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Potential Protective Effects of Poinciana (*Delonix regia*) Flower Extracts Against Benzo[*a*]pyrene Induced-hepatotoxicity in Rats

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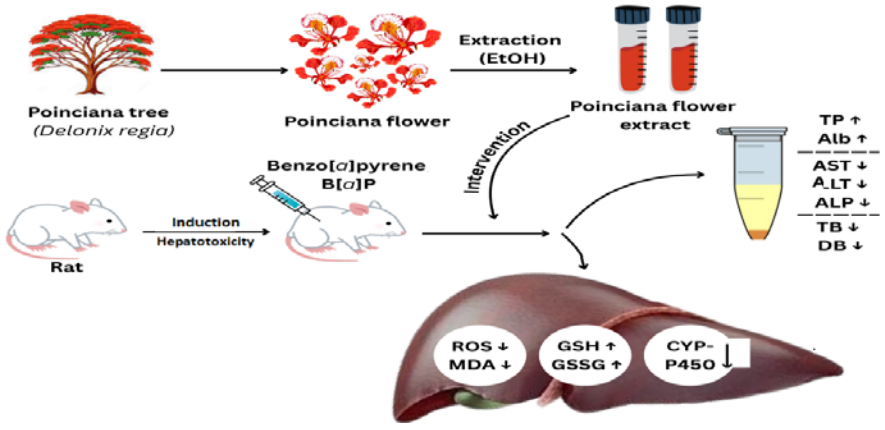
Abstract During the research, the goal was to test the hepatoprotective ability of a *Delonix regia* flower ethanol extract (DRFE) on benzo[*a*]pyrene (B[*a*]P)-induced hepatic toxicity in rats. Thirty rats in this paper consisted of a normal control group and a hepatotoxic group, which was subjected to DRFE at doses of 200, 400, or 600 mg/kg over the 28 days. B[*a*]P treatment resulted in significant reductions in body weight (-35.16%), feed intake (-37.48%), feed efficiency ratio (-27.36%), and liver weight (- 24.61%), and an increase in serum liver enzyme (AST, +187%, ALT, +164%, ALP, +88.6%), total bilirubin (+208.9%) and direct bilirubin (+234.2%), and the reduction in total protein (-25.3%). There was a violation of the balance of glutathione (GSH, -65.76%, GSSG, +31.08%, GSH/GSSG +73.88%), and an increase in the number of ROS (+241.79%) and MDA (+70.87%). The dose-dependent treatment of DRFE restored nutritional and hepatic parameters, the highest dose (600 mg/kg) improving body weight (+47.46%), liver weight (+24.20%), AST (−53.2%), ALT (−44.5%), ALP (−38.6%), total bilirubin (−51.1%), direct bilirubin (−47.8%), total protein (+20.52%), albumin (+32.78%), GSH (+109.97%). Such protective activities are attributed to the DRFE flavonoids, phenolic acids, tannins, sterols, and triterpenoids that have antioxidant, anti-inflammatory and membrane-stabilizing properties, repairing liver functioning, redox, and protein production. In general, DRFE had a good hepatoprotective effect against the B[a]P-induced toxicity with increasing doses which also indicates that it has a high potential in the use as a natural and plant-based agent in reducing the liver damage caused by environmental or dietary hepatotoxins.



Keywords: *Liver enzyme, bilirubin, glutathione, cytochrome P450, lipid peroxidation, malondialdehyde*

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1. Introduction

Benzo[*a*]pyrene (B[*a*]P) is a polycyclic aromatic hydrocarbon (PAH) and its chemical formula is C_{10} H_{12}. It is produced in the partial burning of organic (carbon-bearing) substances at between 300 and 600 ^{0}C. This substance is abundantly spread all around the environment and could be found in domestic wood burning, coal tar and motor vehicle emissions, especially those found in diesel-powered engines. B[*a*]P can also be found in smoke formed during burning of organic substances, such as cigarette smoke, and in a variety of food, particularly that cooked by high-temperature processes such as

charbroiling [1,2,3,4,5]. Food has been identified as one of the greatest sources of human exposure to B[*a*]P. Elhassaneen and Tawfik, [1] have reported that the levels of B[*a*]P in the food cooked to a well-done state by barbecues were significantly higher in steaks, chicken with skin and hamburgers. Data provided by other researchers has suggested that cooked meat products could have as much as 4 ng/g of B[a]P and fried chicken could be as much as 5.5 ng/g.

