

Benzo[a]pyrene Induced Toxicity and Molecular Damage in Liver of Rats: Possible Protective Effects of Onion (*Allium cepa*) Skin Extract

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Abstract Benzo[a]pyrene (B[a]P) is the main representative of polycyclic aromatic hydrocarbons (PAHs), and has been repeatedly found in the air, surface water, soil, sediments and foods. Exposure to this ubiquitous environmental and food contaminants is associated with the development of several diseases including liver cancer. The current work was conducted to investigate the possible protective effects of onion (*Allium cepa*) skin extract (OSE) against toxicity and molecular damage in liver of rats induced by B[a]P. Injected of rats with B[a]P caused a significant increased ($p \leq 0.05$) in serum liver enzymes functions (AST, 60.16%, ALT, 111.31%, and ALP, 145.15%), serum lipid profile (total cholesterol, 8.54% and LDL-c, 52.61%), biological oxidants levels in serum such Malondialdehyde, (MDA), serum inflammation parameters (TNF-, 89.17, nitric oxide, 82.42%), hepatic CYP450, 81.41%, oxidative stress parameters (MDA, 59.54% and ROS, 283.67%), and a significant decreased ($p \leq 0.05$) in serum triglycerides (-9.09%), serum HDLC-c (-38.69%), and hepatic glycogen (-68.10%). Also, hepatic apoptosis biomarkers were altered, increase in caspase-3 and Bax (67.57 and 104.20%), and decrease in Bcl-2 (-69.43%) compared to normal control rats. Treatment with OSE by a concentrations (50, 100, 150 and 200 mg/kg bw day) led to improve all of these biological parameters by different rates and positively manipulated B[a]P-related histopathological changes in liver tissues. The rate of prevention was recorded a dose-dependent manner as a result of OSE treatment. These findings supported our hypothesis that such plant part extract (OSE) contains several classes of bioactive compounds with different biological activities that are able to prevent and/or inhibit hepatotoxicity induced by B[a]P through one or more of the biochemical and molecular pathways. In conclusion, our present findings suggested that OSE could be used as antihepatotoxic complement of liver disease patients. This may be related to it's improve the liver functions, serum lipid profile, and antioxidant and inflammatory defense systems as well as the anti-apoptotic effect.

Keywords: Liver functions, serum lipid profile, TNF- α , CYP450, nitric oxide, ROS, MDA, apoptosis

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1. Introduction

Benzo[a]pyrene (B[a]P) is a polycyclic aromatic hydrocarbon (PAH) with the formula $C_{20}H_{12}$ and the result of incomplete burning of organic (carbon-containing) items at temperatures between 300 °C and 600 °C. It is a ubiquitous compound can be found in residential wood burning, coal tar, automobile exhaust fumes especially from diesel engines), all smoke resulting from the combustion of organic material including cigarette smoke and many foods, especially charbroiled items [1,2,3,4,5]. For foods, the study of Kazerouni [6] found levels of B[a]P to be significantly higher in foods that were cooked well-done on the barbecue, particularly steaks, chicken with skin, and hamburgers. Also, cooked meat products

have been shown to contain up to 4 ng/g of BaP, up to 5.5 ng/g in fried chicken and 62.6 ng/g in overcooked charcoal barbecued beef [7,8,9]. Furthermore, Elhassaneen and Tawfik, [10] stated that fumes generated from the frying process contains B[a]P and could be threaten the public health. The World Health Organization [11] stated that 99% of the oral intake of PAH including B[a]P contributed by food, 0.9% by inhalation and 0.1-0.3% by drinking water.

Several decades ago, many studies indicated that B[a]P have been shown to be toxic, mutagenic and/or carcinogenic by extensive experiments *in vivo* and *in vitro* systems [12,13,14,15,16,17,18]. Also, numerous studies since the 1970s have documented links between B[a]P and liver cancer in mammals from fish to human [12,13,14,16,19,20,21,22]. All previous studies and others stated that the toxic and carcinogenic effects of B[a]P

comes through three enzymatic reactions as follow: 1) It is first oxidized by cytochrome P450 1A1 to form a variety of metabolites, including (+) B[a]P-7,8-epoxide, 2) This product is metabolized by epoxide hydrolase, opening up the epoxide ring to yield (–) B[a]P-7,8-dihydrodiol, and 3) the ultimate carcinogen is formed after another reaction with cytochrome P450 1A1 to yield the (+) B[a]P-7,8-dihydrodiol-9,10-epoxide that covalently binds to DNA [12,14,23,24]. On the other side, B[a]P reactive intermediate metabolites (arene oxides, phenols, quinones, dihydrodiols, and epoxides) induced several cytotoxic effects to the liver cells through oxidative stress pathways such as mitochondria and lysosomes dysfunctions, and defects in the cell wall membrane integrity [2,5,14,20,22,25,26,27].

All previous studies and others emphasize the need to search for treatments used to prevent or treat the harmful effects of B[a]P on the liver. In this direction, the modern pharmaceutical therapy is costly and associated with multiple side effects resulting in patient non-compliance. For that, there is a need to explore alternative therapies particularly from natural sources as these are cost effective and possess minimal side effects. Many of authorities and academic centers of research pay more attention towards the area of hepatotoxicity 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 hepatotoxicity. Many of these classes are lies in an enlarged group of compounds called phytochemicals. Scientists have identified thousands of phytochemicals, including phenolics, flavonoids, carotenoids, glucosinolates, alkaloids, terpenes, and phytoestrogens, in vegetables, fruits, grains, legumes, and other plant sources. A vast variety of phytochemicals that are present in the daily human diet have been found to possess substantial antitoxic, antimutagenic and anticarcinogenic properties [17,18,21,22,27,28,29,30]. The results of these and other studies were encouraging to search for more of these plant parts that are highly effective in treating liver damage resulting from exposure to toxic substances.

Onion (*Allium cepa* L.) belongs to the *Lilliaceae* family and is grown all over the world. Dehydrated onions in the form of flakes or powder are in extensive demand in several parts of the world such as United Kingdom, Japan, Russia, Germany, Netherlands, Spain etc. Thus, dehydrated onions are a product of considerable importance in world trade. The predicted size of the global dehydrated onions market in 2022 was close to US\$ 1.1 billion (2.25 million kg) (www.osti.gov/etdweb/servlets/purl/895137). The global dehydrated onion market is growing due to reasons such as increased consumer demand for fast food as well as ready-to-eat convenience foods. Such food industry produces a large amount of onion wastes, making it necessary to search for possible ways for their utilization. The main onion wastes include onion skins, two outer fleshy scales and roots generated during industrial peeling. One way could be to use these onion wastes as a natural source of high-value functional ingredients, since onion are rich in several groups of compounds [31,32,33,34,35,36,37]. Processing and stabilizing onion wastes could solve the environmental problem derived from a great onion wastes

disposal [38]. Several studies have been established for using the onion skin in different applications include pectin production, pigments extraction, natural antioxidants, vinegar production, biogas productions, natural fertilizers, food products etc. [39–48]. In a trial to open new avenue for the utilization of onion wastes in some new therapeutic applications, the current study was conducted to investigate the possible protective effects of onion (*Allium cepa*) skin extract against toxicity and molecular damage in liver of rats induced by B[a]P.

2. Materials and Methods

2.1. Materials

Onion skins were obtained from New Bani Suef Company for Preservation, Dehydration and Industrialization of Vegetables, Bani Suef El-Goudida City, Nile east, Bani Suef, Egypt. Benzo[a]pyrene (B[a]P) was purchased from Sigma Chemical Co. (St. Louis, MO, Company agent, Cairo, Egypt). Casein was obtained from Morgan Chemical Co., Cairo, Egypt. All organic solvents, buffers and other chemicals of analytical grade were purchased from El-Ghomhorya Company for Trading in Drug, Chemicals and Medical Instruments, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of Onion Skin Powder (OSP)

Red onion skin were washed and dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 60 °C until arriving by the moisture in the final product to about 7%. The dried skin was ground into a fine powder in high mixer speed (Toshiba, ElAraby Co., Benha, Egypt). The material that passed through an 80 mesh sieve was retained for the basil diet blending purpose.

