant activity and total phenolics of selected plant parts methanolic extracts.

Selected plant parts	Antioxidant activity AA (%)		Total phenolics (mg GAE. g ⁻¹ extract)	
Prickly pear peel (PPP)	57.51±	4.18 ^c	11.29±	2.77 ^c
Turmeric rhizome (TR)	65.32±	4.66 ^d	19.71±	5.87 ^c
Red onion skin (ROS)	90.73±	12.10 ^b	211.45±	42.89 ^a
Eggplant peel (EP)	74.14±	8.88 ^c	63.20±	14.67 ^b
α -tocopherol, 50 mg/L	98.77±	4.13 ^a	-----	-----

^c Each value represents the mean of ten replicates \pm SD. Mean values with the different letters in the same column mean significantly different at level $p \leq 0.05$.

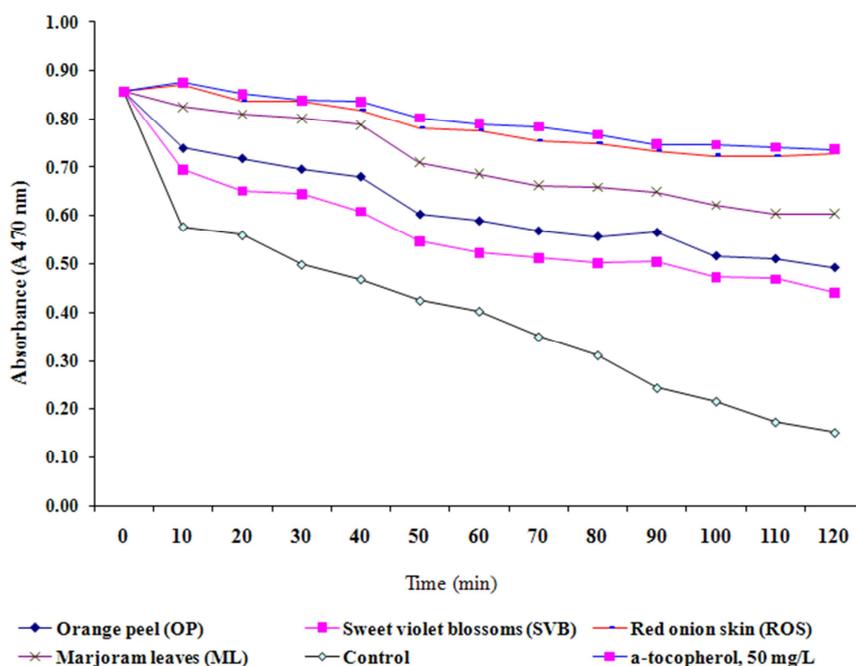


Figure 1. Activity of selected plant parts methanolic extracts assayed by the β -carotene bleaching method (α -tocopherol at 50 mg.L⁻¹ concentration was used as a reference).

3.2.2. Relationship Between Total Phenolics Content and Antioxidant Activity

Data in Table (2) investigated the total phenolics content of the selected plant parts methanolic extracts which varied from 11.29 to 211.45 mg GAE.g⁻¹. The results also indicated that when all selected plant parts methanolic extracts were included in the statistical analysis, there was a positive and highly significant ($r^2=0.597-0.919$, $p \leq 0.05$) relationship between total phenolics and antioxidant activity (Figure 2).

The highest value was recorded for ROS ($r^2=0.919$, $p \leq 0.05$) followed by EP ($r^2=0.869$, $p < 0.05$), TR ($r^2=0.735$, $p < 0.05$), and PPP ($r^2=0.597$, $p < 0.05$), respectively. This indicates that phenolic compounds can play a major role in the antioxidant activity of some selected plant parts methanolic extracts such ROS, EP and TR and partially role for the other such PPP. Such data means the antioxidant activity of PPP extract was depended on the occurrence of other bioactive compounds beside the phenolics one. The bioactive compounds could be

included vitamins (ascorbic acid and tocopherols), sterols, pigments (betaxanthins and betacyanins) and minerals [43]. In similar study, Velioglu *et al.*, [42] reported that the correlation coefficient between total phenolics and antioxidant activities of 28 plant products, including plant by-products was

statistically significant. Also, many studies indicated that there was a positive and significant ($p \leq 0.05$ or $p < 0.01$) relationship between total phenolics and antioxidant activity in different plant parts extracts [23, 39, 44-45].

