Effect of poly-plant parts preparations on the liver functions, oxidant/antioxidant status and lipid metabolism parameters during benzo[a]pyrene-induced experimental hepatotoxicity

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Abstract:

Liver toxicity, or hepatotoxicity, occurs when the liver is damaged by harmful substances, like benzo[*a*]pyrene (B[*a*]P), an environmental and food contaminant. While many studies have explored the protective effects of plant compounds against B[*a*]P toxicity, most focused on single plant sources. The present study aims to evaluate the potential hepatoprotective effects of a combination of plant extracts against B[a]P-induced liver damage. Forty-eight rats were divided into two primary groups: group 1 (6 rats) maintained on a basal diet (BD) as a normal control, and group 2 (42 rats) injected with B[*a*]P to induce liver toxicity. The B[*a*]P-treated rats were further divided: Group 2 received no treatment, groups 3–5 received ethanol extracts of formula 1 at doses of 50, 100, and 200 mg/kg body weight (bw)/day, and groups 6–8 received formula 2 at the same doses. B[*a*]P administration significantly ($p \leq 0.05$) increased hepatic glucose-6-phosphatase (G6Pase) activity by 297.33%, total cholesterol by 19.62%, LDL-c by 59.01%, hepatic reactive oxygen species by 109.21%, and cytochrome P450 expression by 74.51%. Conversely, it significantly reduced serum triglycerides by 48.65%, serum HDL-c by 38.62%, body weight gain by 10.37%, hepatic glycogen by 63.34%, G6PD activity by 61.26%, and antioxidant enzyme activities like glutathione peroxidase (-48.31%), catalase (-29.84%), and superoxide dismutase (-42.94%). Supplementation with the extracts (50, 100, and 200 mg/kg bw/day) over 28 days significantly improved liver function, antioxidant status, lipid metabolism, and liver histopathology. These results suggest that the tested plant-based formulae may serve as potential hepatoprotective agents for liver disorders.

Keywords: Liver functions, serum lipid profile, antioxidant enzymes, CYP450, reactive oxygen species, histopathology.

1. Introduction

The liver is a vital organ in all vertebrates, playing a crucial role in numerous essential bodily functions. Some of its key responsibilities include metabolizing carbohydrates, proteins, and fats, as well as processing and regulating substances such as glucose, plasma proteins, cholesterol, specialized proteins that assist in fat transport, urea, and hemoglobin. Additionally, the liver acts as a storage site for iron and converts surplus glucose into glycogen for future energy use, ensuring glucose levels remain balanced. It also produces clotting factors necessary for preventing excessive bleeding, secretes bile into the intestine to aid nutrient absorption, and stores essential compounds like glycogen, fats, and vitamins within its parenchymal cells (Lee *et al.*, 2007). Moreover, the liver plays a fundamental role in detoxification and the biotransformation of drugs and foreign substances, helping to neutralize potentially harmful compounds (Elhassaneen, 1996; Kebamo *et al.*, 2015; Sayed-Ahmed and Elhassaneen, 2020; Yu *et al.*, 2020; Elhassaneen *et al.*, 2021a; Mahran and Elhassaneen, 2023). Due to its high functional capacity, early damage to the liver often goes unnoticed as it continues to perform efficiently. However, as liver disease advances—especially when bile flow is disrupted—serious health complications can arise, potentially becoming life-threatening (Lawrence and Emmett, 2012).

Liver diseases rank among the most severe health conditions and are primarily triggered by exposure to various toxic substances, including certain antibiotics, chemotherapy drugs, oxidized oils, aflatoxins, carbon tetrachloride, polycyclic aromatic hydrocarbons (PAHs), pesticides, and other harmful chemicals (Elhassaneen, 1996; Kumar *et al.*, 2011; Mahran and Elhassaneen, 2023). Among these toxins, PAHs are particularly significant due to their widespread presence in the environment. These

organic compounds consist of two or more fused aromatic rings and are of great concern to researchers and professionals in the field, as they represent some of the most prevalent environmental pollutants (Emerole *et al.*, 1982; Elhassaneen, 1996; Bassiouny, 1999; Elhassaneen *et al.*, 2016-a).

One notable PAH is benzo[a]pyrene (B[*a*]P), which serves as a key representative of this compound group. It is produced as a by-product of incomplete combustion of carbon-based materials such as tobacco, gasoline, and wood. Additionally, B[a]P is commonly found in grilled, broiled, smoked, and fried foods, as well as in emissions from various industrial activities (Elhassaneen and Tawfik, 1998; Elhassaneen, 2004; Elhassaneen and El-Badawy, 2013). It is also present in both outdoor and indoor air, along with certain water sources (U.S. Environmental Protection Agency, 2005). Furthermore, research by Elhassaneen and Tawfik, (2001) highlighted that fumes generated during frying contain multiple PAH compounds, including B[*a*]P, which may pose significant risks to public health. According to the World Health Organization, approximately 99% of PAH ingestion, including B[a]P, occurs through food, with inhalation accounting for 0.9% and drinking water contributing between 0.1% and 0.3% (WHO, 1984).

Several decades ago, research established that benzo[a]pyrene (B[a]P) exhibits toxic, mutagenic, and carcinogenic properties, as demonstrated through extensive in vivo and in vitro studies (Harvey 1985; Hawkins *et al.*, 1990; Elhassaneen 1996; Elhassaneen *et al.*, 1997; Elhassaneen *et al.*, 2002; Elhassaneen *et al.*, 2016 b; Mahran *et al.*, 2018; Mahran and Elhassaneen 2023). Exposure to B[a]P has also been linked to liver cancer in mammals, rodents, and fish (Harvey, 1985; Hawkins *et al.*, 1988 and 1990; Elhassaneen 1996; 2002; Elhassaneen *et al.*, 2023). The harmful impacts of B[a]P, including toxicity, tumor formation, and cancer development, are closely related to its metabolic transformation within cells. This process results in the production of reactive intermediates, such as arene oxides, phenols, quinones and dihydrodiols which can form covalent bonds with DNA, leading to the creation of DNA adducts (Weinstein, 1978; Harvey, 1985; Elhassaneen, 1996; Bukowska *et al.*, 2022). Additionally, cell proliferation plays a role in fixing these biochemical alterations. B[a]P also induces cytotoxic effects in liver cells by triggering oxidative stress pathways, contributing to mitochondrial and lysosomal dysfunction, as well as compromising cell membrane integrity (Elhassaneen *et al.*, 2002; 2024).

For decades, researchers have been investigating ways to prevent and treat liver toxicity and cancer caused by exposure to various harmful substances, primarily through the use of synthetic chemical compounds. However, this approach has faced significant challenges, including serious side effects and high costs, which often result in poor patient compliance (Martin *et al.*, 2002). Consequently, there has been a growing interest in exploring alternative treatments, particularly those derived from natural sources, which are more affordable and have fewer adverse effects. In response to this, many academic institutions and regulatory bodies have shifted their focus toward studying different plant-based materials rich in bioactive compounds. These natural substances have demonstrated promising biological activities in both the prevention and treatment of liver diseases (El-Sayed et al., 2012; El-Sayed et al., 2013; Elhassaneen *et al.*, 2016a and b; Sayed-Ahmed *et al.*, 2020; Elhassaneen *et al.*, 2021a and b,; Abd Elalal *et al.*, 2022; Mahran and Elhassaneen, 2023; Elhassaneen *et al.*, 2023, 2024). Encouraging findings from these studies have sustained interest in this research direction. In this study, we have chosen to focus on plant materials obtained as by-products or waste from food processing, as well as certain medicinal and

aromatic plant components, with the goal of developing formulations that offer both nutritional and therapeutic benefits. Furthermore, most previous studies have primarily examined the effects of individual plant parts, making it difficult to assess the potential benefits of combining multiple plant components for toxicity treatment. Therefore, the present study aims to investigate the potential protective effects of specific plant-based preparations-including roots, leaves, flowers, seeds, kernels, and food industry by-products-against liver toxicity induced by benzo[*a*]pyrene.