2.2.2. Preparations of Onion Skin Powder Extract (OSE)

Onion skin powder (OSE) was prepared according to the method mentioned by Gharib et al., [49] with some modifications. Briefly, each 100g of onion skin powder were extracted with 1000 ml of hydro-ethanolic solution (methanol and water; 80:20%) on an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) at 50 °C for 6 h. The extract was filtered (Whatman No. 5 filter paper) on a Buchner funnel. The collected filtrate was evaporated to complete dryness under reduced pressure at 40°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). The resulted extract was stored at 4 °C for biological experiments.

2.2.3. Biological Experiments

2.2.3.1. Animals

Animals used in this study, adult male albino rats (170± 7.5 g per each) were obtained from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt. Rats were kept under similar management and hygiene

conditions. They were housed individually in wire cages in a room maintained at 25 ± 3.0 °C, relative humidity ($56 \pm 4\%$), and kept under normal healthy conditions. Rats were fed a basal diet (BD) for one week before starting the experiment for acclimation.

2.2.3.2. Basal Diet

The basic diet prepared according to the following formula as mentioned by Reeves et al., [50] 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 compositions of salt and vitamin mixtures used in the BD were prepared according to the same reference.

2.2.3.3. Induction of Hepatotoxicant in Rats

Thirty male albino rats were administrated by intraperitoneal (IP) injection of B[a]P (125 mg/kg/b.wt. dissolved in 0.9% NaCl solution containing 0.1% Tween 20), on the seventh day only following the method described by Shahid et al., [51]. Liver intoxication was confirmed by taking a random sample of experimental animals (three rats) and biochemical (liver functions) examined.

2.2.3.4. 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 [52]. Rats ($n=36$ rats) were divided into two main groups, the first main group (Group 1, 6 rats, as a negative control group) still fed on basal/standard diet and injected with the vehicle alone (5 ml/kg body weight) and the other main group (30 rats, hepatotoxic) then classified into five sub- groups as follow: Group 2, fed on standard diet only as a positive control, model control, and Groups 3 (OSET1), 4 (OSTE2), 5 (OSTE3) and 6, (OSET4), fed on BD and treated with OSE at 50., 100, 150, 200 mg/kg bw/day, respectively. The tested concentrations of OSE suggested for current study were based on several previous studies [45,21]. For 28 days, each of the above groups was housed in a single cage. To determine the biological evaluation of body weight gain (BWG, %), food intake (FI) and food efficiency ratio (FER), the diet consumed was recorded every day and body weight was recorded every week during the experimental period (32 days). The BWG (%), FI and FER were calculated according to the Chapman et al., [53] equations as follow: $BWG (\%) = (Final\ weight - Initial\ weight) / Initial\ weight \times 100$, $FER = Grams\ gain\ in\ body\ weight\ (g/32\ day) / Grams\ feed\ intake\ (g/32\ day)$.

2.2.3.5. Blood and Liver Sampling

For blood and liver samples, rats were deprived of food overnight at the end of the experiment (28 days) and sacrificed by decapitation. Blood samples were collected using the abdominal aorta and the livers were immediately dissected out, washed in ice-cold saline, blotted dry and divided into two parts weight. The first part used for preparation liver homogenates such as mentioned by El-Khawaga et al., [54] for measuring various biochemical

parameters, and the second part used for histological studies. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 4000 rpm to separate the serum according to Drury and Wallington, [55]. Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at $-18^{\circ}C$ until analysis.

2.2.3.6. Hematological Analysis

Liver functions

Serum glutamic pyruvic transaminase (ALT) and serum glutamic oxaloacetic transaminase (AST) activities were measured in serum using the modified kinetic method of Tietz et al., [56] by using kit supplied by Biocon Company. Alkaline Phosphatase (ALP) activity was determined using modified kinetic method of Vassault et al., [57] by using kit supplied by Elitech Company.

Liver glycogen level

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).

Serum lipids profile

Triglycerides (TGs), total cholesterol (TC), HDL-Cholesterol and LDL-cholesterol were determined in serum according to the methods of Ahmadi et al., [58], Fossati and Prenape [59], Lopes-Virella et al., [60], and Richmond, [61], respectively.

Drug Metabolizing Enzymes (Cytochrome P450)

Cytochrome P-450 was measured by the carbon monoxide difference spectrophotometry of dithionite-reduced samples by using the method of Omura and Sato [62].

Malonaldehyde (MDA) and Reactive oxygen species (ROS)

Malonaldehyde (MDA) content was measured using the colorimetric method described by Buege and Aust, [63] based on the reaction of thiobarbituric acid (TBA) with MDA, one of the aldehyde products of lipid peroxidation. Reactive oxygen species (ROS) was determined by a colorimetric method described by Erel, [64].

Nitric oxide determination

Nitric oxide (NO) determination was done as the sum of NO_2 and NO_3 as mentioned by Miranda et al., [65].

TNF- α assay

TNF- α was determined by a sandwich enzyme-linked immunosorbent assay (ELISA), utilizing two monoclonal antibodies directed against separate antigenic determinants on rat TNF- α according to the manufacturer's instructions. The kits for the assay were provided by Adlitteram Diagnostic Laboratories Inc. (San Diego, CA, USA).

Liver cell apoptosis assays

Liver samples were prepared and analyzed for B-Cell Lymphoma 2 (Bcl-2), Bcl-2-like Protein 4 (Bax), and caspase-3 by using of flow cytometer instrument (Becton Dickinson, San Jose, CA, USA) according to the method of Gong et al., [66].

2.2.3.7. Histopathological Examination

Liver samples were collected immediately after slaughtering animals and submerged in 10% neutral buffered formalin. Fixed specimens were then trimmed

and dehydrated with increasing concentrations of alcohol, cleared in xylene, embedded in paraffin, sectioned (4-6 μm thickness), stained with hematoxylin and eosin and examined microscopically [67].

2.3. Statistical Analysis

All measurements were done in triplicate and recorded as mean $\pm$ SD. A significance of differences was determined by one-way ANOVA followed by Duncan's test for multiple comparisons using an MINITAB 12 computer program (Minitab Inc., State College, PA). A probability level of $P \leq 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Effect of Four Weeks Treatment With Onion Skin Ethanolic Extract (OSE) on BWG, FI and FER of Normal and Benzo[A]Pyrene-Hepatotoxic Rats

Data in Table 1 and Table 2 illustrated the effect of treatment with onion skin ethanolic extract (OSE) on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of normal and benzo[a]pyrene (B[a]P)-hepatotoxic rats. A significant ($p \leq 0.05$) increase in BWG, FI and FER level were observed in B[a]P- treated rats compared with that of normal rats. The rate of decreasing was recorded -18.08, -15.66 and -18.86% for all of these parameters, respectively. However, treatment the hepatotoxic rats with OSE (50, 100, 150 and 200 mg/kg body weight) for 28 days led to significantly ($p \leq 0.05$) increase the levels of BWG, FI and FER by the rate of

0.94, 3.80, 11.72 and 14.24%, 0.67, 6.36, 7.71 and 9.22%, 2.88, 6.29, 12.64 and 15.94% compared to the hepatotoxic rats, respectively. The rate of increasing in BWG, FI and FER recorded a dose-dependent manner as a result of OSE treatment. The present data are compatible with those reported by Elhassaneen *et al.* [68] who found that injection of B[a]P in male albino rats led to a decrease in body weight compared with normal rats. The same results were noticed by Tag Al Deen and Ghozy [69], significant decrease in body weight and feed intake in rats injected with B[a]P compared to normal rats. In contrary, other studies reported that treatment male rats with B[a]P had no significant impact on the body weight as related to the normal group rats [70,71]. Such variation may be related to differences in B[a]P dosage concentration, administration route, age/weight of the animals, experimental duration. The present data exhibited that OSE treatment at high concentrations resulted in a significant improvement in FI, BWG, and FER than at low OSE concentrations in B[a]P-treated rats. The improvement in BWG, FI, and FER of hepatotoxic rats treated with OSE at high concentrations may be due to its high antioxidant capacity, which scavenges different free radicals subsequently alleviates oxidative stress induced by B[a]P. In this direction, several studies reported that hepatotoxicity led to decrease in BWG, FI and FER which were improved by consumption of plant parts contains active secondary metabolites such as found in OSE [30,72,73]. Other related studies have shown that liver diseases can lead to malnutrition, which may be one of the main causes of malnutrition in people with these diseases, some of whose effects may be poor food intake, poor digestion, poor absorption, metabolic disorders and the level of storage of macro and micronutrients [74,75].