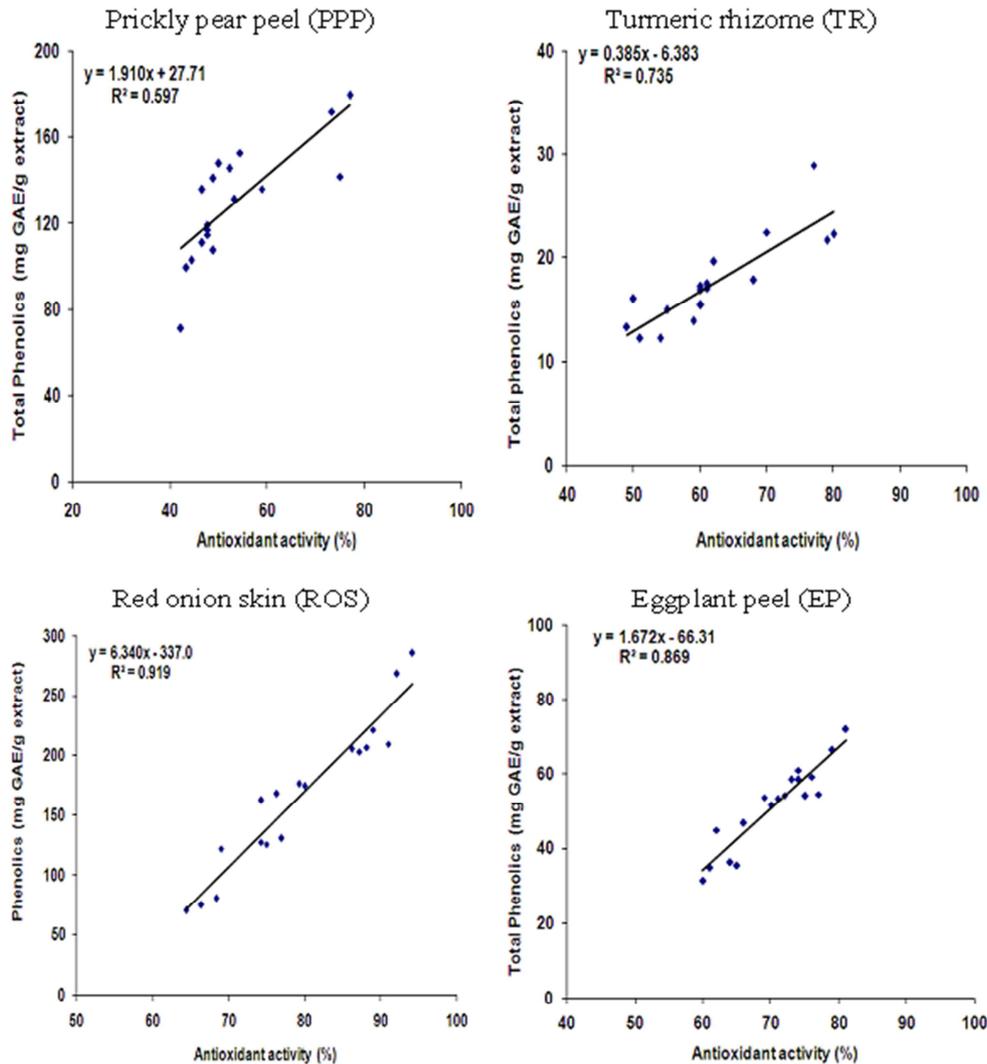


Figure 2. Relationship between total phenolic content and antioxidant activity (AA) of selected plant parts methanolic extracts.

The data of this study with the others proved the importance of using all selected plant parts methanolic extracts as natural antioxidants in both therapy and food technology. For examples, Majid *et al.*, [46] found feeding of phenolic acid (ellagic, found in PPP) significantly increased the levels of reduced glutathione (GSH) and glutathione reductase (GSH-Rd) in liver and lungs of male and female mice as well as increase in inhibition of NADPH-dependent lipid peroxidation. The antioxidant activity of four phenolic acids, representative of three chemical groups, present in the all tested plant by-products, upon low density lipoprotein peroxidation was studied *in vitro* in a low density lipoprotein (LDL) oxidation model by Laranjinha *et al.*, [47]. 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, conditions of aging including neurodegenerative diseases such as Parkinson's and Alzheimer's disease. Serum glucose concentration of alloxane-induced diabetic rats consumed the tested plant by-product powder were studied by Shalaby [48]. It was noticed that treatment of animals with alloxane caused a significant increased ($p \leq 0.05$) in serum glucose concentration (41.49%) compared to normal controls. Supplementation of the rat diets with meatballs (20%) decreased the rise of mean serum glucose by the ratio 9.64%. The rate of decreasing was increased with the supplementation of the meatballs with 0.25% w/w/ by pomegranate peel powder (PGPP), ROSP, PPP and their

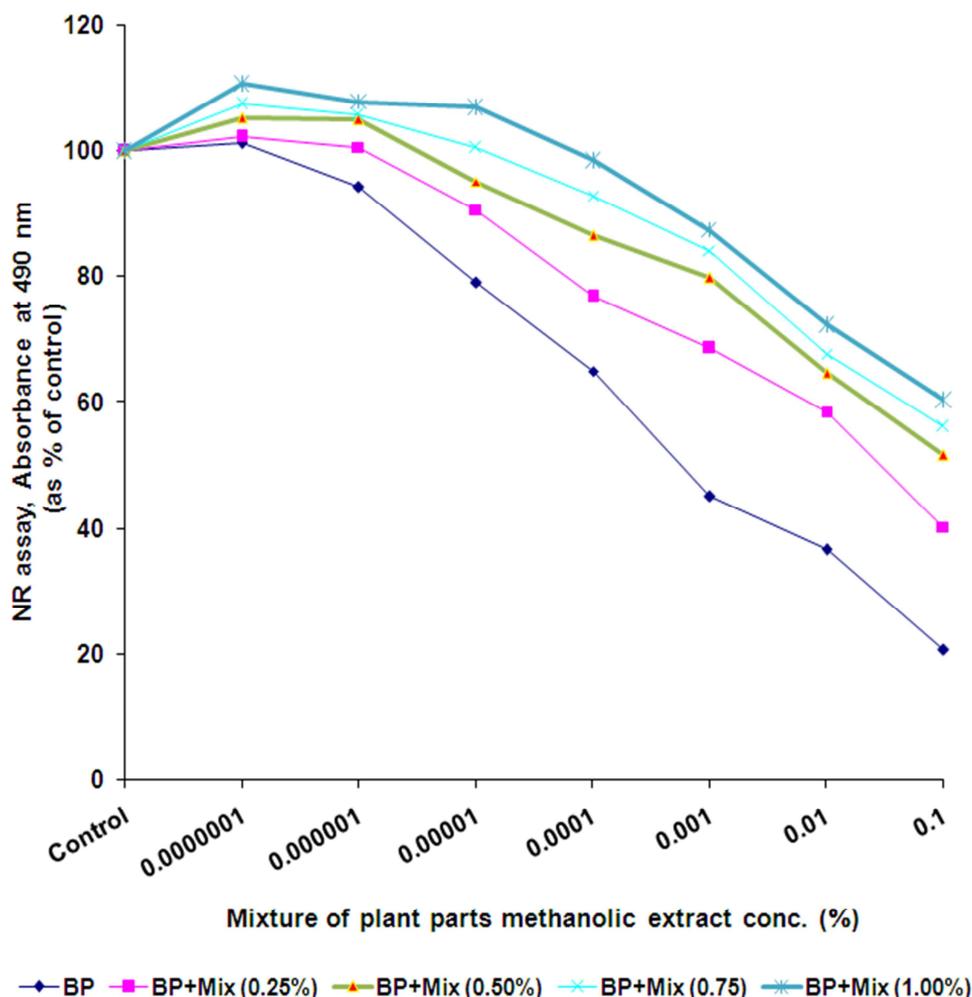
mixture by 23, 28.6, 26.25 and 35.55%, respectively. The mixture treatment gave maximum hypoglycemic yield when compared with the tested plant parts separated. It could be mean that a combination of different plant parts may be more efficient for reducing the serum glucose level because the interactive effects occurred by different categories of bioactive compounds of plant parts used. The same findings were observed by Sayed Ahmed, [45] when using mixture of potato peel powder (PPP), red onion skin powder (ROSP), cauliflower leaves powder (CLP) and mango peel powder (MPP). Recently, Elhassaneen *et al.*, [30] reported that some plant parts extracts including sweet violet blossoms (SVB), marjoram leaves (ML), red onion skin (ROS) and orange peel (OP), individually or to work together, improve the liver injuries (cytotoxic, immunotoxic and genotoxic effects) induced by CCL₄ *in vitro*.