Overcooked Charcoal-barbecued beef has been found to have even greater concentrations up to 62.6 ng/g [6,7,8]. Besides this, Elhassaneen and Tawfik, [9] also found that the fumes generated during the frying process might be contaminated with B[*a*]P and this might pose a potential hazard to the populace. The World Health Organization estimates that about 99 percent of human food oral intake of PAHs, including B[a]P, comes through food and only 0.9 percent through inhalation with only 0.1-0.3 percent through drinking water [10]. Numerous experimental studies have been conducted over the last few decades that have established that B[*a*]P has toxic, mutagenic and carcinogenic properties in both in vivo and in vitro systems [11,12,13,14,15,16]. Strong association of B[a]P exposure with liver cancer development in mammals, including fish, to humans, has also been reported in numerous investigations that started since the 1970s [11,12,13,14,15] [16,17,18,19,20].

B[*a*]P is toxic and cancer causing due to its metabolic activation in the body. It has been demonstrated that this process is accomplished by three large scale enzymatic reactions in the past. Initial B[a]P is oxidized at cytochrome P450 1A1 to form a number of metabolites, such as (+) B[a]P-7,8-epoxide. Second, this compound can be hydrolyzed by epoxide hydrolase that cleaves the epoxide ring to produce (−) B[*a*]P-7,8-dihydrodiol. Third, another reaction catalyzed by cytochrome P450 1A1 forms the final carcinogenic product, the ultimate carcinogenic metabolite, the covalent binding product to DNA, is the ultimate carcinogenic metabolite, (+) B[*a*]P-7,8-dihydrodiol-9,10-epoxide [11,13,21]. Besides causing DNA damage, reactive intermediate metabolites of B[a]P, e.g. arene oxides, phenols, quinones, dihydrodiols, and epoxides, can cause cytotoxic effects in liver cells via oxidative stress effects. They are mitochondrially dysfunctional, lysosomally damaged, and cell membrane disrupted [2,13,22] [23,24,25,26].

Accordingly, B[*a*]P can cause serious liver damage and cause liver diseases due to exposure to the substance. Considering these devastating impacts, there have been numerous investigations highlighting the necessity to find the effective strategies to prevent or mitigate the liver toxicity caused by B[a]P. Traditional pharmaceutical interventions can be quite costly and result in several side effects, thus, decreasing the levels of compliance in patients. Thus, more interest has been paid to other types of therapeutic methods and especially natural ones. Natural products are cheaper in general, but the effects are usually not as adverse. In that connection, the chemoprevention of hepatotoxicity has been the object of attention of numerous research institutions and scientific authorities. Among the most outstanding discoveries in the area is the vast amount of compounds that have been shown to have protective effects against liver damage. A large number of these compounds fall into a wide category referred to as phytochemicals. Plant-based foods contain thousands of phytochemicals such as phenolics, flavonoids, carotenoids, glucosinolates, alkaloids, terpenes and phytoestrogens. These substances are basically available in vegetables, fruits, grains, legumes and other vegetable products. Strength of many phytochemicals found in the human diet has been reported to have potent antitoxic, antimutagenic, and anticarcinogenic properties [27,28,29,30] [31,32,33,34].

These results have prompted more studies to determine other plant materials that would assist in the prevention of the liver against poisonous substances. One example of such plants is *Delonix regia*. The Raf. or the Royal Poinciana or Flame Tree has received growing scientific attention due to its exceptional phytochemical constitution and numerous ethnomedicinal applications [35]. The plant is a member of the family *Fabaceae* and it is commonly produced in the tropical and subtropical areas. Conventionally, various components of *Delonix regia* have been utilized in herbal medicine to treat a number of diseases including inflammation, microbial infections, and digestive disorders. Recent phytochemical research showed that flowers of the *Delonix regia* plant are abundant sources of biologically active compounds such as flavonoids, phenolic acids, tannins, anthocyanins, sterols, and triterpenoids [35,36]. These substances have a strong antioxidant and anti-inflammatory effect and are related to various pharmacological actions. The flowers were found to contain major flavonoid, which include quercetin, quercitrin, rutin, and isoquercitrin. They are known to efficiently neutralize free radicals and prevent biological tissues against oxidative damage; furthermore, they are well known [37,38].