Table 1. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on BWG, FI and FER of normal and benzo[a]pyrene (B[a]P)-hepatotoxic rats

Group	BWG (%)		FI (g/day/rat)		FER	
	Range	Means $\pm$ SD	Range	Means $\pm$ SD	Range	Means $\pm$ SD
Normal control	0.891-1.076	0.981 $\pm$ 0.089 ^a	9.98-11.45	10.99 $\pm$ 0.62 ^a	0.061-0.087	0.075 $\pm$ 0.010 ^a
Model control (Hepatotoxic)	0.743-0.985	0.804 $\pm$ 0.089 ^c	8.65-9.94	9.27 $\pm$ 0.61 ^c	0.052-0.068	0.061 $\pm$ 0.009 ^b
OSET1	0.890-0.841	0.811 $\pm$ 0.022 ^c	7.67-9.97	9.33 $\pm$ 0.77 ^c	0.056-0.073	0.063 $\pm$ 0.008 ^b
OSET2	0.761-0.923	0.834 $\pm$ 0.079 ^c	9.20-10.43	9.86 $\pm$ 0.66 ^c	0.061-0.077	0.065 $\pm$ 0.007 ^{ab}
OSET3	0.834-0.959	0.898 $\pm$ 0.07 ^{bc}	9.56-10.33	9.98 $\pm$ 0.41 ^{bc}	0.060-0.075	0.069 $\pm$ 0.007 ^a
OSET4	0.947-0.955	0.918 $\pm$ 0.033 ^b	9.92-10.73	10.12 $\pm$ 0.57 ^b	0.061-0.074	0.071 $\pm$ 0.006 ^a

Results are expressed as means $\pm$ SD (n = 6). Different superscripts including in the same column indicate a significant difference ($P \leq 0.05$). Normal control: healthy rats without treatment; Model control: B[a]P-induced hepatotoxicity rats without treatment; OSE, Onion skin ethanol extract, bw, body weight; OSET1, OSET2, OSET3 and OSET4, hepatotoxic rats treated with OSE at 50., 100, 150, 200 mg/kg bw/day, respectively.

Table 2. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on BWG, FI and FER of normal and benzo[a]pyrene (B[a]P)-hepatotoxic rats

Group	BWG (as a % of change)*	FI (as a % of change)*	FER (as a % of change)*
Normal control	-----	-----	-----
Model control (Hepatotoxic)	-18.08 $\pm$ 0.88	-15.66 $\pm$ 1.21	-18.86 $\pm$ 0.93
OSET1	0.94 $\pm$ 0.10	0.67 $\pm$ 0.05	2.88 $\pm$ 0.32
OSET2	3.80 $\pm$ 0.52	6.36 $\pm$ 0.69	6.29 $\pm$ 0.42
OSET3	11.72 $\pm$ 0.39	7.71 $\pm$ 0.54	12.64 $\pm$ 0.77
OSET4	14.24 $\pm$ 0.66	9.22 $\pm$ 0.43	15.94 $\pm$ 0.23

Results are expressed as means $\pm$ SD (n = 6). *Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with OSE compared to the hepatotoxic group. The guides of the experimental groups are as shown in Table 1.

3.2. Effect of Four Weeks Treatment with Onion Skin Ethanolic Extract (OSE) on Liver Functions of Normal and Benzo[a]Pyrene-Hepatotoxic Rats

Effect of four weeks treatment with onion skin ethanolic extract (OSE) on liver functions of normal and benzo[a]pyrene-hepatotoxic rats were shown in Table 3 and Table 4. Such data indicated that a significant ($p \leq 0.05$) increase in AST, ALT and ALP levels were observed in B[a]P-treated rats compared with that of normal rats. The rate of decreasing was recorded 60.16, 111.31 and 145.15% for all of these parameters, respectively. However, treatment the hepatotoxic rats with OSE (50, 100, 150 and 200 mg/kg body weight) for 28 days led to significantly ($p \leq 0.05$) increase the levels of AST, ALT and ALP by the rate of -7.82, -14.83, -23.71 and -26.64%, -9.27, -13.54, -27.81 and -42.67%, and -11.82, -15.93, -35.12 and -43.07% compared to the hepatotoxic rats, respectively. The rate of increasing in AST, ALT and ALP levels recorded a dose-dependent manner as a result of OSE treatment. Several decades ago, B[a]P was commonly used as a hepatotoxic agent in the experimental studies of liver diseases. The hepatotoxicity induced by B[a]P are extremely large due to the binding of its activated metabolites with the different cellular macromolecules rich in polyunsaturated fatty acids such as cell wall membrane, mitochondria and lysosomes, and induce peroxidative degradation of their membrane lipids [14,15,18]. Such degradation induced in

cellular membranes represents one of the main causes of hepatotoxicity of B[a]P [2,18]. This is confirmed by the raising recorded in the serum marker enzymes namely AST, ALT and ALP. Elevated levels of such enzymes illustrated liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury [76]. In the same context, Elhassaneen and Al-Badawy, [5] found that elevations in liver functions enzymatic activities including AST, ALT and ALP in human samples as the result of B[a]P consumption in charcoal broiled meat diet. Several previous studies reviewed that onion skin is rich in bioactive compounds including phenolics, carotenoids, phytosterols and organosulfur compounds [77,78]. These phytochemicals have the ability to protect the B[a]P-treated animals from hepatotoxicity through their various biological activities that they induce [17,79]. The effect of many plant parts on manipulation the serum liver function enzymes activity could be attributed to their high level content of bioactive compounds such as found in OSE [21,29,76,80,81,8283]. The possible mode of action of liver serum enzymes-lowering activity of the OSE could be explained by one or more of the following process: 1) block the hepatocellular uptake of bile acids [21,81,84], 2) improve the antioxidant capacity of the hepatocytes [5,45,85], 3) treating the defect in the enzyme systems responsible for the phases of biotransformation processes, 4) improve the of antioxidant defense system in red blood cells [2, 86] and 5) attenuating molecular damage in liver cells [20,86].

Table 3. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on liver functions of normal and benzo[a]pyrene-hepatotoxic rats

Group	AST (IU/L)		ALT (IU/L)		ALP (IU/L)	
	Range	Means $\pm$ SD	Range	Means $\pm$ SD	Range	Means $\pm$ SD
Normal control	51.11-66.45	57.01 $\pm$ 6.21 ^c	29.00-40.07	34.53 $\pm$ 5.88 ^d	105.67-129.67	119.18 $\pm$ 13.28 ^e
Model control (Hepatotoxic)	84.87-96.89	91.31 $\pm$ 5.19 ^a	59.54-80.12	72.98 $\pm$ 10.66 ^a	242.44-329.39	292.17 $\pm$ 39.89 ^a
OSET1	73.67-92.12	84.17 $\pm$ 9.22 ^a	55.33-74.06	66.21 $\pm$ 10.18 ^a	239.10-266.83	257.65 $\pm$ 11.31 ^b
OSET2	70.89-75.90	77.77 $\pm$ 5.57 ^{ab}	54.72-67.39	63.10 $\pm$ 6.63 ^a	220.62-272.78	245.64 $\pm$ 29.86 ^b
OSET3	60.65-77.43	69.66 $\pm$ 8.76 ^b	45.12-57.32	52.68 $\pm$ 6.31 ^b	167.32-207.45	189.57 $\pm$ 19.42 ^c
OSET4	56.87-78.92	66.99 $\pm$ 11.06 ^{bc}	33.89-52.22	41.84 $\pm$ 10.68 ^{cd}	149.45-182.90	166.32 $\pm$ 15.45 ^d

Results are expressed as means $\pm$ SD (n = 6). Different superscripts including in the same column indicate a significant difference ($P \leq 0.05$). OSE, Onion skin ethanol extract. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase. Details of groups are as shown under Table 1.