Finally, data of the present work with that carried out by the others could be represent the mile stone towards the extension of using those selected plant parts methanolic extracts, as natural antioxidants in many different therapeutic nutrition applications instead of the synthetic antioxidants/food supplements/drugs. Restrictions on the use of the last synthetic antioxidants/drugs, however, are being imposed

because of their carcinogenicity [35]. Thus, the interest in natural/nutritional antioxidants has increased considerably.

3.3. Determination of the Ideal Concentrations of the Selected Plant Parts Extract for Applying in Co-treatment with BP

Catfish liver cells homogenate were incubated with serial dilutions of BP alone and in co-treatment with mixture of the selected plant parts methanolic extract by different concentrations (0.25-1.0%). Neutral red (NR) assay which determined the lysosomal activity was used to make a dose response toxicity of BP. The data were standardized by expressing absorbance data in the presence of each mixture concentration as a percentage of that in the control medium. Such as shown in Figure (3), the absorbance measurements of NR assay (as % of control) were 20.70-101.22, 40.30-102.34, 51.56-105.33, 56.12-107.56 and 60.32-110.74 for the BP alone and co-treatment with mixture of the selected plant parts (prickly pear peel + turmeric rhizome + red onion skin + eggplant peel, by equal amounts) methanolic extract by different concentrations (0.25, 0.50, 0.75 and 1.0%), respectively.



* BP, Benzo(a)pyrene; Mix, mixture of prickly pear peel + turmeric rhizome + red onion skin + eggplant peel extracts by equal amounts.

Figure 3. Effect of mixture selected plant parts methanolic extract on the cytotoxicity of BP in liver cells as determined by neutral red (NR) assay*.

Such data indicated that BP induced highly adverse cytotoxic effects including lysosomal dysfunctions of liver cells which expressed by NR assay. Co-treatment of liver cell with BP and mixture of the selected plant parts methanolic extract exhibited therapeutic effects through decreasing the cytotoxic effect. That decreasing in cytotoxic effect was depending on the concentration of the mixture applied. The therapeutic effect was gradually increased with the increasing.

3.4. Effect of Selected Plant Parts Methanolic Extracts on the Metabolic Disorders Induced by BP in Liver Cells

3.4.1. Reduced Glutathione (GSH) Synthesis

The influence of selected plant parts methanolic extracts on the metabolic disorders induced by BP in liver cells through determination of the GSH synthesis (Figures 4). The GSH

content (as % of control) of liver cells were 41.20-108.13, 55.79-117.01, 49.80-115.91, 63.24-120.18, 60.68-115.06 and 65.87-123.35% for the BP and BP plus the four selected plant parts extract PPP, TR, ROS, EP as well as their mixture, respectively. Such data indicated that BP induced some metabolic disorders in liver cells which expressed by decreasing the GSH synthesis. Co-treatment of liver cell with BP and the tested selected plant parts extracts as well as their mixture exhibited therapeutic effects through decreasing the metabolic disorders i.e. increasing the GSH synthesis. That decreasing in the metabolic disorders of liver cells was depending on the type of the plant parts applied. The highest therapeutic effect was recorded for the mixture of the selected plant parts extracts followed by ROS, EP, TR and PPP, respectively.