Some of the satudies have indicated high antioxidant activity of extracts obtained by using *Delonix regia* flowers because they contain high amounts of phenolic compounds and flavonoids [39]. The bioactive compounds could also increase the action of natural antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase thus improving cellular protection against oxidative stress [35,40]. It has been also found that *Delonix regia* extracts of flowers have hepatoprotective effects. As an illustration, ethanolic extracts and flavonoid-containing fractions of the flowers were found to provide significant hepatotoxicity protection to liver tissues in experimental models caused by chemicals [37,41].

These protective mechanisms are largely explained by the antioxidant activity of flavonoids like quercetin and rutin that have the potential to reduce the lipid peroxidation, stabilize the cellular membranes, and improve the activity of antioxidant enzymes in the liver tissues [42]. Thus, based on the rising exposure to environmental toxicants like benzo[a]pyrene and the rising concern in the use of plant-based chemoprotective plants, the current study had aimed to test the hepatoprotective effect of *Delonix regia* flower extract in preventing the B[a]P-induced liver toxicity. It was theorized that the antioxidant and protective properties of rich phytochemical composition of Delonix regia flower especially phenolic and flavonoid compounds can be of great benefit against hepatic oxidative stress. Based on this, this research paper sought to examine the effect of

the *Delonix regia* flower extract on liver functional biomarkers, oxidative stress and antioxidant defense mechanism after benzo[a]pyrene-induced exposure. The collected results of this study can support the scientific argument in favor of the possible application of Delonix regia as a natural hepatoprotective factor and help to create the more harmless approaches to the dietary/therapeutic interventions to decrease the risk of the negative impact of various environmental carcinogens.

2. Materials and Methods

2.1. Materials

2.1.1. The Flowers of Poinciana (*Delonix regia*)

Flowers of the mature Poinciana (*Delonix regia*) were taken at the trees at the densely spaced trees that were found along the main road running between the cities, Banha and Shibin El Kom, Shibin El Kom, Menoufia Governorate, Egypt, with the help of some local agricultural workers who own farmlands around Poinciana trees. The Department of Agricultural Plant, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt did the botanical identification and taxonomic verification of the pods.

2.1.2. Chemicals, Kits and Instrumentation

B[*a*]P was acquired at Sigma Chemical Co. (St. Louis, MO, Company agent, Cairo, Egypt). Casein which was employed in the experimental protocols was obtained in Morgan Chemical Co., Cairo, Egypt. Organic solvents, buffers and other chemical of analytical grade were bought at El-Ghomhorya Company that deals with Trading in Drug, Chemicals and Medical Instrument, Cairo, Egypt. The diagnostic assay kits of the evaluation of liver enzyme alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and malondialdehyde (MDA) are acquired at BIODIAGNOSTIC, Dokki, Giza, Egypt. Alb and TP testing kit were acquired in El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. GSH and oxidized glutathione (GSSG) were measured with the kits offered by MyBioSource, Inc., the company located in San Diego, CA, USA. The entire absorbance data in the course of the experimental assays was measured with a UV-160A spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

2.2. Methods

2.2.1. Delonix Regia

Poinciana (*Delonix regia*) flower powder preparation. The flowers of Poinciana (Delonix regia) were separated manually and sorted to extract any spoilt, discolored, or contaminated flowers. The flowers were then dried in a hot-air oven at the temperature of 50°C over four hours. After drying, they were finely grounded in a high speed grinder (Moulinex Egypt, Al-Araby Co., Egypt). The powder that resulted was sieved using an 80-mesh screen and the resulting fine fraction that was able to pass through was collected and stored to undergo an additional analysis.

2.2.2. Poinciana Flowers Extract (DRFE) Preparation

The powder of poinciana flower was made in line with the method cited by Gharib et al., (2022), but with slight changes. Pound into 100g of Poinciana flower powder were dried in a mixer at 50°C to a dry powder and extracted with 1000 ml of hydro-ethanolic solution (ethanol and water, 80:20) in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) at 50°C in 6 h. It was filtered using Whatman No. 5 filter paper using a Buchner funnel. The filtrate obtained was dried under reduced pressure at 40°C on a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). The extract obtained was stored at 4°C to be used in a biological experiment. It was estimated that the overall extractive yield was about 5.89 percent (w/w).

2.3. Biological Study

2.3.1. Ethical Considerations

All the activities on the handling and treatment of laboratory animals were conducted in compliance with the ethical regulations and got the Scientific Research Ethics Committee of Animal Care and Use Approval at the Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval Number: 11-SREC-06-2024).