Table 4. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on liver functions of normal and benzo[a]pyrene-hepatotoxic rats

Group	AST (as a % of change)*	ALT (as a % of change)*	ALP (as a % of change)*
Normal control	-----	-----	-----
Model control (Hepatotoxic)	60.16 $\pm$ 5.34	111.31 $\pm$ 4.76	145.15 $\pm$ 6.98
OSET1	-7.82 $\pm$ 0.51	-9.27 $\pm$ 0.45	-11.82 $\pm$ 0.99
OSET2	-14.83 $\pm$ 0.14	-13.54 $\pm$ 0.56	-15.93 $\pm$ 0.68
OSET3	-23.71 $\pm$ 2.73	-27.81 $\pm$ 1.53	-35.12 $\pm$ 3.21
OSET4	-26.64 $\pm$ 1.49	-42.67 $\pm$ 2.94	-43.07 $\pm$ 5.10

Results are expressed as means $\pm$ SD (n = 6). *Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with OSE compared to the hepatotoxic group. The guides of the experimental groups and abbreviations are as shown in Table 1.

Table 5. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on liver glycogen concentration of normal and benzo[a]pyrene-hepatotoxic rats

Group	Glycogen concentration (mg/g wet tissue)		
	Range	Means $\pm$ SD	As a percent of change (%) [*]
Normal control	10.04-13.78	12.76 $\pm$ 1.11 a	-----
Model control (Hepatotoxic)	3.56-5.37	4.07 $\pm$ 0.98 e	-68.10 $\pm$ 5.11
OSET1	3.13-4.26	4.13 $\pm$ 1.01 e	1.47 $\pm$ 0.04
OSET2	4.12-5.86	5.02 $\pm$ 0.97 d	23.34 $\pm$ 3.18
OSET3	5.01-6.78	5.75 $\pm$ 0.93 cd	41.28 $\pm$ 4.55
OSET4	7.59-8.97	7.89 $\pm$ 0.58 b	93.86 $\pm$ 7.10

Results are expressed as means $\pm$ SD (n = 6). ^{*}Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with OSE compared to the hepatotoxic group. OSE, Onion skin ethanol extract. Details of groups are as shown under Table 1.

3.3. Effect of Four Weeks Treatment with Onion Skin Ethanolic Extract (OSE) on Liver Glycogen Concentration of Normal and Benzo[A]Pyrene-Hepatotoxic Rats

Effect of four weeks treatment with onion skin ethanolic extract (OSE) on liver glycogen concentration of normal and benzo[a]pyrene-hepatotoxic rats were shown in Table 5. From such data it could be noticed that B[a]P induced significantly ($p \leq 0.05$) decreasing in liver glycogen content by the ratio of -68.10%. However, treatment the hepatotoxic rats with OSE (50, 100, 150 and 200 mg/kg body weight) for 28 days led to significantly ($p \leq 0.05$) increase the levels of glycogen by the rate of 1.47, 23.34, 41.28 and 93.86 compared to the hepatotoxic rats, respectively. The rate of increasing in glycogen levels recorded a dose-dependent manner as a result of OSE treatment. In general, glycogen is represents a complex sugar necessary for production of nutrients and energy in the body. In liver hepatotoxicity, the disorders of carbohydrates play significant roles in hepatotoxic disorders [21,29,87]. This fact has been observed in the present results in which decreased the levels of hepatic glycogen content when compared to normal-control group. Perhaps this decrease in glycogen content in the liver homogenates of hepatotoxic rats may be due to a decrease of glycogenesis pathway in the liver as a result of B[a]P toxicity [19,29]. Such hepatoprotective effect of OSE in B[a]P-induced hepatotoxic rats may be related to its high content of active secondary metabolites including phenolics, carotenoids, anthocyanins, pigments, essential oils and glycosides [46,88,89]. Such active secondary metabolites are known for their biological activities including antioxidant and scavenging activities, and inhibition of lipid oxidation which plays an important role in protecting the liver from many complications resulting from many diseases including hepatotoxicity [17,80,89]. In similar study, Hasegawa *et al.*, [87] found that previous drinking of green tea clearly protected against the changes in liver glycogen content. 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.

3.4. Effect of Four Weeks Treatment with Onion Skin Ethanolic Extract (OSE) on Serum Lipid Profile of Normal and Benzo[A]Pyrene-Hepatotoxic Rats

Effect of four weeks treatment with onion skin ethanolic extract (OSE) on serum lipid profile of normal and benzo[a]pyrene-hepatotoxic rats were shown in Table 6 and Table 7. Such data indicated that B[a]P induced significantly ($p \leq 0.05$) decreasing in serum TG and HDL levels by the ratio of -9.09 and -38.69% whereas, the serum total cholesterol and LDL-c concentration were exhibited the opposite direction. As the result of OSE, serum TGs and HDL were significantly ($p \leq 0.05$) decreased and cholesterol was significantly ($p \leq 0.05$) elevated. The rate of decreasing in TGs and HDL as well as elevation in total cholesterol and LDL-c exhibited a dose-dependent increase with the OSE treatment. Such data are in agreement with the reported by Fayez [29] and Badawi, [90] as the result of treatment of liver injuries inducing by B[a]P with turmeric powder and reishi mushroom (*Ganoderma lucidum*) ethanol extract, respectively. Also, the hypocholesterolemic effect of OSE in B[a]P-treated animals is consistent with the results of Mahram and Sayed Ahmed [91] with mulberry leaves (*Morus alba* L.), and stated that such protective effect could be attributed to its bioactive compounds content, which were partially measured similar to OSE. In general, the possible hypocholesterolemic effects of several dietary components, such as found in OSE including, flavonoids, phenolics, alkaloids, carotenoids etc., have attracted much interest [32,92,93,94]. These bioactive compounds exerts their beneficial effects on cardiovascular health by antioxidant, radicals scavenging and anti-inflammatory activities.

On the other side, serum TGs levels was decreased as the result of P[a]P subjection. Such data are consistent with that reported by several authors [87,29,91,21]. All of these studies with the present data may interpreted the decreasing of the serum TGs and HDL as the B[a]P injection by secretion of their components 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.

Table 6. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on serum lipid profile of normal and benzo[a]pyrene-hepatotoxic rats

Group	TGs (mg/dL)		TC (mg/dL)		HDL-c (mg/dL)		LDL-c (mg/dL)	
	Range	Means $\pm$ SD	Range	Means $\pm$ SD	Range	Means $\pm$ SD	Range	Means $\pm$ SD
Normal control	1.24-1.38	1.32 $\pm$ 0.06 ^a	2.80-3.10	2.93 $\pm$ 0.15 ^b	1.29-1.43	1.37 $\pm$ 0.09 ^a	0.94-1.02	0.98 $\pm$ 0.03 ^c
Model control (Hepatotoxic)	1.17-1.28	1.20 $\pm$ 0.05 ^b	3.05-3.30	3.18 $\pm$ 0.10 ^a	0.76-0.99	0.84 $\pm$ 0.09 ^c	1.30-1.56	1.49 $\pm$ 0.17 ^a
OSET1	1.17-1.30	1.21 $\pm$ 0.04 ^b	3.00-3.25	3.15 $\pm$ 0.12 ^a	0.90-0.96	0.92 $\pm$ 0.04 ^c	1.34-1.60	1.45 $\pm$ 0.13 ^a
OSET2	1.21-1.27	1.23 $\pm$ 0.04 ^b	2.91-3.20	3.10 $\pm$ 0.14 ^a	0.90-1.02	0.94 $\pm$ 0.06 ^c	1.34-1.58	1.40 $\pm$ 0.12 ^a
OSET3	1.20-1.28	1.25 $\pm$ 0.02 ^{ab}	2.81-3.10	3.02 $\pm$ 0.13 ^{ab}	0.98-1.16	1.07 $\pm$ 0.07 ^{bc}	1.22-1.51	1.34 $\pm$ 0.14 ^{ab}
OSET4	1.26-1.31	1.29 $\pm$ 0.02 ^a	2.80-3.17	2.97 $\pm$ 0.12 ^b	1.14-1.20	1.19 $\pm$ 0.07 ^b	1.09-1.29	1.18 $\pm$ 0.09 ^b

Results are expressed as means $\pm$ SD (n = 6). Different superscripts including in the same column indicate a significant difference ($P \leq 0.05$). OSE, Onion skin ethanol extract. TGs, total glycerides; TC, total cholesterol; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol. Details of groups are as shown under Table 3.