2. Materials and Methods

2.1. Materials

2.1.1. Plant parts

Plant parts used in formulae/preparations were obtained from the Egyptian companies as follow: from the herbs merchandize, local markets, Egy. Plant parts include: Onion skin "*Allium Cepa* L." and tomato (*Lycopersicon esculentum* L.) pomace, which was obtained as a donation from New Bani Suef Company, a company located in Bani Suef El-Goudida City, Bani Suef Governorate, for specializes in the preservation, dehydration, and industrial processing of vegetables. Potato peels "*Solanum tuberosum*" was obtained as a donation from Safco Company, El-Nigella Village, Beheira Governorate; Apricot "*Prunus armeniaca*" kernels from Amar Village , Al-Qalyubia Governorate; and Marjoram "*Origanum majorana* " leaves, Molokheiya "*Corchorus capsularis* Linn" leaves, Sweet fennel "*Foeniculum vulgare* ", Ginseng "*Panax ginseng* ", Black seeds "*Nigella sativa* L. ", Eucalyptus "*Eucalyptus globules* " leaves, Cinnamon "*Cinnamomum verum* ", Spearmint "*Mentha spicata* " leaves Lemon peels "*Citrus limon*", Orange "*Citrus sinensis*" peels, turmeric *(Curcuma longa* L.*)* rhizomes from Agricultural Seeds, Spices and Medicinal Plants Company (Harraz), El-Darb El-Ahmar, Cairo; Governorate. Dried fruits of milk thistle (*Silybum marianum L.*) were collected from wild plant populations growing in public irrigation canals, specifically from Mit Ghoarb Village in Sinbillawin, Dakahlia Governorate; Egy. Gum Arabic (GA) "*Acacia senegal* L." (Specifications: powder, colouroff white and purity, 98.34±0.67%) was obtained as a donation from the SAVANNA Companies Group (Processing Gums, Juices and Confectionery), Khartoum, Sudan. Taxonomic confirmation for plant parts were completed by the assistance of Agricultural Plant Department staff, Faculty of Agriculture, Menoufia Uni., Shebin El-Kom, Egy.

2.1.2. Chemicals and kits

Benzo[a]pyrene (B[a]P) and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Company in Cairo, Egy. Casein was sourced from Morgan Chemical Co., also in Cairo, Egy. Assay kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and malondialdehyde (MDA) were provided by BIODIAGNOSTIC, Dokki, Giza, Egy. Kits for glutathione (GSH) and glutathione disulfide (GSSG) were supplied by MyBioSource, Inc., San Diego, CA, USA. Other chemicals, including food-grade vitamins, salt mixtures, and analytical-grade reagents and solvents, were purchased from El-Ghomhorya Company for Trading Drug, Chemicals, and Medical Instruments in Tanta and Mansoura, Egy. Assay kits for glucose-6-phosphate dehydrogenase (G6Pase), glucose-6-phosphatase (G6PD) activity, albumin, total protein, and total bilirubin were obtained from BIODIAGNOSTIC, Dokki, Giza, Egy. Superoxide dismutase (SOD) activity assay kits were from Creative BioLab, NY, USA. Kits for triglycerides (TGs), total

cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were supplied by El-Nasr Pharmaceutical Chemicals, Cairo, Egypt, and glycogen assay kits were from Abcam, USA.

2.2. Methods

2.2.1. Preparation of plant parts powder

The plant materials selected for formula preparation were carefully inspected by hand to eliminate any foreign substances, as well as broken or damaged pieces. These plant parts were then dried using a hot air oven (Metkorp Equipments PVT. LTD., Egypt) at temperatures ranging from 45°C to 65°C, depending on the specific plant type, until the final product reached a moisture content of approximately 7%. Once dried, the materials were finely ground using a high-speed mixer (Toshiba, ElAraby Co., Benha; Egy.). The resulting powder was then sieved through an 80-mesh screen, with only the fine particles retained for further formulation and analysis.

2.2.2. Preparation of the suggested formulae

The suggested formulae were prepared by weighing the components of each blend separately as follow: Formula I, milk thistle , black seeds, tomato pomace, ginseng, cinnamon, eucalyptus, marjoram, molokhia, sweet fennel, black pepper, turmeric and gum arabic by 20, 4, 8.5, 8.5, 8.5, 7, 9.5, 11, 7.5, 1.5, 4, and 10% respectively; Formula II, milk thistle , spearmint leaves, marjoram leaves, sweet fennel, lemon peels, potato peels, orange peels, onion skin, apricot kernels, turmeric and gum Arabic by 20, 9, 7.5, 9.5, 8, 8, 8.5, 8.5, 7, 4 and 10%, respectively. Components of each formula were mixing and homogenizing by a high-speed blender (Toshiba, ElAraby Co., Benha, Egypt).The resulting formulae were placed in glass jars and kept in refrigerator at 4°C until used for further studies.

2.2.3. Preparation of the suggested formulae extracts

The extraction of the suggested formulae was carried out by the method of Gharib *et al.*, (2022). Briefly, 100 grams of each formula powder were mixed with 900 ml of an aqueous ethanol solution (20% water and 80% ethanol) and subjected to extraction using an orbital shaker at 60°C for 4 hours. The resulting mixture was then filtered through Whatman No. 5 filter paper using a Buchner funnel. To remove any remaining solvents, the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator (Heidolph Instruments GmbH & Co., Germany). The extraction yields for formulae I and II were found to be 7.25% and 7.02%, respectively. The final extracts were stored at 4°C until further use.

2.2.4. Biological experiments

2.2.4.1. Animals

The study utilized adult male albino rats, weighing approximately 177.45±7.21g, obtained from the Laboratory Animal Unit at the College of Veterinary Medicine, Cairo Uni., Egypt.

2.2.4.2. Basal diet (BD)

The standard diet was formulated based on the composition outlined by Reeves *et al.*, (1993) and included the following ingredients: 10% protein, 10% corn oil, 1% vitamin mix, 4% mineral mix, 0.2% choline chloride, 0.3% methionine, and 5% cellulose, with the remaining 69.5% consisting of corn starch. The vitamin and mineral

mixtures used in this formulation were prepared following the guidelines established by Reeves *et al.*, (1993).

2.2.4.3. Induction of hepatotoxicant in rats

Following the protocol of Shahid *et al.*, (2016), forty-two male albino rats were administered benzo[a]pyrene (B[a]P) at a dose of 125 mg/kg body weight via intraperitoneal IP injection in corn oil. On day seven, liver intoxication was confirmed through biochemical assessment of liver function in a randomly selected group of three rats.

2.2.4.4. Experimental design

All biological experiments adhered to the guidelines of the Institute of Laboratory Animal Resources, Commission on Life Sci., National Research Council (NRC, 1996). A total of 48 rats were individually housed in wire cages under controlled conditions, maintaining a temperature of 24 ± 3°C and ensuring a healthy environment. Prior to the experiment, all rats were fed a basal diet (BD) for one week to allow for acclimatization. Following this period, the rats were divided into two main groups: the first group (Group 1, consisting of 6 rats) continued on the basal diet, while the second group (42 rats) was injected with B[a]P to induce hepatotoxicity. This group was then subjected to dietary treatments using the formulated preparations for further study.

- Group (2): fed on BD only as a positive control, model control.
- Group (3): fed on BD and treated with 50 mg/kg bw/day of formulae 1 ethanol extract (F1EE).
- Group (4): fed on BD and treated with 100 mg/kg bw/day of formulae 1 ethanol extract (F1EE).
- Group (5): fed on BD and treated with 200 mg/kg bw/day of formulae 1 ethanol extract (F1EE)
- Group (6): fed on BD and treated with 50 mg/kg bw/day of formulae 2 ethanol extract (F2EE).
- Group (7): fed on BD and treated with 100 mg/kg bw/day of formulae 2 ethanol extract (F2EE).
- Group (8): fed on BD and treated with 200 mg/kg bw/day of formulae 2 ethanol extract (F2EE).

The tested concentrations of suggested formulae for the current study were proposed based on our primary experiments. Each group was individually housed in a cage for 28 days.

2.2.4.5. Biological evaluation

To assess the biological evaluation parameters, including body weight (BW, g), food intake (FI), and food efficiency ratio (FER), daily food consumption was recorded, while body weight measurements were taken weekly throughout the 28-day experimental period. The percentage of body weight gain (BWG), food intake (FI), and food efficiency ratio (FER) were calculated using the following formulas:

BWG (%) = [(Final weight – Initial weight) / Initial weight] × 100

FER = (Total body weight gain in grams over 28 days) / (Total food intake in grams over 28 days)

2.2.4.6. Blood and liver sampling

At the end of the 28-day experimental period, blood samples were collected following a 12-hour fasting period. The rats were anesthetized with ether, and blood

was drawn from the abdominal aorta. The samples were placed in clean centrifuge tubes to clot at room temperature (25°C) and then centrifuged at 3,500 rpm for 10 minutes to separate the serum following the method described from Stroev and Makarova, (1989). The resulting serum was carefully transferred into sterile tubes and stored at -18°C until further analysis. Additionally, liver tissue samples were collected immediately after euthanasia and preserved in 10% neutral buffered formalin for histological examination.