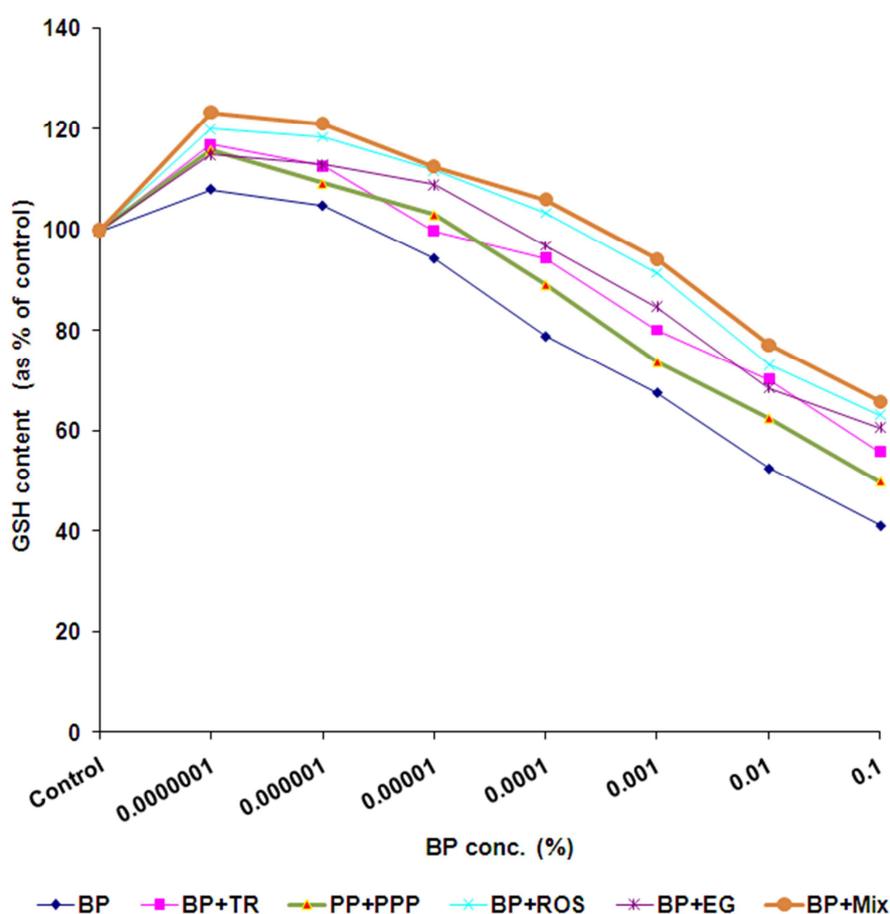


Figure 4. The influence of selected plant parts methanolic extracts on the metabolic disorders induced by BP in liver cells through determination of the GSH synthesis*.

* BP, Benzo(a)pyrene; PPP, Prickly pear peel extract; TR, Turmeric rhizome extract; ROS, red onion skin extract; EP, Eggplant peel extract; Mix, mixture of PPP+TR+ROS+EP by equal amounts.

Reduced glutathione (GSH) is a tripeptide-thiol (γ -glutamylcysteinyl-glycine) that has received considerable attention in terms of its biosynthesis, regulation, and various intracellular functions [49-50]. Among these functions are two constructing roles in detoxifications: (1) as a key conjugate of electrophilic intermediates, principally via

glutathione-S-transferase activities in phase II metabolism, and (2) as an important biological antioxidant. The antioxidant functions of GSH includes its role in the activities of the antioxidant enzymes such glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rd). In addition, GSH can apparently serve as a non-enzymatic scavenger of oxyradicals [51].

A fall in GSH observed by BP treatment accompanied by a concomitant increase in the liver lipid peroxidation (MDA content). Such data with the others (Hasegawa *et al.*, 1995) suggested that secretion of GSH from liver to blood might be blocked by BP because of intracellular structural failure, elevation of the lipid peroxide content and/or the energy depletion suggested by the marked decrease in glycogen content [52]. Co-treatment of liver cell with BP and the tested selected plant parts extracts was significantly ($p \leq 0.05$) removed some of the metabolic disorders induced by BP in liver cells through increasing the GSH synthesis. PPP, TR, ROS and EP methanol extracts was described by its higher content of different classes of bioactive compounds including flavonoids and betacyanin such as betanin in PPP, polyphenolic curcuminoids such as curcumin in TR, flavonoids such as quercetin and organosulfur compounds in ROS, and Phenolic acids (chlorogenic and caffeic acids) and anthocyanins (nasunin and delphinidin) in EP [52-57]. Several reports have documented the potent antioxidant capacity [57] and anticarcinogenic effects [56, 58-61] of such bioactive compounds in experimental animals. Such effects lead to increase glutathione content and stimulate the activity of antioxidant enzymes i.e.

GSH-Px, GSH-Rd and catalase [52, 62].

3.4.2. Albumin (Alb) Secretion into the Culture Medium

Effect of selected plant parts methanolic extract on the metabolic disorders induced by BP in liver cells by measurement of albumin (Alb) secretion into the culture medium (Figures 5). The Alb content (as % of control) of culture medium were 119.68-296.10, 111.91-212.54, 115.54-249.11, 107.87-172.23, 108.91-181.83, 105.01-151.67% for the BP and BP plus the four selected plant parts extract PPP, TR, ROS, EP as well as their mixture, respectively. Such data indicated that BP induced some metabolic disorders in liver cells which expressed by increasing the Alb into the culture medium. Co-treatment of liver cell with BP and the tested selected plant parts extracts as well as their mixture exhibited therapeutic effects through decreasing the metabolic disorders i.e. decreasing the Alb secretion into the culture medium. That decreasing in the metabolic disorders of liver cells was depending on the type of the plant parts applied. The highest therapeutic effect was recorded for the mixture of the selected plant parts extracts followed by ROS, EP, TR and PPP, respectively.