Table 7. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on serum lipid profile of normal and benzo[a]pyrene-hepatotoxic rats

Group	TGs (as a % of change)*	TC (as a % of change)*	HDL-c (as a % of change)*	LDL-c (as a % of change)*
Normal control	-----	-----	-----	-----
Model control (Hepatotoxic)	-9.09 $\pm$ 0.29	8.53 $\pm$ 0.56	-38.69 $\pm$ 2.11	52.04 $\pm$ 5.87
OSET1	0.83 $\pm$ 0.04	-0.94 $\pm$ 0.63	9.52 $\pm$ 0.66	-2.68 $\pm$ 0.32
OSET2	2.50 $\pm$ 0.08	-2.52 $\pm$ 0.16	11.90 $\pm$ 0.94	-6.04 $\pm$ 0.23
OSET3	4.17 $\pm$ 0.34	-5.03 $\pm$ 0.34	27.38 $\pm$ 1.45	-10.07 $\pm$ 0.45
OSET4	7.50 $\pm$ 0.78	-6.60 $\pm$ 0.09	41.67 $\pm$ 3.11	-20.81 $\pm$ 2.05

Results are expressed as means $\pm$ SD (n = 6). *Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with OSE compared to the hepatotoxic group. The guides of the experimental groups and abbreviations are as shown in Table 1.

3.5. Effect of Four Weeks Treatment with Onion Skin Ethanolic Extract (Ose) on Hepatic Malonaldehyde (Mda) and Reactive Oxygen Extract (Ros) of Normal and Benzo[A]Pyrene-Hepatotoxic Rats

Effect of four weeks treatment with onion skin ethanolic extract (OSE) on hepatic malonaldehyde (MDA) and reactive oxygen extract (ROS) of normal and benzo[a]pyrene-hepatotoxic were shown in Table 8 and Table 9. Such data indicated that injection of animals with B[a]P induced a significant increased ($p \leq 0.05$) in ROS and MDA by 283.67 and 59.54% compared to normal control rats, respectively. Treatment of hepatotoxic rats with OSE by 50, 100, 150 and 200 mg/kg bw was significantly ($p \leq 0.05$) decreased the mean serum ROS and MDA by the rate of -7.45, -18.62, -39.36 and -54.79%, and -6.94, -16.53, -22.53 and -27.13%, respectively. This means, the rate of decreasing in both serum ROS and MDA exhibited a dose- dependent manner with SOE treatment. Increasing the ROS and MDA in model control rats means B[a]P could induced hepatotoxicity through oxidative stress pathways. Oxidative stress defined as "a disturbance in the prooxidant - antioxidant balance in favor of the former, leading to potential damage" [95]. In the cells, oxidative stress can cause several toxic effects through the production of free radicals (reactive oxygen species, ROS) that damage different cell components including proteins, lipids, and DNA [14,86]. The oxidative stress as the result of oxidative metabolites causes base damage and strand breaks in DNA [96,97]. Base damage in DNA is mostly indirect and caused by ROS generated, e.g. O_2^- (superoxide radical), OH (hydroxyl radical) and H_2O_2 (hydrogen peroxide) [86,96,98]. Also, oxidative

stress induced other cytotoxic effects in cells. With this context, increased lipid peroxidation diminishes the membrane functions through decreasing membrane fluidity subsequently changing the activity of membrane bound enzymes and receptors [99]. Several studies reported that lipid peroxidation products are more cytotoxic and steady than ROS that reacts with cellular organelles such as mitochondria, lysosomes and cell wall membrane inducing dysfunction [42,100]. Also, Grune et al., [101] reported that MDA is a modulator of signal transduction pathways that disturb cellular activities. In similar studies, clinical evidences for B[a]P-associated oxidative stress have been provided by measurement of either biomarkers or end-products of free radical-mediated oxidative processes [2,18]. With this context, lipid peroxidation marker such as MDA, one of the most important and major product of the oxidation of polyunsaturated fatty acids, lipid hydroperoxides and conjugated dienes is found to be increased in plasma patients exposed to/ingested B[a]P [2]. The possible significance of MDA on human health has been stimulated several decades ago by reports that are mutagenic and carcinogenic compound [102]. Data of the present study indicated that four weeks treatment of B[a]P-hepatotoxic rats with OSE leads to decrease MDA and ROS in hepatic cells. Such effect could be attributed to the its high content of bioactive compounds including phenolics, anthocyanin's, carotenoids, essential oil etc. Several studies have documented the potent antioxidant capacity of these compounds where by mitigation of lipid peroxidation and oxidative stress in several tissues [18,77,86,89]. Therefore, data of this study prove that OSE is useful in the prevention of oxidative stress and lipid peroxidation caused by exposure of cells to B[a]P.

Table 8. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on hepatic malonaldehyde (MDA) and reactive oxygen extract (ROS) of normal and benzo[a]pyrene-hepatotoxic rats

Group	ROS (nmol/g wet tissue)		(MDA, nmol/g wet tissue)	
	Range	Means $\pm$ SD	Range	Means $\pm$ SD
Normal control	0.41-0.57	0.49 $\pm$ 0.04 ^e	610.56-709.54	651.15 $\pm$ 52.14 ^e
Model control (Hepatotoxic)	1.80-1.94	1.88 $\pm$ 0.07 ^a	939.15-1167.33	1038.86 $\pm$ 153.49 ^a
OSET1	1.60-1.78	1.74 $\pm$ 0.07 ^{ab}	829.56-1045.23	966.81 $\pm$ 111.38 ^b
OSET2	1.35-1.60	1.53 $\pm$ 0.18 ^b	750.56-960.54	867.18 $\pm$ 103.59 ^c
OSET3	1.07-1.16	1.14 $\pm$ 0.05 ^c	812.03-901.83	804.81 $\pm$ 89.26 ^{cd}
OSET4	0.71-0.89	0.85 $\pm$ 0.09 ^d	689.90-823.65	756.99 $\pm$ 74.04 ^d

Results are expressed as means $\pm$ SD (n = 6). Different superscripts including in the same column indicate a significant difference ($P \leq 0.05$). OSE, Onion skin ethanol extract. Details of groups are as shown under Table 3.

Table 9. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on hepatic malonaldehyde (MDA) and reactive oxygen extract (ROS) of normal and benzo[a]pyrene-hepatotoxic rats

Group	ROS (as a % of change)*	MDA (as a % of change)*
Normal control	-----	-----
Model control (Hepatotoxic)	283.67 $\pm$ 11.34	59.54 $\pm$ 5.23
OSET1	-7.45 $\pm$ 0.65	-6.94 $\pm$ 0.65
OSET2	-18.62 $\pm$ 1.04	-16.53 $\pm$ 0.93
OSET3	-39.36 $\pm$ 2.15	-22.53 $\pm$ 1.55
OSET4	-54.79 $\pm$ 3.97	-27.13 $\pm$ 2.05

Results are expressed as means $\pm$ SD (n = 6). * Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with OSE compared to the hepatotoxic group. The guides of the experimental groups and abbreviations are as shown in Table 1.