2.2.4.7. Hematological analysis

Liver functions

Glycogen levels in tissue homogenates were measured following the method described by Damsbo *et al.*, (1991). Hepatic glucose-6-phosphate dehydrogenase (G6PD) and glucose-6-phosphatase (G6Pase) activities were determined using the methods of Chan *et al.*, (1965) and Rossetti *et al.*, (1993), respectively.

Serum lipids profile

Serum levels of triglycerides (TGs), total cholesterol (TC), HDL-cholesterol, and LDL-cholesterol were determined using assays based on the methods of Ahmadi *et al.*, (2008), Fossati and Prenape, (1982), Lopes-Virella *et al.*, (1977), and Richmod, (1973).

Redox Status parameters

The activity of red blood cell (RBC) glutathione peroxidase (GSH-Px) and catalase (CAT) was assessed following the protocols outlined by Splittgerber and Tappel, (1979) and Aebi, (1974), respectively. The activity of superoxide dismutase (SOD) was assayed using a colorimetric kit (Creative BioLab, NY) based on the method of Mett and Müller, (2021). Glutathione reductase (GSH-Rd) activity was determined using the ICSH (1979) recommended assay. Reactive oxygen species (ROS) levels were quantified using the colorimetric assay of Erel, (2005).

Drug Metabolizing Enzyme (Cytochrome CYP450)

The method of Omura and Sato, (1964) was used to measure CYP450 via carbon monoxide difference spectrophotometry of dithionite-reduced samples.

2.2.4.8. Histopathological Examination

Liver specimens were processed through a series of steps: trimming, dehydration with increasing alcohol concentrations, clearing in xylene, embedding in paraffin, sectioning to a thickness of 4-6 µm, staining with hematoxylin and eosin, and finally, microscopic examination (Carleton, 1978).

2.2.5. Statistical analysis

All measurements were performed in triplicate and are expressed as mean ± standard deviation SD. Statistical significance was evaluated using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons, using MINITAB 12 software (Minitab Inc. State College; PA). A p-value of ≤ 0.05 was deemed statistically significant.

3. Results and Discussion

3.1. Body weight

The impact of the proposed formula extracts on body weight in hepatotoxic rats is illustrated in Figure (1) and Table (1). After 28 days of the experiment, the B[a]P-exposed group exhibited a notable ($p \leq 0.05$) body weight increase of 22.88%. However, this rise was relatively lower than that of the normal control group, which reached 35.95%. Administration of the suggested formula extracts at 50, 100, and 200 mg/kg bw/day resulted in a significant ($p \leq 0.05$) enhancement in body weight. The increments recorded were 26.38%, 30.73%, and 35.81% for formula I and 23.55%, 27.99%, and 32.75% for formula II, respectively. These findings indicate that formula I demonstrated a more pronounced effect on body weight gain compared to formula II. Furthermore, both formulations exhibited a dose-dependent response, with weight gain progressively increasing alongside higher extract concentrations. The consistent weight gain in treated rats suggests good palatability of these formulae. These results align with prior research showing that B[*a*]P exposure significantly reduces body weight gain, feed intake, and feed efficiency in rats (Owumi *et al.*, 2021; Badawi, 2023; Mahran and Elhassaneen, 2023; Elhassaneen and Mahran, 2024; El-Nagar, 2024). Other studies have also reported that liver-compromised rats experience significant declines in body weight and feed intake (Hamzawy *et al.*, 2013; Abd El-Rahman, 2021). This phenomenon has been explained by Dickerson and Lee, (1988) and Morresion and Hark, (1999) linked liver dysfunction, including hepatotoxicity, to malnutrition resulting from poor food intake, digestive inefficiencies, impaired absorption, and metabolic imbalances. Moreover, Mahran and Elhassaneen, (2023) suggested that body weight reduction in affected rats could be attributed to their deteriorating health, including changes in the absolute and relative weights of vital organs such as the liver and kidneys. Conversely, the present study demonstrates that treatment with the proposed formulae significantly improved body weight in hepatotoxic rats. This improvement is likely due to the presence of bioactive secondary metabolites in the extracts, including phenolics, polysaccharides, terpenoids, flavonoids, saponins, triterpenoids, oxalate, kaempferol, and lycopene. These compounds possess antioxidant properties, lipid oxidation inhibition, and free radical scavenging activities, all of which play a crucial role in body weight regulation (Aly *et al.*, 2017; Abd Elalal *et al.*, 2021). Several previous studies have confirmed that B[a]P exposure leads to reduced body weight gain, which can be mitigated through the consumption of plant-based bioactive compounds, as demonstrated by the formulae used in this study (Bedawy, 2008; Abd El-Fatah, 2012; El-Sayed *et al.*, 2013; Elbanna, 2014; Mansour, 2017; Tahoon, 2019; Elhassaneen *et al.*, 2021a and b; Mahran and Elhassaneen, 2023).

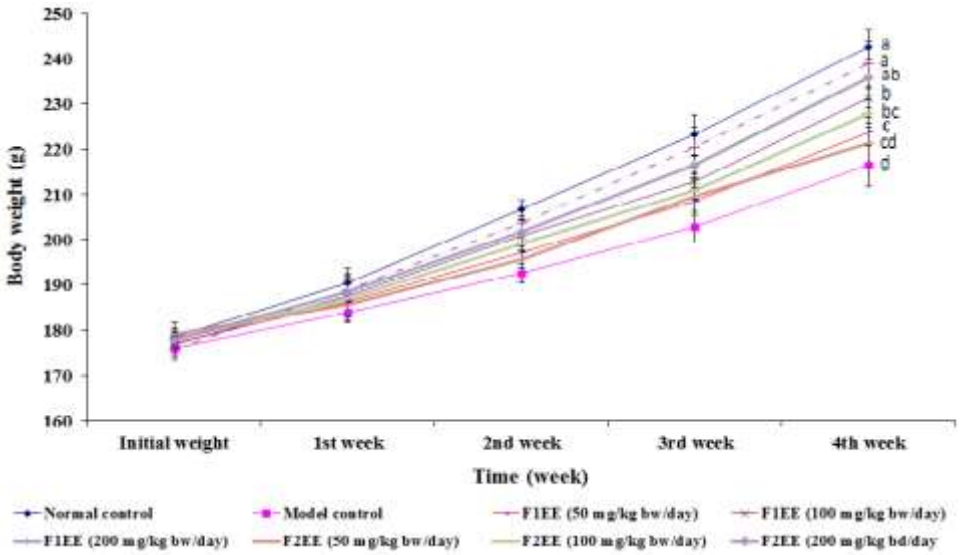

Figure 1. Body weight in control and differently treated rats.
Data presented as mean ± SD of six rats in each group. Means with different letters indicates significant at $p \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3 to G8, model group treated with Formulae extract; F1EE, formula 1 ethanol extract, F2EE, formula 2 ethanol extract.

Table 1. Effect of treatment with suggested formulae extracts on body weight (BW, as a percent of change from the base line)*

Groups	Time (week)				
	0	1	2	3	4
G1: Normal	0.00	6.71± o.54 ^{a}	15.80± 0.89 ^{a}	25.06± 2.07 ^{a}	35.95± 1.94^{a}
G2: Model	0.00	4.39± 0.47 ^{b}	9.32± 1.02 ^{c}	15.12± 1.11 ^{d}	22.88± 2.10 ^{d}
G3: F1EE (50 mg/kg bw/day)	0.00	5.17± 0.72 ^{ab}	11.39± 0.39 ^{bc}	17.79± 1.67 ^{cd}	26.38± 2.09 ^{c}
G4: F1EE (100 mg/kg bw/day)	0.00	6.08± 0.31 ^{a}	13.60± 1.35 ^{a}	20.27± 0.74 ^{bc}	30.73± 3.17 ^{b}
G5: F1EE (200 mg/kg bw/day)	0.00	7.38± 0.86 ^{a}	15.75± 2.17 ^{a}	25.24± 2.11 ^{a}	35.81± 4.19 ^{a}
G6: F2EE (50 mg/kg bw/day)	0.00	3.68± 0.09 ^{b}	9.28± 1.08 ^{c}	17.03± 1.59 ^{cd}	23.55± 1.52 ^{cd}
G7: F2 EE (100 mg/kg bw/day)	0.00	4.96± 0.60 ^{b}	11.86± 1.08 ^{ab}	18.50± 0.78 ^{c}	27.99± 2.83 ^{bc}
G8: F2EE (200 mg/kg bw/day)	0.00	6.09± 0.91 ^{a}	13.56± 0.94 ^{a}	21.81± 2.00 ^{b}	32.75± 3.33 ^{ab}

* Data presented as mean ± SD of six rats in each group. Means under the same column with different superscript letters indicates significant at $p \leq 0.05$. The significant change at $P \leq 0.05$ (n=6). G1, normal group; G2, model group (hepatotoxic); G3 to G8, model group treated with Formulae extract; F1EE, formula 1 ethanol extract, F2EE, formula 2 ethanol extract.