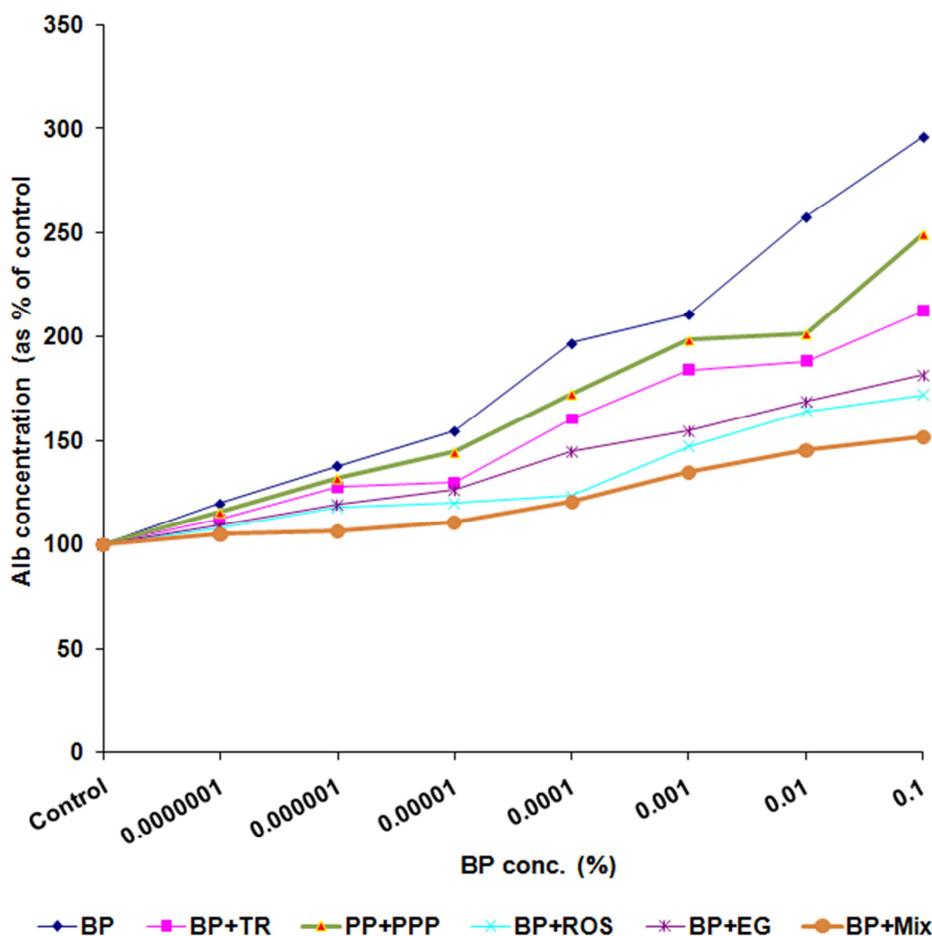


Figure 5. Effect of selected plant parts methanolic extract on metabolic disorders induced by BP in liver cells by measurement of albumin (Alb) secretion into the culture medium*.

* BP, Benzo(a)pyrene; PPP, Prickly pear peel extract; TR, Turmeric rhizome extract; ROS, red onion skin extract; EP, Eggplant peel extract; Mix, mixture of PPP+TR+ROS+EP by equal amounts.

Albumin production is often employed as a marker of hepatocyte metabolic activity *in vitro* and is generally recognized as an indicator of liver-specific functions [63]. Earlier work on the synthesis of albumin reported the rate to be $4\mu\text{g} (\text{min.mg DNA})^{-1}$ which is understood to be several fold greater than the rate of albumin secretion by cultured hepatocytes [64]. The metabolic activity of liver cells was assessed by determination of the level of albumin secretion. The present data indicated that secretion of Alb from liver to culture media was increased by BP. It could be corresponding well with the study of Fayez [62] which suggested that secretion of Alb from liver to blood might be blocked by BP because of intracellular structural failure. Also, throughout cytotoxic challenge of different toxic agent including BP, higher levels of albumin secretion were noted in liver cells culture media [65]. However, co-treatment of liver cell with BP and the tested selected plant parts extracts exhibited partially reducing in albumin secretion by cells cultured on media. These data illustrate that hepatocyte cell function was improved in the presence of tested plant parts extracts in a dose-dependent manner and cells grown in media supplemented with those extracts appeared more tolerant to the cytotoxin, i.e. BP.

3.5. Effect of Selected Plant Parts Methanolic Extract on the Oxidative Stress i.e. Malondialdehyde (MDA) Formation Induced by BP in Liver Cells

Effect of selected plant parts methanolic extract on the oxidative stress induced by BP in liver cells by measurement of malondialdehyde (MDA) formation in cells (Figures 6). The MDA content (as % of control) of cells were 117.68-226.55, 108.91-179.31, 111.55-204.91, 105.00-143.23, 105.91-166.31, 102.67-134.20% for the BP and BP plus the four selected plant parts extract PPP, TR, ROS, EP as well as their mixture, respectively. Such data indicated that BP induced oxidative stress in liver cells which expressed by increasing the MDA liver cells. Co-treatment of liver cell with BP and the tested selected plant parts extracts as well as their mixture exhibited therapeutic effects through decreasing the oxidative stress i.e. decreasing the MDA formation in liver cells. That decreasing in oxidative stress of liver cells was depending on the type of the plant parts applied. The highest therapeutic effect was recorded for the mixture of the selected plant parts extracts followed by ROS, EP, TR and PPP, respectively.