3.6. Effect of Four Weeks Treatment with Onion Skin Ethanolic Extract (OSE) on Hepatic on Serum Tumor Necrosis Factor- α (TNF- α) and Hepatic Nitric Oxide (NO) Content of Normal and Benzo[A]Pyrene-Hepatotoxic Rats

Effect of four weeks treatment with onion skin ethanolic extract (OSE) on hepatic on serum Tumor necrosis factor- α (TNF- α) and hepatic nitric oxide (NO) content of normal and benzo[a]pyrene-hepatotoxic rats were shown in Table 10 and Table 11. Such data indicated that injection of animals with B[a]P induced a significant increased ($p \leq 0.05$) in TNF- α and NO by 89.17 and 82.42% compared to normal control rats, respectively. Treatment of hepatotoxic rats with OSE by 50, 100, 150 and 200 mg/kg bw was significantly ($p \leq 0.05$) decreased the mean serum TNF- α and NO by the rate of -1.01, -8.42, -21.21 and -33.67%, and -6.29, -22.50, -31.17 and -37.72%, respectively. This means, the rate of decreasing in both serum TNF- α and NO exhibited a dose- dependent

manner with SOE treatment. Increasing the TNF- α and NO in model control rats means B[a]P could induced hepatotoxicity through Inflammation/ immunological pathways. This observation is corresponding with reported by many authors, exposure to B[a]P induces oxidative stress and inflammation in experimental animals [86,103,104]. B[a]P exposure triggers the activation of different transcription factors, particularly nuclear factor kappa B (NF- κ B), which triggers the release of pro-inflammatory cytokines such as TNF- α and interleukin-6 (IL-6) and the production of ROS [86,105,106]. Also, excessive ROS generated during B[a]P metabolism, after B[a]P-quinone metabolites formation, results in elevated lipid peroxidation and a drop in the antioxidant defense system. This shift in the balance forces B[a]P metabolism towards toxification, resulting in the generation of more reactive metabolites and DNA adducts formation [86,107]. Furthermore, TNF, a cytotoxic pro-inflammatory cytokine, is thought to be involved in the onset of liver damage. High levels of circulating TNF- α activate TNF- α receptors on the cell surface, which induces the stress-related proteins kinase c-Jun N-terminal kinase (JNK) and I κ B kinase (IKK). This cascade effect leads to further production of additional inflammatory cytokines [108]. Thus, inhibition of TNF- α is considered a therapeutic approach to alleviate liver injury. In this context, the co-treatment of rats with B[a]P + OSE (50 to 200 mg/kg bw/day) showed a marked decrease in TNF- α levels which was statistically significant ($p \leq 0.05$) compared to the model control group. This finding indicate a potential anti-inflammatory role for OSE contain diverse phytochemicals. This effect is attributed to its dual capability as a free radical scavenger and inhibitor of lipid peroxidation, as demonstrated in both *in vitro* and *in vivo* studies [88,89]. On the other side, nitric oxide (NO) is a small molecule that plays an important role in the communication among liver cells and regulates great liver functions [109]. It is generated from catalyzes the conversion of L-arginine to citrulline and highly reactive free radical species, NO by nitric oxide synthase. NO can react with different ROS and cell components as follow: O_2 and H_2O_2 to form nitrite (NO_2^-) and nitrate (NO_3^-), with superoxide anion ($O_2^{\cdot -}$) to make nitrate, with the amino and thiol groups of protein to form nitrosylated species, and with hemoglobin to form iron-nitrosyl adducts and/or NO_3 in blood, [109,110]. The excess production of NO has been implicated in the pathogenesis and tissue destruction of a growing number of immunological and inflammatory diseases including hepatotoxicity [86,89]. Present data indicated that OSE selected in the present study for treatment experiments is rich in bioactive compounds including phenolics, carotenoids, flavonoids, alkaloids and essential oil which exhibited antioxidant and scavenging activities in different biological systems [3,4,88,111]. Such antioxidant properties are important in the manipulation of hepatotoxicity and the development of its complications through nitric oxides scavenging processes and enhancing the immunological and inflammation parameters in liver cells.

Table 10. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on hepatic on serum Tumor necrosis factor- α (TNF- α) and hepatic nitric oxide (NO) content of normal and benzo[a]pyrene-hepatotoxic rats

Group	Serum tumor necrosis factors-alpha (TNF- α , ng/L)		Hepatic nitric oxide (NO, μ mol/g wet tissue)	
	Range	Means $\pm$ SD	Range	Means $\pm$ SD
Normal control	1.37-1.83	1.57 $\pm$ 0.24 ^c	15.03-22.57	18.66 $\pm$ 3.24 ^c
Model control (Hepatotoxic)	2.81-3.30	2.97 $\pm$ 0.17 ^a	30.03-36.21	34.04 $\pm$ 3.40 ^a
OSET1	2.80-3.26	2.94 $\pm$ 0.18 ^a	28.34-34.15	31.90 $\pm$ 2.67 ^a
OSET2	2.42-3.12	2.72 $\pm$ 0.39 ^{ab}	23.15-29.43	26.38 $\pm$ 2.40 ^{ab}
OSET3	2.11-2.70	2.34 $\pm$ 0.21 ^b	19.08-26.98	23.43 $\pm$ 3.81 ^b
OSET4	1.87-2.09	1.97 $\pm$ 0.06 ^{bc}	18.03-24.65	21.20 $\pm$ 2.30 ^{bc}

Results are expressed as means $\pm$ SD (n = 6). Different superscripts including in the same column indicate a significant difference ($P \leq 0.05$). OSE, Onion skin ethanol extract. Details of groups are as shown under Table 3.

Table 11. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on hepatic on serum Tumor necrosis factor- α (TNF- α) and hepatic nitric oxide (NO) content of normal and benzo[a]pyrene-hepatotoxic rats

Group	Serum tumor necrosis factors-alpha (TNF- α , as a % of change) *	Hepatic nitric oxide (No, as a % of change) *
Normal control	-----	-----
Model control (Hepatotoxic)	89.17 $\pm$ 5.21	82.42 $\pm$ 3.75
OSET1	-1.01 $\pm$ 0.01	-6.29 $\pm$ 0.56
OSET2	-8.42 $\pm$ 0.98	-22.50 $\pm$ 2.91
OSET3	-21.21 $\pm$ 1.22	-31.17 $\pm$ 1.89
OSET4	-33.67 $\pm$ 3.17	-37.72 $\pm$ 4.21

Results are expressed as means $\pm$ SD (n = 6). *Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with OSE compared to the hepatotoxic group. The guides of the experimental groups and abbreviations are as shown in Table 1.

Table 12. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on hepatic cytochrome p450 (CYP450) concentration content of normal and benzo[a]pyrene-hepatotoxic rats

Group	CYP450 (nmol/mg protein)		
	Range	Means $\pm$ SD	Percent of change (%) *
Normal control	1.47-1.62	1.56 $\pm$ 0.07 ^d	-----
Model control (Hepatotoxic)	2.59-3.01	2.83 $\pm$ 0.22 ^a	81.41 $\pm$ 4.17
OSET1	2.68-2.83	2.76 $\pm$ 0.09 ^a	-2.47 $\pm$ 0.11
OSET2	2.43-2.90	2.64 $\pm$ 0.19 ^{ab}	-6.71 $\pm$ 0.69
OSET3	2.10-2.81	2.40 $\pm$ 0.37 ^b	-15.19 $\pm$ 1.26
OSET4	1.52-2.17	1.92 $\pm$ 0.35 ^c	-32.16 $\pm$ 2.64

Results are expressed as means $\pm$ SD (n = 6). Different superscripts including in the same column indicate a significant difference ($P \leq 0.05$). OSE, Onion skin ethanol extract. *Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with OSE compared to the hepatotoxic group. Details of groups are as shown under Table 3.

3.7. Effect of Four Weeks Treatment with Onion Skin Ethanolic Extract (OSE) on Hepatic Cytochrome P450 (CYP450) Concentration Content of Normal and Benzo[A]Pyrene-Hepatotoxic Rats

Effect of four weeks treatment with onion skin ethanolic extract (OSE) on hepatic cytochrome p450 (CYP450) concentration content of normal and benzo[a]pyrene-hepatotoxic rats was shown in Table 12. From such data it could be noticed that injection of rats with B[a]P caused a significant increased ($p \leq 0.05$) in cytochrome P450 (81.41%) compared to the normal control rats. Treatment of rats with OSE by 50, 100, 150 and 200 mg/kg bw/day was significantly ($p \leq 0.05$) decreased the mean liver cytochrome P450 activity by the rate of -2.47, -6.71, -15.19 and -32.16%, respectively. This means, the rate of decreasing in liver cytochrome P450 showed a dose- dependent manner increase with the OSE treatment. Cytochromes P450 are a subfamily of enzymes that contain heme as a cofactor and act as

monooxygenases [112]. These enzymes are found in highest concentrations in the liver, while they are found in lower concentrations in other organs throughout the body, especially in the small intestinal mucosal enterocytes. P450 enzymes have versatile substrate specificities that evolved as a primary defense against xenobiotics and in this process are also responsible for the biotransformation of foreign substances (drugs, toxins and carcinogens) and endogenous compounds into more reactive intermediates [14,113,114]. Thus, measuring the activity level of such enzymes is one of the important biological indicators of the presence of xenobiotic substrates in the liver of all vertebrates. For example, Elhassaneen, [14] reported that B[a]P incubated brought about a significant ($p \leq 0.05$) increase in the activities of drug metabolizing enzymes (cytochrome P450 and b5) in liver hepatocytes. Also, Liu *et al.*, [115] found that B[a]P treatment inducing a significant increase in the activities of cytochrome P450 in lungs of mice and the activities of these enzymes were markedly decreased by the treatment with phytochemicals including curcumin and phenolic compounds. Furthermore, Mahram and Sayed Ahmed [91] reported

that B[a]P injection induce significant increasing in cytochrome P450 in rats and treatment with mulberry leaves (*Morus alba* L.) powder stated protective effects. They attributed such protective effect to its different bioactive compounds content, which were measured almost in OSE.