3.2. Effect of treatment with the suggested formulae extracts on liver functions of hepatotoxic rats induced by B[*a*]P

The impact of the suggested formulae extracts on liver function in hepatotoxic rats induced by B[*a*]P is presented in Table (2). By the conclusion of the 28-day experiment, rats exposed to B[*a*]P exhibited a significant ($p \leq 0.05$) decline in glycogen content (-63.34%) and G6PD activity (-61.26%), while G6Pase activity markedly increased by 297.33% in comparison to the normal control group. However, administering the proposed formulae extracts at doses of 50, 100, and 200 mg/kg bw/day significantly ($p \leq 0.05$) restored glycogen content and G6PD activity while reducing G6Pase activity, with varying degrees of effectiveness. Notably, formula I demonstrated a superior impact on these biochemical parameters compared to formula II. Additionally, both formulae exhibited a dose-dependent response, with improvements in glycogen content and G6PD activity, alongside a decline in G6Pase activity, becoming more pronounced with higher doses. These findings align with previous research highlighting the detrimental effects of B[*a*]P on liver function, leading to its progressive impairment in experimental models (Manibusan *et al.*, 2007; Fayez, 2016; Mahran *et al.*, 2018; Elhassaneen *et al.*, 2018; Susilo *et al.*, 2019; Ajami, 2022; Elhassaneen *et al.*, 2022-a; Mahran and Elhassaneen, 2023; Elhassaneen and Mahran, 2024). The liver damage associated with B[a]P exposure arises due to its metabolic conversion into reactive intermediates, including arene oxides, phenols, quinones, and dihydrodiols, which interact with DNA and form genotoxic adducts (Weinstein, 1978; Harvey.1985; Elhassaneen.1996; Hassan *et al.*, 1996; Elhassaneen

et al., 1997; 2024; Elhassaneen. 2000-a; Bukowska *et al.*, 2022). Additionally, oxidative stress mechanisms contribute to cytotoxic effects on liver cells, leading to mitochondrial and lysosomal dysfunction, as well as compromised cell membrane integrity (Elhassaneen.1996; Elhassaneen *et al.*, 1996; Elhassaneen. 1999; Elhassaneen. 2000-b; Elhassaneen.2004; Elhassaneen and El-Badawy.2013; Badawy. 2017; Elhassaneen *et al.*, 2002; Elhassaneen *et al.*, 2022-b; Elhassaneen *et al.*, 2024). Furthermore, the present study suggests that B[*a*]P-induced hepatotoxicity contributes to disruptions in carbohydrate and lipid metabolism, which play a crucial role in liver function deterioration (Fayez, 2016; Mahran *et al.*, 2018; Elhassaneen *et al.*, 2018; Ajami, 2022; Elhassaneen *et al.*, 2022a and b). This was evident from the observed decrease in hepatic glycogen content, G6PD activity, and HDL-cholesterol levels, alongside an increase in G6Pase activity, total cholesterol, and LDL-cholesterol compared to normal-control rats. The substantial depletion of hepatic glycogen in the hepatotoxic group suggests impaired glycogenesis due to liver dysfunction (Fayez, 2016; Elhassaneen *et al.*, 2022a and b). G6Pase, an enzyme predominantly expressed in the liver, plays a critical role in gluconeogenesis and glycogenolysis by catalyzing the hydrolysis of glucose 6-phosphate into free glucose and a phosphate group (D-glucose 6-phosphate + H2O = D-glucose + phosphate). This study indicates in the findings that increased G6Pase activity in hepatotoxic rats may contribute to excessive glucose production while reducing its utilization (Aiston *et al.*, 1999). Conversely, G6PD, a cytoplasmic enzyme essential for the pentose phosphate pathway, regulates redox balance by generating NADPH (D-glucose 6-phosphate + $NADP^+$ + H2O ⇌ 6-phospho-D-glucono-1,5-lactone + NADPH + H^+). In the present study, B[*a*]P exposure led to a significant decline in G6PD activity in hepatotoxic rats compared to healthy controls. The protective effects observed with the proposed formulae may be attributed to their rich content of bioactive secondary metabolites, which are abundant in the constituent plant-based ingredients. For instance, onion skin and potato peel contain diverse polyphenolic compounds and flavonoids (Singh *et al.*, 2002; Badawy, 2008; El-Wazeer, 2011; El-Abasy, 2013; Elhassaneen and Sayed, 2015; Elhassaneen *et al.*, 2016a and b; Hallabo *et al.*, 2018). Marjoram is noted for its essential oils, phenolic components, and volatile compounds (Novak *et al.*, 2002; El-Safty, 2008). Fennel contains bioactive constituents such as trans-anethole, estragole, fenchone, and polyphenolics (Badgujar *et al.*, 2014), while black seed is rich in phenolic compounds and volatile oils (Aber and Afa, 2011). Additionally, cinnamon is a source of condensed tannins, essential oils, coumarins, cinnamaldehyde, and flavonoids (Lee *et al.*, 1999; El-Nashar, 2007). Milk thistle seeds are recognized for their flavonolignan content, collectively referred to as silymarin, which has hepatoprotective properties (Kvasnicka *et al.*, 2003; Kroll *et al.*, 2007; Elhassaneen *et al.*, 2023; Mahran and Elhassaneen. 2023). Turmeric contains curcumin, a well-documented bioactive compound with antioxidant and anti-inflammatory effects (Chattopadhyay *et al.*, 2004; Elhassaneen *et al.*, 2018). Furthermore, molokhia is abundant in phenolics, tannins, saponins, and carotenoids (El-Safty, 2012; Tekram, 2016; Nour ElDeen, 2023; Elhassaneen *et al.*, 2023), while apricot kernels provide alkaloids and phenolic compounds (Hassan, 2011). Gum arabic is notable for its polysaccharide and phenolic composition (Nasir *et al.*, 2010; Elhassaneen *et al.*, 2014; Domma *et al.*, 2016; ElSamoty.2021), and tomato pomace is particularly rich in lycopene (Baysal *et al.* 2000*;* Aly *et al.*, 2017). Numerous studies have demonstrated that these bioactive compounds exhibit antioxidant, free radical scavenging, lipid oxidation inhibition, anticancer, and antimutagenic properties. The hepatoprotective effects of the tested formulae could also be linked to their ability to regulate bilirubin metabolism by modulating enzyme activity, transporter synthesis, and bilirubin clearance pathways (Fati, 2017; Arthur *et al.*, 2012; Coria-Tellez *et al.*, 2018; Elhassaneen *et al.*, 2018; Hamza. 2020; ElSamouny, 2021; Elhassaneen *et al.*, 2021a and b). Furthermore, the outcomes of this study suggest that the proposed formulae may exert therapeutic effects on B[*a*]P-induced hepatotoxicity by modulating key metabolic enzymes involved in carbohydrate and lipid metabolism, particularly G6PD and G6Pase.

Table 2. Effect of treatment with suggested formulae extracts on liver functions of hepatotoxic rats induced by B[*a*]P

Groups	Glycogen content (mg/g wet tissue)		Glucose-6-phosphate dehydrogenase activity (G6PD, U/g wet tissue)		Glucose-6-phosphatase activity (G6Pase, µmole/min/g wet tissue)	
	Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change
G1: Normal	14.24 ± 0.64 [a]	0.00	18.51 ± 2.11 [a]	0.00	2.25 ± 0.83 [c]	0.00
G2: Model	5.22 ± 0.39 [d]	-63.34	7.17 ± 1.38 [d]	-61.26	8.94 ± 1.04 [a]	297.33
G3: F1EE (50 mg/kg bw/day)	9.01 ± 0.83 [bc]	72.61	10.19 ± 1.65 [c]	42.12	4.11 ± 0.67 [b]	-54.03
G4: F1EE (100 mg/kg bw/day)	11.64 ± 1.11 [b]	122.99	12.93 ± 2.17 [b]	80.34	3.53 ± 0.49 [b]	-60.53
G5: F1EE (200 mg/kg bw/day)	12.43 ± 1.05 [ab]	138.12	14.81 ± 0.98 [b]	106.56	2.89 ± 0.32 [c]	-67.67
G6: F2EE (50 mg/kg bw/day)	8.27 ± 0.36 [c]	58.43	9.88 ± 0.80 [cd]	37.80	4.20 ± 0.77 [b]	-53.02
G7: F2 EE (100 mg/kg bw/day)	10.67 ± 0.58 [b]	104.41	12.3±9 ± 1.2 [bc]	72.80	3.82 ± 0.28 [b]	-57.27
G8: F2EE (200 mg/kg bw/day)	11.78 ± 1.01 [b]	125.67	15.01 ± 1.42 [ab]	109.34	3.47 ± 0.58 [bc]	-61.19

Each value represents the mean value of six rats ± SD. Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with formulae compared to the hepatotoxic group. The guidelines for statistical analyses and experimental groups are shown in Table 1.