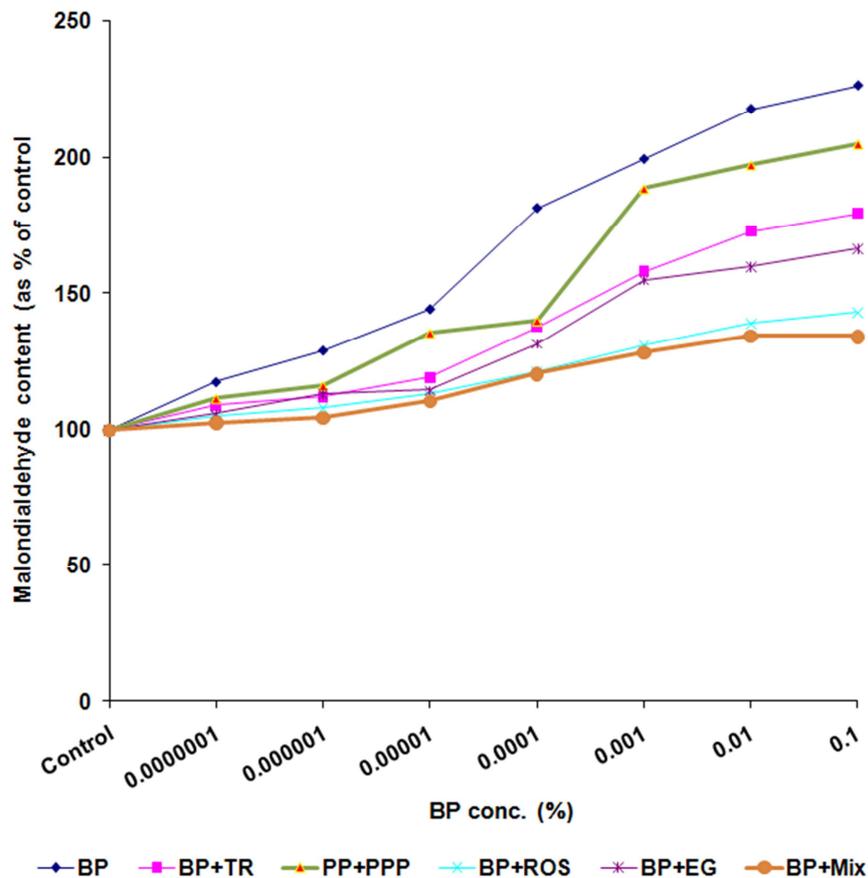


Figure 6. Effect of selected plant parts methanolic extract on the oxidative stress induced by BP in liver cells by measurement of malondialdehyde (MDA) formation in cells*.

* BP, Benzo(a)pyrene; PPP, Prickly pear peel extract; TR, Turmeric rhizome extract; ROS, red onion skin extract; EP, Eggplant peel extract; Mix, mixture of PPP+TR+ROS+EP by equal amounts.

Several reports have documented the potent antioxidant capacity of curcumin where by mitigation of lipid peroxidation and oxidative stress in several tissues were demonstrated [62]. Flavonoids isolated from eggplant showed potent antioxidant activity in experimental animals. It lowers the concentrations of malondialdehyde, hydroperoxides and conjugated dienes significantly [52]. Also, chlorogenic acid and nasunin isolated from eggplant alls showed antioxidant activates including protecting blood cholesterol from peroxidation, preventing cellular damage that can promote cancer, and lessening free radical damage in joints, which is a primary factor in rheumatoid arthritis [53]. Furthermore, Fayeze [62] found that liver lipid peroxide levels increased in all BP-treated animals and previous feeding of turmeric powder clearly protected against those changes.

4. Conclusion

In conclusion, throughout cytotoxic challenge of BP, a fall in GSH content in liver cells and higher levels of Alb secretion in liver cells culture media were noted. Such cytotoxic effects observed by BP treatment accompanied by a concomitant increase in the liver lipid peroxidation (MDA content). However, liver cells functions were improved as the co-treatment with BP and the tested selected plant parts extracts in a dose-dependent manner and cells grown in media supplemented with those extracts appeared more tolerant to the cytotoxin, i.e. BP. The highest preventive/improving effects was recorded by the mixture of the selected plant parts extracts which could be attributed to the antagonism effects as the result of different phytochemicals categories including. Therefore, we recommended those tested plant parts, individually or mixture case, by a moderate amounts to be included in our daily diets.

Acknowledgements

The present study was partially financed by the Research Supporting Fund (RSF), Minoufiya University, Shebin El-Kom, Egypt. Also, the authors acknowledge the assistance and comments of Prof. Dr. Sobhy Hassab El-Nabi, Department of Zoology, Faculty of Science, Minoufiya University.