3.8. Effect of Four Weeks Treatment with Onion Skin Ethanolic Extract (OSE) on Liver Apoptosis Biomarkers of Normal and Benzo[A]Pyrene-Hepatotoxic Rats

Effect of four weeks treatment with onion skin ethanolic extract (OSE) on liver apoptosis biomarkers of normal and benzo[a]pyrene-hepatotoxic rats were shown in Table 13 and Table 14. Such data indicated that injection of animals with B[a]P induced a significant increased ($p \leq 0.05$) in Caspase-3 (a cell apoptosis executor) and Bax (a pro-apoptosis marker) by 67.57 and 104.20% compared to normal control rats, respectively. In contrast, Bcl-2 (an anti-apoptosis marker) protein expression was significantly diminished by a rate of change (-69.43%). On the other side, OSE treatment attenuated these changes significantly ($p \leq 0.05$) in a dose-dependent manner by either upregulating the protein expression of Bcl-2 (14.60, 43.58, 91.33 and 151.34 %) or downregulating the expressions of caspase-3 (-6.10, -13.14, -24.66 and -31.28%) and and Bax (-4.79, -16.61, -25.71 and -29.07%) effectively in OSE treated groups (50, 100, 150 and 200 mg/kg bw.day), respectively when compared to the B[a]P-injected control group. Data of the present study confirmed that B[a]P activates apoptosis by altering the expression of proteins related to apoptosis. These findings are in according with those observed by Khattab *et al.*, [116], who noticed a highly marked elevation in Bax and caspase-3 and a highly significant decrease in Bcl-2 in kidney tissues of rats injected with B[a]P. Also, B[a]P in the cellular environment causes a variety of molecular changes, including lipid peroxidation and protein oxidation, leading to oxidative stress [27,117]. Oxidative stress triggers multiple apoptotic signaling pathways due to increased ROS production or reduced antioxidant enzymes activities. ROS is closely linked to the activation of cell apoptosis through changing the redox status of the cell and creating an imbalance in the protective mechanism that results in damage to cellular molecules like lipids, proteins, and DNA, ultimately leading to cell death via necrotic and apoptotic processes [20,86,118,119]. Also, ROS plays an important role in triggering apoptosis by promoting caspase activation. The study of Bratton and Cohen [120] reported that caspases, specifically caspase-3, are the key apoptotic effectors that cause cytoskeletal degradation, nuclear demise, and other apoptotic cellular alterations. Furthermore, Kim *et al.* [121] reported that B[a]P may induce apoptosis through increasing the ROS levels and activating the endoplasmic reticulum stress response. Data of the present study demonstrated that OSE attenuates B[a]P-induced hepatic apoptosis which could be attributed to its content of bioactive compounds including phenolics, flavonoids and carotenoids. These compounds exhibit the potential to inhibit ROS formation, which may explain the extract's anti-hepatic apoptotic

action [3,86,88]. The same study mentioned that phenolics and flavonoids attenuates B[a]P-induced hepatic apoptosis through inhibiting the apoptotic proteins caspase-3 and Bax and activating the anti-apoptotic mediator Bcl-2. Several studies reported to be effective in treating a variety of liver diseases, including hepatocellular carcinoma, chronic liver diseases, and cirrhosis, due to its remarkable antioxidant, anti-inflammatory, and antifibrotic, and anti-apoptotic properties [86,122,123,124,125].

Table 13. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on liver apoptosis biomarkers of normal and benzo[a]pyrene-hepatotoxic rats

Group	Caspase-3 (%) (a cell apoptosis executor)		Bax (%) (a pro-apoptosis marker)		Bcl-2 (%) (an anti-apoptotic marker)	
	Range	Means $\pm$ SD	Range	Means $\pm$ SD	Range	Means $\pm$ SD
Normal control	28.11-39.67	33.98 $\pm$ 5.36 ^c	25.34-35.76	30.69 $\pm$ 4.31 ^c	50.12-61.99	57.34 $\pm$ 5.08 ^a
Model control (Hepatotoxic)	48.10-64.28	56.95 $\pm$ 7.11 ^a	55.76-71.04	62.67 $\pm$ 8.24 ^a	14.21-20.32	17.53 $\pm$ 2.34 ^d
OSET1	48.90-61.21	53.47 $\pm$ 6.84 ^a	51.34-64.79	59.67 $\pm$ 6.31 ^a	17.67-21.50	20.09 $\pm$ 1.76 ^d
OSET2	45.78-56.34	49.46 $\pm$ 5.31 ^{ab}	46.02-58.90	52.26 $\pm$ 5.98 ^{ab}	21.34-29.38	25.17 $\pm$ 3.97 ^{cd}
OSET3	33.45-53.67	42.90 $\pm$ 9.25 ^b	39.56-55.23	46.56 $\pm$ 7.19 ^b	29.38-39.90	33.54 $\pm$ 5.34 ^c
OSET4	31.23-44.98	39.13 $\pm$ 6.54 ^{bc}	38.23-49.28	44.45 $\pm$ 4.24 ^b	42.34-49.84	44.06 $\pm$ 4.32 ^b

Results are expressed as means $\pm$ SD (n = 6). Different superscripts including in the same column indicate a significant difference ($P \leq 0.05$). OSE, Onion skin ethanol extract. Details of groups are as shown under Table 3.

Table 14. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on liver apoptosis biomarkers of normal and benzo[a]pyrene-hepatotoxic rats

Group	Caspase-3 (a cell apoptosis executor) (as a % of change)*	Bax (a pro-apoptosis marker) (as a % of change)*	Bcl-2 (an anti-apoptotic marker) (as a % of change)*
Normal control	-----	-----	-----
Model control (Hepatotoxic)	67.57 $\pm$ 3.34	104.20 $\pm$ 5.21	-69.43 $\pm$ 4.21
OSET1	-6.10 $\pm$ 1.02	-4.79 $\pm$ 0.56	14.60 $\pm$ 1.99
OSET2	-13.14 $\pm$ 0.89	-16.61 $\pm$ 2.34	43.58 $\pm$ 4.02
OSET3	-24.66 $\pm$ 2.45	-25.71 $\pm$ 3.20	91.33 $\pm$ 5.34
OSET4	-31.28 $\pm$ 3.23	-29.07 $\pm$ 3.06	151.34 $\pm$ 6.87

Results are expressed as means $\pm$ SD (n = 6). *Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with OSE compared to the hepatotoxic group. The guides of the experimental groups and abbreviations are as shown in Table 1.

3.9. Histology

3.9.1. Effect of Four Weeks Treatment with Onion Skin Ethanolic Extract (OSE) on Liver Histology of Normal and Benzo[A]Pyrene-Hepatotoxic Rats

The effect of four weeks treatment with onion skin ethanolic extract (OSE) on liver histology of normal and benzo[a]pyrene-hepatotoxic rats was illustrated in Figure (1). Microscopically, liver sections of rats from normal control group revealed the normal histoarchitecture of hepatic parenchyma (Photo 1). In adverse, hepatic tissue of rats from model control (hepatotoxic) group showed histopathological changes characterized by dilatation and congestion of central veins (Photo 2), Kupffer cells proliferation and focal hepatocellular necrosis associated with inflammatory cells infiltration (Photo 3). Meanwhile, liver of rats from OSET1 group described proliferation of Kupffer cells and dilatation of hepatic sinusoids (Photo 4). On the other hand, some liver sections of rats from OSET2 group exhibited slight proliferation of Kupffer cells and inflammatory cells infiltration in the portal triad (Photo 5). Otherwise, liver of rats from OSET3 group revealed slight congestion of central vein and slight cytoplasmic vacuolization of some hepatocytes (Photo 6). Furthermore, liver of rats from OSET4 group demonstrated small focal hepatocellular necrosis (Photo 7). In similar studies, Kiruthiga *et al.* [126] found that the liver sections of rats that injected by B[a]P showed hepatic necrosis, hepatocyte degeneration, loss of hepatic plate architecture, and infiltration of mononuclear cells.