3.3. Effect of intervention with suggested formulae extracts on serum lipid profile (mg/dl) of hepatotoxic rats induced by B[*a*]P

The influence of the proposed formulae extracts on the serum lipid profile, including triglycerides (TGs), cholesterol (Cho), low-density lipoprotein cholesterol (LDL-c), and very low-density lipoprotein cholesterol (VLDL-c), in hepatotoxic rats exposed to B[*a*]P is presented in Table 3. By the end of the four-week experiment, rats subjected to B[*a*]P exhibited a significant ($p \leq 0.05$) decrease in TGs (-48.65%) and HDL-c (-38.62%), along with a marked increase in Cho (19.62%) and LDL-c (59.01%) in comparison to the controls. Yet, dietary supplementation with the tested formulae extracts at doses of 50, 100, and 200 mg/kg bw/day over four weeks resulted in a significant ($p \leq 0.05$) elevation in TGs and HDL-c levels, while reducing Cho and LDL-c in comparison to the hepatotoxic model group. The observed percentage changes for formula I were 13.86, 21.13, -6.34, and -9.36%; 41.26, 31.61, -11.06, and -24.51%; and 48.49, 44.74, -13.89, and -25.42%, respectively. For formula II, the corresponding percentage changes were 11.01, 18.66, -3.18, and -8.56%; 30.66, 19.52, -7.48, and -11.88%; and 38.92, 28.97, -10.83, and -21.17%, respectively. These results indicate that formula I had many pronounced impacts on improving lipid profile parameters compared to formula II. Moreover, both formulae exhibited a dose-dependent effect, with higher doses producing greater improvements in lipid metabolism. Previous studies have similarly reported that B[*a*]P exposure significantly reduces serum TGs and HDL-c while increasing cholesterol levels, likely as a consequence of liver dysfunction (Fayez, 2016; Badawi, 2023; Elhassaneen and Mahran, 2024; El-Nagar, 2024). The protective effects of the tested formulae in improving serum lipid profiles, with the exception of TGs, may be attributed to their potential hypercholesterolemic properties. This effect is linked to various biological activities, including antioxidative properties, free radical scavenging, and inhibition of LDL-c peroxidation. The beneficial properties of these formulae can likely be traced to their rich composition of bioactive compounds derived from their plant-based ingredients. These compounds include polysaccharides, phenolics, volatile compounds, flavonoids, saponins, oxalates, carotenoids, kaempferols, turmeric, alkaloids, and lycopene (Chattopadhyay *et al.*, 2004; EL-Nashar, 2007; El-Safty, 2008; Nasir *et al.*, 2010; Aber and Afa, 2011; Hassan, 2011; Aly *et al.*, 2017; Hallabo *et al.*, 2018). Phenolic compounds, for instance, are known to reduce cholesterol absorption (Usunobun *et al.*, 2015). Also, phenolics have a strong affinity for proteins, binding to albumin and preventing their incorporation into LDL particles. Moreover, the

hypocholesterolemic effects of alkaloids, phenolics, carotenoids, polysaccharides, and kaempferol have attracted significant research interest (Elhassaneen *et al.*, 2023). These compounds contribute to cardiovascular health by improving serum lipid profiles through antioxidant, anti-inflammatory, and scavenging activities (Kuhlmann *et al.*, 1998; Poma *et al.*, 2011; Correa-Gordillo *et al.*, 2012; Elhassaneen *et al.*, 2023). Additionally, LDL-c oxidation and endothelial cell damage are believed to play key roles in the early stages of atherosclerosis (Kaneko *et al.*, 1994). Several studies have demonstrated that phenolics, polysaccharides, and alkaloids significantly reduce LDL-c oxidation in vitro by various oxidases (Aviram *et al.*, 1999; Kaneko *et al.*, 1994; Aly *et al.*, 2017; Boraey, 2023). On the other hand, findings from this study, in alignment with previous research, suggest that the decline in serum TGs and HDL-c following B[a]P administration is due to impaired secretion of these components from the liver into the bloodstream. This impairment likely results from intracellular structural damage to the liver and/or energy depletion, as indicated by a significant reduction in glycogen content (Fayez, 2016; Elhassaneen *et al.*, 2024; Elhassaneen and Mahran, 2024; El-Nagar, 2024).

Table 3. Effect of intervention with suggested formulae extracts on serum lipid profile (mg/dl) of hepatotoxic rats induced by B[*a*]P

Groups	TGs (mg/dL)		T-Cho (mg/dL)		HDL-c		LDL-c	
	Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change
G1: Normal	78.56± 2.21 ^{a}	------	151.78± 3.87 ^{c}	------	50.61± 3.78 ^{a}	------	101.65± 5.31 ^{d}	------
G2: Model	40.34± 4.28 ^{c}	-48.65	181.56± 5.67 ^{a}	19.62	31.06± 5.11 ^{c}	-38.62	161.63± 6.11 ^{a}	59.01
G3: F1EE (50 mg/kg bw/day)	45.93± 3.7 ^{c}	13.86	170.05± 4.21 ^{b}	-6.34	37.62± 2.56 ^{b}	21.13	146.50± 2.89 ^{b}	-9.36
G4: F1EE (100 mg/kg bw/day)	56.98± 5.16 ^{b}	41.26	161.48± 6.32 ^{bc}	-11.06	40.88± 3.17 ^{ab}	31.61	122.01± 3.77 ^{c}	-24.51
G5: F1EE (200 mg/kg bw/day)	59.90± 4.79 ^{b}	48.49	156.34± 5.29^{c}	-13.89	44.96± 3.17 ^{a}	44.74	120.54± 8.90 ^{c}	-25.42
G6: F2EE (50 mg/kg bw/day)	44.78± 3.22 ^{c}	11.01	175.78± 4.22 ^{ab}	-3.18	36.86± 2.90 ^{bc}	18.66	147.80± 4.32 ^{b}	-8.56
G7: F2 EE (100 mg/kg bw/day)	52.71± 6.10 ^{bc}	30.66	167.98± 5.80 ^{b}	-7.48	37.13± 6.07 ^{b}	19.52	142.43± 4.72 ^{b}	-11.88
G8: F2EE (200 mg/kg bw/day)	56.04± 4.45 ^{b}	38.92	161.89± 4.05 ^{bc}	-10.83	40.06± 2.98 ^{ab}	28.97	127.42± 5.09 ^{c}	-21.17

Each value represents the mean value of six rats ± SD. Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with formulae compared to the hepatotoxic group. The guidelines for statistical analyses and experimental groups are shown in Table 1. TGs, Triglycerides, T-Cho, Cholesterol HDL, High-density lipoproteins cholesterol, LDL, Low-density lipoproteins cholesterol.

3.4. Effect of intervention with suggested formulae extracts on RBCs antioxidant enzymes activity of hepatotoxic rats induced by B[*a*]P