References

- [1] 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.
- [2] Lehman, E. M. (2008). *Dynamics of liver disease in Egypt: Shifting paradigms of a complex etiology*, PhD Thesis, The University of Michigan, MI.
- [3] 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.
- [4] Kebamo, S.; Shibiru T. and Bekesho G. (2015). *The Role of Biotransformation in Drug Discovery and Development* 6 J Drug Metab Toxicol:5: 1-13.
- [5] Lawrence, S. F. and Emmet B. K. (2012). *Handbook of Liver Disease*, 3rd ed. Elsevier Saunders, Philadelphia, PA.
- [6] Harvey, R. G. (1982). *Polycyclic hydrocarbons and cancer.* Am. Sci., 80: 386.
- [7] David, K. M.; Bowman, E. D.; Parker, N. B.; Caporaso, N. E. and Weston, A. (1992). *Determinants of polycyclic aromatic hydrocarbon-DNA adducts in human placenta.* Cancer Res.52:1499-503.
- [8] 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.
- [9] WHO, World Health Organization. (1984). *World Health Organization guidelines for drinking water quality*, Geneva, 130.
- [10] Harvey, R. G. (1985). *Polycyclic hydrocarbons and carcinogenesis.* ACS Symp. Ser. 283, American Chemical Society, Washington, D.C.
- [11] 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.
- [12] Elhassaneen, Y. A.; Khater, O. M. and Morsey, A. A. (1997). *The effect of vitamins on the cancer initiation induced by some food chemical carcinogenic pollutants in vitro.* The Second Conference: The Role of Women and Egyptian Associations in Environment Protection and Community Development (25-26 August), Dept. of Home Economics, Faculty of Agriculture, Alexandria University, Egypt, pp. 252-282.
- [13] Elhassaneen, Y. (2002). *New and quickly biological method for detection the potential chemical toxins and/or carcinogens in foods.* Proceedings of 2nd scientific Conference on Foodborne Contamination and Egyptian's Health (24-24 April), Faculty of Agriculture, Mansoura University, Mansoura, Egypt, pp 371-394.
- [14] 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.
- [15] Mackenzie, K.; and Angevine, D. M. (1981). *Infertility in mice exposed in utero to benzo(a)pyrene.* Biol. Reprod., 24(1): 183-191.
- [16] Minjun C.; Ayako S.; Jürgen B.; Ra'ol J. and Isabel L. (2015). *Drug-induced liver injury: Interactions between drug properties and host factors* Journal of Hepatology63:503-514.
- [17] Elizabeth I. O. and Magali C. (2014). *Culinary herbs and spices: their bioactive properties, the contribution of polyphenols and the challenges in deducing their true health benefits.* Int. J. Mol. Sci. 15: 19183-19202.
- [18] Rathaur, P., Waseem, R., Ramteke, P. W., & John, S. A. (2012). *Turmeric: The golden spice of life.* International Journal of Pharmaceutical Sciences and Research, 3, 1987-1994.

- [19] Chattopadhyay, I.; Biswas, K.; Bandopadhyay, U.; and Banerjee, R. K. (2004): Turmeric and curcumin: Biological actions and medicinal applications. *Curr Sci.*, 87: 44-53.
- [20] Payton, F.; Sandusky, P.; and Alworth, W. L. (2007). NMR study of the solution structure of curcumin. *J. Nat. Prod.*, pp: 143-146.
- [21] Aggarwal, B. B.; Sundaram, C.; Malani, N.; and Ichikawa, H. (2007): Curcumin the Indian solid gold. *Adv. Exp. Med. Biol.*, 595: 1-75.
- [22] Schieber, A.; Stintzing, F. C. and Carle, R. (2001). By-products of plant food processing as a source of functional compounds—recent developments. *Trends Food Sci. Technol.*; 12; 401-413.
- [23] Elhassaneen, Y.; Safaa El-Waseef, Naglaa Fathy and Sayed Ahmed, S. (2016-a). Bioactive Compounds and Antioxidant Potential of Food Industry By-products in Egypt. *American Journal of Food and Nutrition*, 4 (1): 1-7.
- [24] Singh, N. P.; McCoy, M. T.; Tice, R. R. and Schneider, E. L. (2009). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*; 175: 184-191.
- [25] Cao, G.; Sofic, E. and Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.*, 44: 3426-3431.
- [26] Mazza, G.; Cacace, J. E. and Kay, C. D. (2004). Methods of analysis for anthocyanins in plants and biological fluids. *J. AOAC Int.*, 87: 129-145.
- [27] Seung-Cheol Lee (2011). Antioxidant activity of different parts of eggplant. *Journal of Medicinal Plants Research* 5(18): 4610-4615.
- [28] 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.
- [29] Tesoriere LM, Butera D, Pintaudi AM, Allegra M, Livrea MA (2004) Supplementation with cactus pear (*Opuntia ficus-indica*) fruit decreases oxidative stress in healthy humans: a comparative study with vitamin C. *Am J Clin Nutr* 80(2): 391-395.
- [30] Elhassaneen, Y.; Sherif, S. Ragab and Saleh, A. (2016-b). Effect of Selected Plant Parts Extracts on Liver Injuries Induced by CCl₄ in vitro. *Pyrex Journal of Medicinal Plant Research*, 2 (2): 8-20.
- [31] Cardador-Martínez, A.; Jiménez-Martínez, C. and Sandoval, G. (2011). Revalorization of cactus pear (*Opuntia spp.*) wastes as a source of antioxidants. *Ciê e Tecn. Alim*, 31: 782-788.
- [32] Abou-Elella, F. and Ali, R. (2014). Antioxidant and Anticancer Activities of Different Constituents Extracted from Egyptian Prickly Pear Cactus (*Opuntia Ficus-Indica*) Peel. *Biochem Anal Biochem*, 3: 158.
- [33] Amin, I.; Zamaliah, M. and Chin, W. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry*; 87(4): 581-586.
- [34] Marco, G. (1968). A rapid method for evaluation of antioxidants. *J. Am. Oil Chem. Soc.*, 45: 594-598.
- [35] Al-Saikhan, M.; Howard, L. and Miller, J. (1995). Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum*, L.). *J. Food Sci.*, 60 (2): 341-343.
- [36] Singleton, V. and Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 16: 144-158.
- [37] Tietze F. (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal. Biochem.* 27, 502-522.
- [38] Geoff D. and Brian S. (2002). *A Handbook of Statistical Analyses using SAS*, Chapman & Hall/CRC, Washington, D.C.
- [39] Saleh, A. M. (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.
- [40] Montenegro, M. A., Marya L. Boiero, Lorena Valle and Claudio D. Borsarelli (2012). Gum Arabic: More Than an Edible Emulsifier, In *Tech Europe*, University Campus STePRi, Slavka Krautzeka 83/A, 51000 Rijeka, Croatia.
- [41] Ahmed, S. K. (2015). Utilization of by-products of food industries in the production of snacks with high nutritional value and healthy safe " Ph.D. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt.
- [42] Velioglu, Y. S.; Mazza, G.; Gao, L. and Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem.*, 46 (10): 4113-4117.
- [43] Kunyanga, C. N.; Imungi, J. K.; Okoth, M. W.; Biesalski, H. K. and Vellingiri, V. (2012). Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients. *LWT—Food Sci. Technol.*, 45(2): 269-276.
- [44] El-Mokadem, K. (2010). The effect of technological treatments on phytochemical properties of some foods. M.Sc. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt.
- [45] Sayed Ahmed, S. (2016). Nutritional and technological studies on the effect of phytochemicals on obesity injuries and their related diseases by using experimental animals Ph.D. Thesis in Home Economics (Nutrition and Food Science), Faculty of Specific Education, Port Said University, Egypt.
- [46] Majid, S.; Khanduja, K. L.; Gandhi, R. K.; Kapur, S. and Sharma, R. R. (1991). Influence of ellagic acid on antioxidant defense system and lipid peroxidation in mice. *Biochemistry and Pharmacology journal*, 42(7): 1441-1445.
- [47] Laranjinha, J.; Almeida, L. and Madeira, V. (1994). Reactivity of dietary phenolic acids with peroxy radicals: antioxidant activity upon low-density lipoprotein peroxidation. *Biochem. Pharmacol*, 48(3): 487-494.
- [48] Shalaby, H. H. (2015). The effect of some food products mixed with plant parts on blood sugar levels of rats " Ph.D. Thesis in Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Egypt.
- [49] Beatty, P. W. and Reed, D. J. (1980): Involvement of the cystathionine pathway in the biosynthesis of glutathione by isolated rat hepatocytes. *Arch Biochem Biophys*, 204: 80-87.