Also, Rangi *et al.* [127] noticed that the hepatocytes experience apoptosis, characterized by pyknotic nuclei, along with the manifestation of ballooning degeneration, which was evident through the swollen wispy cytosol and the presence of eosinophilic councilman bodies in B[a]P-injected rats. Furthermore, Elhassaneen and Mahran [21] reported that ROS instigates damage to the membranes of hepatocytes, resulting in destroying, which causes collagen accumulation in the hepatocytes and leads to the development of liver. In additional, hepatic cells subjected to lipoapoptosis due to incomplete biomolecule oxidation and trigger immune reactions in the liver. In contrast, in B[a]P-hepatotoxic rats injected with OSE, liver tissues showed varying degrees of improvement in histopathological changes depending on OSE dose, indicating that the degenerative alterations were mitigated and the liver's histological architecture was improved significantly. These improvements in liver tissue histoarchitecture may be attributed to the antioxidant properties of OSE. Phenolic compounds, an extract from the onion skin, can diminish oxidative stress and subsequent cytotoxicity, thus protecting intact hepatocytes or cells that have not yet sustained irreversible damage [86,22].

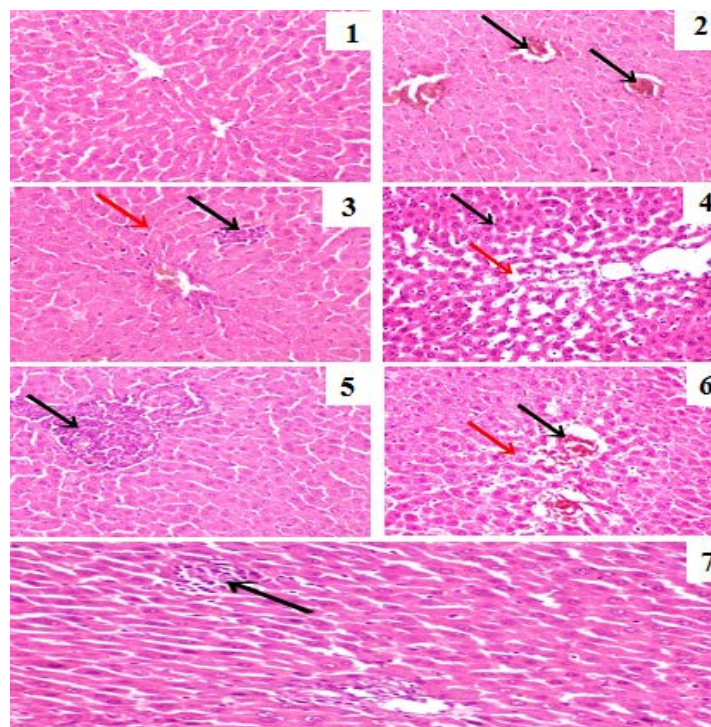


Figure 1. Effect of four weeks treatment with onion skin ethanolic extract (OSE) on liver histology of normal and benzo[a]pyrene-hepatotoxic rats (H & E X 400)

Photo 1, Photomicrograph of liver of rat from control normal group showing the normal histoarchitecture of hepatic parenchyma, **Photo 2,** Photomicrograph of liver of rat from model (hepatotoxic) group showing dilatation and congestion of central veins (black arrow), **Photo 3,** Photomicrograph of liver of rat from model (hepatotoxic) group showing Kupffer cells proliferation (red arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (black arrow), **Photo 4,** Photomicrograph of liver of rat from OSET1 group showing Kupffer cells

proliferation (black arrow) and dilatation of hepatic sinusoids (red arrow), **Photo 5,** Photomicrograph of liver of rat from OSET2 group showing inflammatory cells infiltration in the portal triad (black arrow), **Photo 6,** Photomicrograph of liver of rat from OSET3 group showing slight congestion of central vein (black arrow) and slight cytoplasmic vacuolization of some hepatocytes (red arrow), **Photo 7,** Photomicrograph of liver of rat from OSET4 group showing small focal hepatocellular necrosis (black arrow).

4. Conclusion

B[a]P is the main representative of polycyclic aromatic hydrocarbons (PAHs), and has been repeatedly found in the air, surface water, soil, sediments and foods. Exposure to this ubiquitous environmental and food contaminant is associated with the development of several diseases including liver cancer. Findings of the present study has demonstrated the potency of the OSE to partially ameliorate hepatotoxicity and related complications in rats exposed to B[a]P. Also, these findings supported our hypothesis that such plant part extract (OSE) contains several classes of bioactive compounds with different biological activities that are able to prevent or inhibit hepatotoxicity induced by B[a]P through one or more of the following pathways (Figure 2): 1) liver serum enzymes-lowering activity, 2) improving the serum lipid profile, 3) decreasing rate on the formation of serum MDA and nitric oxides, 4)improving the antioxidant and inflammatory defense systems, and 5) exhibiting the anti-apoptotic effect. These findings provide a basis for the use of different plant parts extract such OSE for the prevention and/or treatment of hepatotoxicity and related complications induced by B[a]P.

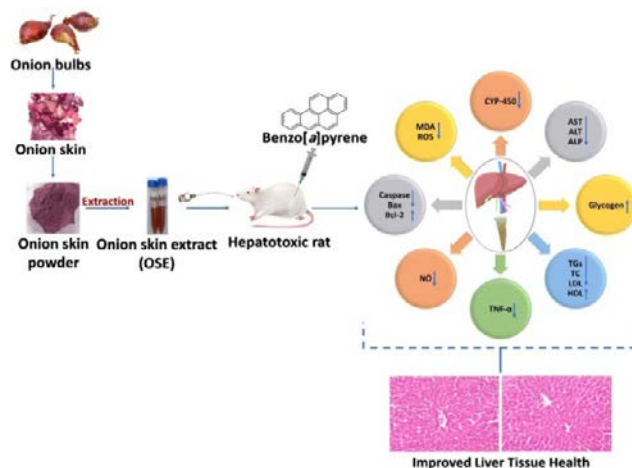


Figure 2. Graphical summary showing the effect of onion skin extract (OSE) on B[a]P-induced hepatic injuries

Author Contributions

Yousif Elhassaneen cooperated in proposing and improving the study protocol, following up the laboratory experimental part, retrieving conceptual information, reviewing and validating the results and statistical analyses, preparing a draft of the manuscript, conducting a critical review to intellectually organize the content and granting approval to publish the final version of the manuscript. Esraa Salama conducted the laboratory experiments, collected, tabulated, analyzed and interpreted the results as well as involved in retrieving conceptual information and preparing the draft of the manuscript. Mai Garib cooperated in proposing the study protocol, retrieving conceptual information, validating the study results, and preparing the draft manuscript. Asmaa Bayomi cooperated in improving the study protocol,

validating the molecular biology results, and submitting contributions to the concept and design of the work.

Ethical Considerations

The ethical issues of this study was reviewed and approved by Scientific Research Ethics Committee (SREC, Approval #19-SREC-03-2023), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt.

Conflicts of Interest

No conflicts of interest are declared by the authors.

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Abbreviations

ALT, Alanine aminotransferase; ALP, alkaline phosphatase, AST; aspartate aminotransferase; B[a]P, benzo[a]pyrene; Bax, a pro-apoptosis marker; Bcl-2, an anti-apoptotic marker; BD, basal diet; Caspase-3, a cell apoptosis executor; GSH, reduced glutathione; HDL-c, high density lipoprotein; MDA: malondialdehyde; NO, nitric oxide, ROS: reactive oxygen species; OSE, onion skin ethanol extract; TC, total cholesterol; TG's, triglycerides, TNF- α , tumor necrosis factor-alpha.

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