The impact of administering the proposed formula extracts on the antioxidant enzyme activity in RBCs of hepatotoxic rats induced by B[a]P is presented in Table (4). The findings indicate that after four weeks, rats exposed to B[a]P experienced a significant ($p \leq 0.05$) decline in GSH-Px (-48.31%), GSH-Rd (-35.54%), CAT (-29.84%), and SOD (-42.94%) in comparison to the normals. Yet, supplementation with the tested formula extracts (50, 100, and 200 mg/kg bw/day) for four weeks significantly ($p \leq 0.05$) enhanced GSH-Px; GSH-Rd; CAT; and SOD levels compared to the model group. The recorded increases for formula I were 56.59, 20.68, 18.28, and 10.71%; 84.10, 30.35, 28.04, and 40.05%; and 92.98, 39.30, 32.53, and 50.77%, respectively. Meanwhile, formula II resulted in increases of 36.36, 8.59, 10.20, and 7.40%; 58.34, 19.83, 19.58, and 21.17%; and 76.61, 31.44, 26.48, and 37.50%, respectively. Formula I demonstrated greater efficacy compared to formula II in enhancing these biochemical parameters. Additionally, both formulas exhibited a dose-dependent effect in restoring antioxidant

enzyme activity in hepatotoxic rats. The present findings align with previous research indicating that exposure to B[*a*]P significantly reduces antioxidant enzyme levels, including GSH-Px, CAT, and SOD, in RBCs. As documented by multiple studies, this decline is attributed to oxidative stress triggered by reactive oxygen species (ROS) generated during B[*a*]P metabolism. The increased levels of oxidative stress markers, such as TBARS and NO2, further disrupt cellular redox balance and deplete antioxidant enzymes (Elhassaneen.2004; Elhassaneen and El-Badawy.2013; Elhassaneen *et al.*, 2016a and b; Shahid *et al.*, 2016; Kumar *et al.*, 2017; Elhassaneen *et al.*, 2018; Aparna and Patri 2021; Bukowska and Duchnowicz 2022; Mahran and Elhassaneen 2023). RBCs are also exposed to oxidants from endothelial and immune cells, including nitric oxide (NO), superoxide, peroxynitrite ($ONOO^-$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl). These oxidants cause oxidative damage to proteins and lipids, leading to hemolysis, mitochondrial dysfunction, and cell membrane degradation (Elhassaneen *et al.*, 1996; Hassan *et al.*, 1996; Elhassaneen, 2000-b and 2008). RBCs contain a complex network of antioxidant enzymes that mitigate oxidative damage by neutralizing oxidant species. Among these are superoxide dismutase (SOD), which catalyzes the conversion of superoxide radicals into hydrogen peroxide and oxygen; glutathione peroxidase (GSH-Px), which reduces hydroperoxides using glutathione; glutathione reductase (GSH-Rd), which regenerates reduced glutathione (GSH) from oxidized glutathione (GSSG) using NADPH; and catalase, which breaks down hydrogen peroxide into water and oxygen (Das and Roychoudhury 2014; Banafsheh and Sirous, 2016; Grigoras, 2017). The current study demonstrated that the proposed formula extracts helped alleviate oxidative stress and partially restored antioxidant enzyme levels towards normal ranges. These effects may be attributed to the high concentration of bioactive compounds present in the formula constituents, which include phenolics, flavonoids, essential oils, volatile compounds, tannins, silymarin, curcumin, saponins, carotenoids, lycopene, alkaloids, and polysaccharides (El-Nashar. 2007; Aber and Afa. 2011; Hassan. 2011; El-Abasy.2013; El-Safty 2012; Aly *et al.*, 2017; Elhassaneen *et al.*, 2018; Hallabo *et al.*, 2018; Elhassaneen *et al.*, 2023; Mahran and Elhassaneen.2023). Multiple studies have shown that these bioactive compounds have many biological activities, such as antioxidant, scavenging, anticarcinogenic, antimutagenic, and antimicrobial effects, as well as inhibition of lipid oxidation. Hamza (2020) demonstrated that mixtures containing various plant parts, similar to those used in the suggested formulas, possess strong antioxidant properties due to their high phenolic and carotenoid content. The hydroxyl groups present in these extracts provide potent free radical scavenging activity, protecting against oxidative liver damage induced by toxins such as carbon tetrachloride (Elhassaneen *et al.*, 2021-a). Moreover, flavonoids found in the proposed formulas have been shown to counteract the effects of B[a]P in erythrocytes by scavenging free radicals, maintaining antioxidant enzyme activity, inhibiting the synthesis of B[a]P metabolites, and preventing excessive ROS formation. These actions contribute to reduced apoptosis rates and improved histological profiles of liver cells (Kiruthiga *et al.*, 2007; Mahran and Elhassaneen, 2023; Elhassaneen *et al.*, 2024).

Table 4. Effect of intervention with suggested formulae extracts on RBCs antioxidant enzymes activity of hepatotoxic rats induced by B[*a*]P

Groups	Glutathione peroxidase (GSH-Px, U/g Hb)		Glutathione reductase (GSH-Rd, U/g Hb)		Catalase (CAT, U/g Hb)		Superoxide dismutase (SOD, U/g Hb)	
	Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change
G1: Normal	24.14± 2.89 ^{a}	-------	12.83± 2.19 ^{a}	-------	180.50± 5.09 ^{a}	------	6.87± 0.56 ^{a}	-------
G2: Model	12.48± 0.87 ^{c}	-48.31	8.27± 1.79 ^{b}	-35.54	126.63± 7.34 ^{e}	-29.84	3.92± 0.34 ^{d}	-42.94
G3: F1EE (50 mg/kg bw/day)	19.54± 2.65 ^{b}	56.59	9.98± 2.72 ^{b}	20.68	149.78± 8.03 ^{c}	18.28	4.34± 0.48 ^{c}	10.71
G4: F1EE (100 mg/kg bw/day)	22.97± 3.21 ^{ab}	84.10	10.78± 2.39 ^{ab}	30.35	162.14± 5.55 ^{b}	28.04	5.49± 0.09 ^{b}	40.05
G5: F1EE (200 mg/kg bw/day)	24.08± 4.17 ^{a}	92.98	11.52± 3.02 ^{a}	39.30	167.82± 6.06 ^{ab}	32.53	5.91± 0.39 ^{ab}	50.77
G6: F2EE (50 mg/kg bw/day)	17.01± 1.89 ^{b}	36.36	8.98± 1.73 ^{b}	8.59	139.55± 4.88 ^{d}	10.20	4.21± 0.28 ^{cd}	7.40
G7: F2 EE (100 mg/kg bw/day)	19.76± 2.92 ^{b}	58.34	9.91± 2.09 ^{b}	19.83	151.42± 7.45 ^{c}	19.58	4.75± 0.61 ^{bc}	21.17
G8: F2EE (200 mg/kg bw/day)	22.04± 3.72 ^{ab}	76.61	10.87± 1.11 ^{a}	31.44	160.16± 6.92 ^{bc}	26.48	5.39± 0.49 ^{b}	37.50

Each value represents the mean value of six rats ± SD. Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with formulae compared to the hepatotoxic group. The guidelines for statistical analyses and experimental groups are shown in Table 1.

3.5. Effect of treatment with suggested formulae on hepatic ROS and CYP-450 of hepatotoxic rats induced by B[*a*]P

The impact of the proposed formulae extracts on hepatic reactive oxygen species (ROS) and cytochrome P450 (CYP-450) levels in hepatotoxic rats induced by B[*a*]P is presented in Table 5. The data revealed that after four weeks of experimentation, rats exposed to B[*a*]P showed a significant ($p \leq 0.05$) raise in hepatic ROS (109.21%) and CYP-450 (74.71%) in comparison to the controls. However, administering the tested formulae extracts (50, 100, and 200 mg/kg bw/day) in the diet for four weeks led to a notable ($p \leq 0.05$) reduction in hepatic ROS and CYP-450 levels relative to the model group. The observed reduction rates for formula I were -16.19%, -27.97%, and -40.27% for ROS, and -25.93%, -37.37%, and -41.41% for CYP-450. Similarly, formula II showed reductions of -14.03%, -24.28%, and -35.25% for ROS, and -23.23%, -33.67%, and -36.70% for CYP-450. These findings indicate that formula I demonstrated a greater efficacy in mitigating oxidative stress markers than formula II. Additionally, both formulae exhibited a dose-dependent trend in decreasing hepatic ROS and CYP-450 levels in hepatotoxic rats. Consistent with previous research, B[a]P is known to induce liver damage by undergoing metabolic activation via hepatic CYP-450, producing reactive intermediates such as hydroxy derivatives, phenolic diols, dihydro-diols, quinones, semiquinones, and epoxide enantiomers (Elhassaneen, 1996). Furthermore, prostaglandin H synthase and lipoxygenase are also implicated in B[a]P metabolism, contributing to the formation of highly reactive oxygen species (ROS) (Fantel, 1996). ROS are highly unstable molecules containing oxygen with unpaired electrons, and their excessive accumulation due to toxic exposure, such as B[*a*]P, leads to oxidative stress (Elhassaneen *et al.*, 2023; Abd Elalal *et al.*, 2022; Mahran and Elhassaneen, 2023; Elhassaneen and Mahran, 2024). This oxidative stress is responsible for cellular damage, including lipid peroxidation, DNA and RNA fragmentation, protein oxidation, and inactivation of critical enzymes due to oxidative modifications of cofactors (Halliwell and Aruoma, 1991). Furthermore, elevated ROS levels have been linked to the progression of liver diseases (Elhassaneen, 1996; Elhassaneen *et al.*, 2012; Abd Elalal *et al.*, 2022; Elhassaneen *et al.*, 2023). The oxidative stress triggered by B[*a*]P also plays a pivotal role in apoptosis (Mahran and

Elhassaneen, 2023; Elhassaneen *et al.*, 2024). The current findings suggest that oxidative stress levels in B[a]P-exposed rats were mitigated by the administration of the suggested formulae, which effectively lowered ROS formation in the liver. This indicates that the hepatoprotective properties of the tested formulae may be attributed to their antioxidant and radical-scavenging activities. Without these biological effects, it would have been unlikely to observe significant reductions in ROS and CYP-450 levels in the treated rats. In agreement with these results, several studies have reported a raise in oxidative stress markers in B[*a*]P-injected rats, while plant-based interventions similar to those used in this study have demonstrated antioxidant and hepatoprotective properties due to their rich content of bioactive secondary metabolites (Mahran *et al.*, 2018; Elhassaneen *et al.*, 2023; Mahran and Elhassaneen, 2023).