- [50] Larsson, B. K.; Sahllerg, G. P.; Eriksson, A. T.; and Busk, L. A. (1983): Polycyclic aromatic hydrocarbons in grilled food. *J. Agric. Fd Chem.*, 31: 867-873.
- [51] Halliwell, B. and Gutteridge, J. M. (1985). Lipid peroxidation a radical chain reaction. In: Halliwell, B., Gutteridge, J. M. C. (Eds.), *Free Radicals in Biology and Medicine*. Clarendon Press, Oxford, pp: 139-205.
- [52] Sudheesh, S.; Sandhya, C. and Sarah Koshy, A. (1999): Antioxidant activity of flavonoids from *Solanum melongena*. *Phytother Res.* 13(5):393-396.
- [53] Matsuzoe, M.; Yamaguchi, S.; Kawanobu, Y.; Watanabe, H. and Sakata, Y. (1999). Effect of Dark Treatment of the Eggplant on Fruit Skin Color and Its Anthocyanin Components. *Journal of the Japanese Society for Horticultural Science*, 68, 1, 138-145.
- [54] 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.
- [55] 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): 141-153.
- [56] Franca, B. and Harri, V. (2009). Allium Vegetables and Organosulfur Compounds: Do They Help Prevent Cancer? *Environmental Health Perspectives*, 109(9): 893-902.
- [57] Albano, C.; Carmine N.; Noemi T.; Carmela G.; Giovanni M.; Antonio M.; Luigi D. and Federica B. (2015). Betalains, Phenols and Antioxidant Capacity in Cactus Pear [*Opuntia ficus-indica*(L.) Mill.] Fruits from Apulia (South Italy) Genotypes. *Antioxidants*, 4, 269-280.
- [58] Boone, C. W.; Steele, V. E.; and Kelloff, G. J. (1992): Screening of chemopreventive (anticarcinogenic) compounds in rodents. *Mut Res.*, 267: 251-255.
- [59] Chauhan, D. P. (2002). Chemotherapeutic potential of curcumin for colorectal cancer. *Current pharmaceutical Design*, 8(19): 1695 -1706.
- [60] Kaur, M.; Kaur, A. and Sharma (2012). R. Pharmacological actions of *Opuntia ficus indica*: A review. *J. Appl. Pharm. Sci.*, 2: 15-18.
- [61] Zheng, A.; Li, H.; Wang, X.; Feng, Z.; Xu, J.; and Cao, K. (2013): Anticancer effect of a curcumin derivative B63: ROS production and mitochondrial dysfunction. *Current Cancer Drug Targets* 14(2):156-166.
- [62] Fayed, S. (2016). The effect of turmeric and curcumin on liver cancer induced by benzo[a]pyrene in rats. M.Sc. Thesis in Home Economics (Nutrition and Food Science), Faculty of Specific Education, Port Said University, Egypt.
- [63] Ranucci, C. S.; Kumar, A.; Batra, S. P. and Moghe, P. V. (2000). Control of hepatocyte function on collagen foams: sizing matrix pores toward selective induction of 2-D and 3-D cellular morphogenesis. *Biomaterials* 21: 783-793.
- [64] Morgan, E. H. and Peters, T. J. (1971). The biosynthesis of rat serum albumin. V. Effect of protein depletion and refeeding on albumin and transferrin synthesis. *J Biol Chem* 246: 3500-3507.
- [65] Bokhari, M.; Ross J.; Neil R. and Stefan, A. (2007). Culture of HepG2 liver cells on three dimensional polystyrene scaffolds enhances cell structure and function during toxicological challenge. *J. Anat.* 211: 567-576.