Table 5. Effect of treatment with suggested formulae on liver ROS and CYP-p450 of hepatotoxic rats induced by B[*a*]P

Groups	ROS (U/ml tissue)		CYP-450 (nmol/mg protein)	
	Mean ±SD	% of change	Mean ±SD	% of change
G1: Normal	423.67 ± 21.54 ^{e}	------	1.70 ± 0.08 ^{c}	------
G2: Model	886.34 ± 30.21 ^{a}	109.21	2.97 ± 0.11 ^{a}	74.71
G3: F1EE (50 mg/kg bw/day)	742.82 ± 19.63 ^{b}	-16.19	2.20 ± 0.05 ^{b}	-25.93
G4: F1EE (100 mg/kg bw/day)	638.45 ± 24.07 ^{c}	-27.97	1.86 ± 0.10 ^{c}	-37.37
G5: F1EE (200 mg/kg bw/day)	529.39 ± 20.56 ^{d}	-40.27	1.74 ± 0.07 ^{c}	-41.41
G6: F2EE (50 mg/kg bw/day)	761.98 ± 12.38 ^{b}	-14.03	2.28 ± 0.14 ^{ab}	-23.23
G7: F2 EE (100 mg/kg bw/day)	671.12 ± 33.23 ^{bc}	-24.28	1.97 ± 0.07 ^{bc}	-33.67
G8: F2EE (200 mg/kg bw/day)	573.87 ± 18.43 ^{d}	-35.25	1.88 ± 0.12 ^{c}	-36.70

Each value represents the mean value of six rats ± SD. Percentage of change (%) for the hepatotoxic (Model) group is calculated compared to the normal group, while it is calculated for the groups treated with formulae compared to the hepatotoxic group. The guidelines for statistical analyses and experimental groups are shown in Table 1.

3.6. Histopathological examination of liver

Effect of treatment with suggested formulae on rat liver histological disorders induced by B[*a*]P was shown in Figure 2. Microscopically, liver of rats from group 1 revealed the normal histological architecture of hepatic lobule (Photos A and B). Conversely, Group 2 livers presented with focal hepatocellular necrosis, accompanied by mononuclear inflammatory cell infiltration (Photo C), Kupffer cell activation, portal triad fibroplasia, and the development of new bile ductules (Photo D). Additionally, further focal hepatocellular necrosis with mononuclear cell infiltration was observed (Photo E). Group 3 livers showed significant congestion of the central vein and hepatic sinusoids (Photo F). Group 4 livers exhibited mild congestion of the hepatic sinusoids and slight vacuolization in some hepatocytes (Photo G). Group 5 livers displayed no histopathological alterations (Photo H). Group 6 livers revealed marked congestion of the hepatoportal vessel and cholangitis (Photo I). Group 7 livers showed slight Kupffer cell proliferation (black arrow) (Photo J). Finally, Group 8 livers presented with mild congestion of the hepatic sinusoids (Photo K). In alignment with our results, Elhassaneen and Mahran (2024) reported that liver tissue in rats exposed to B[a]P exhibited significant histopathological alterations. These changes included vacuolar degeneration of hepatocytes, focal necrosis of liver cells accompanied by inflammatory cell infiltration, activation of Kupffer cells, fibrosis in the portal triad, and the emergence of newly formed bile ductules. Similar

histopathological abnormalities, such as hepatic necrosis, mononuclear cell infiltration, and hepatocyte apoptosis, have been documented by various researchers in rats subjected to B[*a*]P exposure (Kiruthiga *et al.*, 2015; Rangi *et al.*, 2018). Research has shown that the underlying cause of liver damage associated with B[*a*]P exposure is oxidative stress triggered by its reactive metabolites (Sánchez-Valle *et al.*, 2012; Li *et al.*, 2015). This oxidative stress predominantly affects liver parenchymal cells, including Kupffer cells, endothelial cells, and stellate cells, contributing to tissue injury. In this regard, Hajam *et al.*, (2022) highlighted that ROS-induced membrane damage in hepatocytes leads to collagen accumulation, a key factor in the progression of liver fibrosis and cirrhosis. On the other hand, administration of the proposed formulae extracts to B[*a*]P-induced hepatotoxic rats resulted in varying degrees of histopathological improvement in liver tissue. These enhancements in liver architecture may be linked to the bioactive properties of the formulae extracts, including their antioxidant potential, free radical scavenging effects, and ability to inhibit lipid peroxidation. Supporting this, Elhassaneen *et al.*, (2024) and Hussien, (2024) demonstrated that plant-based extracts containing similar bioactive compounds as those in the tested formulae could reduce oxidative stress and protect liver cells that have not yet undergone irreversible damage. Our findings from the current study suggest that the proposed formulae extracts play a significant role in restoring liver function and partially reversing hepatic tissue damage, further supporting their hepatoprotective potential.

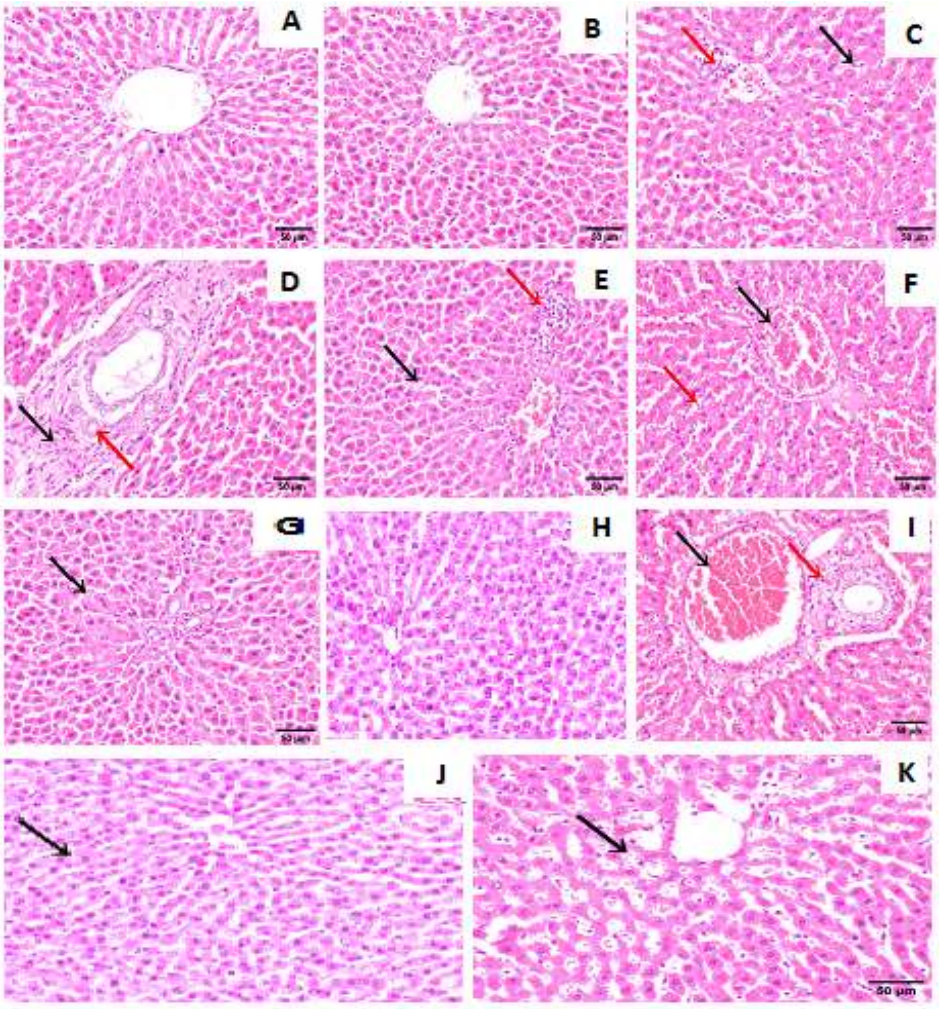

Figure 2. Effect of treatment with suggested formulae on rat liver histological disorders induced by B[a]P

Photomicrographs illustrate liver tissue from various experimental groups**. Photo A and B** depict normal hepatic lobule architecture in Group 1. **Photo C** (Group 2) shows Kupffer cell activation (black arrow) and focal hepatocellular necrosis with mononuclear inflammatory cell infiltration (red arrow). **Photo D** (Group 2) reveals portal triad fibroplasia (black arrow) and the presence of new bile ductules (red arrow). **Photo E** (Group 2) demonstrates hepatocyte vacuolization (black arrow) and focal hepatocellular necrosis with mononuclear cell infiltration (red arrow). **Photo F** (Group 3) exhibits marked congestion of the central vein (black arrow) and hepatic sinusoids (red arrow). **Photo G** (Group 4) shows slight hepatocyte vacuolization (arrow). **Photo H** (Group 5) indicates the absence of histopathological alterations. **Photo I** (Group 6) reveals marked congestion of the hepatoportal vessel (black arrow) and cholangitis (red arrow). **Photo J** (Group 7) displays slight Kupffer cell proliferation (black arrow). **Photo K (**Group 8) shows mild congestion of hepatic sinusoids (arrow). All images are hematoxylin and eosin stained (H&E), scale bar 50 µm, magnification ×200.

Conclusion

Benzo[*a*]pyrene (B[*a*]P), a widespread environmental and food contaminant, is known to cause significant liver damage in rats by triggering oxidative stress. However, intervention with the proposed formulae has shown potential in preventing or mitigating B[*a*]P-induced liver injury. As illustrated in Figure 3, the protective effects of these formulae involve enhancing liver function (such as glycogen content and enzyme activity), regulating lipid metabolism (including triglycerides, total cholesterol, and lipoprotein cholesterol), improving oxidative balance (RBC antioxidant enzymes and ROS levels), modulating biotransformation enzymes (CYP450), and promoting histological recovery. These combined effects contribute to counteracting liver damage and supporting overall biological health. The beneficial impact of the suggested formulae is likely linked to their abundance of bioactive compounds, which possess various protective properties. Based on these findings, the tested formulae may serve as a potential complementary therapy for individuals with liver disorders, offering hepatoprotective benefits.

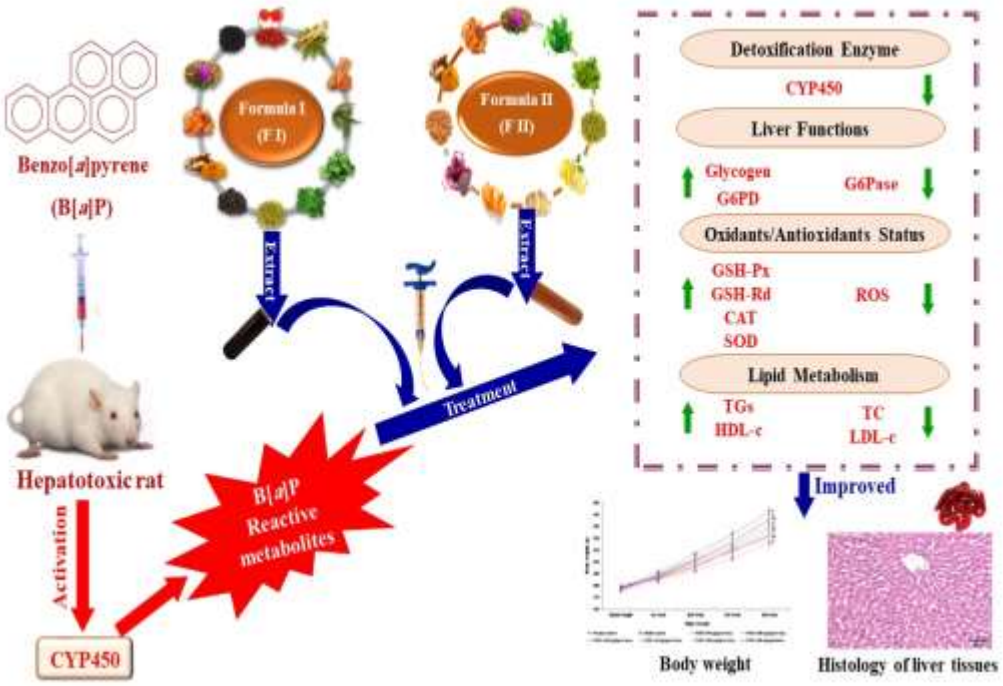

Figure 3. Graphical summary showing the effect of treatment with suggested formulae on B[*a*]P causes severe hepatic damage in rats.

Ethical considerations

The Scientific Research Ethics Committee at the Faculty of Home Economics, Menoufia Uni., granted ethical approval for this study (Approval No. 14-SREC-11-2023) in Shebin El-Kom, Egy.

Conflict of interest statement

The authors confirm that there are no conflicts of interest related to the publication of this work.

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Authors' Contribution

All authors contributed equally to every aspect of this study, including designing, developing, and reviewing the research protocol. They were actively involved in conducting and monitoring the experiments, collecting and analyzing data, organizing and interpreting the findings, and ensuring the accuracy of the results and statistical analyses. Additionally, they participated in gathering relevant conceptual information, drafting the manuscript, critically reviewing and refining its content, and approving the final version for publication.

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تأثير مستحضرات اجزاء نباتية متعددة على وظائف الكبد وحالة الاكسدة/مضادات الاكسدة ومعايير التمثيل الغذائي للدهون أثناء السمية الكبدية التجريبية المستحثة بالبنزوبيرين

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المستخلص:

يحدث التسمم الكبدي عندما يتعرض الكبد للتلف الناتج عن المواد الضارة مثل البنزوبيرين ، وهو ملوث بيئي وغذائي واسع الانتشار.ولقد اجريت العديد من الدراسات على التأثيرات الوقائية للمركبات النباتية ضد سمية البنزوبيرين ، الا ان معظمها قد ركز على مصادر نباتية فردية. لذلك، تهدف الدراسة الحالية إلى تقييم التأثيرات المحتملة لتركيبات من المستخلصات النباتية ضد تلف الكبد الناجم عن البنزوبيرين ، تم تقسيم 48 فأرًا إلى مجموعتين رئيسيتين: المجموعة الأولى (6 فئران) تم تغذيتها على الوجبة الأساسية كمجموعة ضابطة سالبة، والمجموعة الثانية (42 فأرًا) تم حقنها بالبنزوبيرين لتحفيز سمية الكبد ثم تم نقسيمها الى مجموعات فرعية: المجموعة 2 لم تتلقَ أي معاملة، والمجموعات 3–5 تلقت مستخلصات الإيثانول من التركيبة 1 بجرعات 50 و100 و200 ملجم /كجم من وزن الجسم/ يوم، والمجموعات 6–8 تلقت التركيبة 2 بنفس الجرعات. أدى المعاملة بالبنزوبيرين إلى زيادة معنوية في نشاط إنزيم جلوكوز–6–فوسفاتاز بنسبة 297.33%، الكوليسترول الكلي بنسبة 19.62%، LDL–c بنسبة 59.01%، مستويات انواع الاكسيجين النشط فى الكبد بنسبة 109.21%، وانزيم السيتوكروم P450 بنسبة 74.51%. وفي المقابل، حدث انخفاض معنوى في الجليسريدات الثلاثية بنسبة 48.65%، HDL–c بنسبة 38.62%، زيادة الوزن بنسبة 10.37%، الجليكوجين الكبدي بنسبة 63.34%، نشاط G6PD بنسبة 61.26%، وأنشطة الإنزيمات المضادة للأكسدة مثل الجلوتاثيون بيرواكسيداز بنسبة 48,31%، الكاتالاز بنسبة 29,84% والسوبر أكسيد ديسموتاز بنسبة 42,94% . كما أدى المعاملة بالمستخلصات (50، 100 و200 ملجم /كجم وزن الجسم) على مدار 28 يومًا إلى تحسين كبير في وظيفة الكبد، حالة مضادات الأكسدة، استقلاب الدهون، والتركيبات النسجية للكبد. تشير هذه النتائج إلى أن التركيبات النباتية التي تم اختبارها قد تكون عوامل وقائية محتملة ضد أمراض الكبد.

الكلمات المفتاحية: وظائف الكبد، صورة دهون الدم، الإنزيمات المضادة للأكسدة، السيتوكرومب450، أنواع الأكسجين التفاعلية، التغيرات النسيجية.