2.3.2. Animals

The animals that were used in this study were adult male albino rats (155± 7 g per each) which were obtained at the Helwan Station which is found in the Ministry of Health and Population, Helwan, Cairo, Egypt. Rats were maintained under hygienic and similar management conditions. They were kept in single wire cages in a room at 24± 4.0°C, relative humidity (55±3%), and under normal healthy conditions. The acclimatization to rats took place by feeding them basal diet (BD) within one week.

2.3.3. Basal Diet (BD)

The BD that was made following the formula as stated by Reeves et al., [43] as follows protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2), cellulose (5) and the rest is corn starch (69.5). The salt and vitamin mixtures that were used in the BD were made by the same reference.

2.3.4. Hepatotoxicant Induction of Hepatotoxicant in Rats

On the seventh day, only, B[*a*]P (125mg/kg/b.w. dissolved in 0.9per cent NaCI solution with 0.1per cent Tween 20) was intraperitoneally (IP) injected into 30 male albino rats as reported by Shahid et al., [44]. The liver intoxication was also checked by a random sample of the experimented animals (three rats) and biochemical (liver functions) tested.

2.3.5. Experimental Design

Any biological research carried out conformed to the decisions of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council

[45]. A total of 30 rats in two main groups (the first main group (Group 1, 6 rats, as negative control group) remained fed on basal/standard diet and injected with the vehicle alone (5 ml/kg body weight) and the other main group (24 rats, hepatotoxic) then sub-divided as follow: Group 2 was fed on standard diet alone as a positive control, Group 3, 4 and 5 fed on BD and injected with Poinciana (*Delonix regia*) flowers extract (DRFE). at 200, 400 and 600 mg /kg bw/day respectively. The concentrations of DRFE that were being tested and recommended to be used in the current study were founded on multiple previous researches [46,47] During the 28 days, each of the above groups was placed in a single cage. The diet taken was recorded daily and the body weight was estimated weekly in order to calculate the biological assessment of body weight gain (BWG, percentage), food intake (FI), and food efficiency ratio (FER) over the period of experiment (28 days). The equations of Chapman et al., (1959) were used to calculate the BWG (%) by using the following formula: BWG (%) = (Final weight -Initial weight)/ Initial weight×100, FER= Grams gain in body weight (g/32 day)/ Grams feed intake (g/32 day).

2.3.6. Blood and Liver Sampling

In the case of blood and liver samples, rats were starved overnight in the end of the experiment (28 days) and sacrificed by decapitation. Samples of the blood were taken on the abdominal aorta and the livers were promptly dissected away, weighted and washed in ice-cold saline blotted dry and cut into two parts weight. The former was prepared as liver homogenate including those described by El-Khawaga et al., [48] to determine different biochemical parameters and the second segment was used to conduct histological investigations. Blood samples were placed in clean dry centrifuge tubes and allowed to clot at the room temperature [49]. Serum was aspirate and put into clean covet tubes and frozen at -18oC till analyzed.

2.3.7. Hematological Analysis

2.3.7.1. Liver Functions

ALT serum glutamic pyruvic transaminase and AST serum glutamic oxaloacetic transaminase activities were assessed through the modified method of kit by Tietz et al., [50] using kit in Biocon Company. By use of Elitech Company kit, the Alkaline Phosphatase (ALP) activity was identified under modified kinetic method of Vassault et al., [51].

2.3.7.2. Serum Total Protein, Bilirubin and Albumin

The Biuret colorimetric method of determining serum total protein is usually found on the principle that the peptide bonds of the proteins react with copper ions in alkaline pH [52]. At acidic pH, serum albumin is reacted with Bromocresol Green (BCG) to produce a green coloured complex. The albumin concentration measures the intensity of the color which is spectrophotometrically determined at 628 nm. Instead, it can be more specifically used with Bromocresol Purple (BCP) [53]. The total bilirubin has been identified by the colorimetric method as per the Jendrassik-Grof method which is on the basis of reaction of bilirubin with diazotized sulfanilic acid to create azobilirubin. It was spectrophotometrically measured to determine the intensity of formed color at about 540550 nm and the bilirubin concentration was determined by the use of a standard calibration curve [54]. Direct bilirubin was also measured using the colorimetric procedure based on the JendrassikGrof procedure without the use of accelerators to enable the direct reaction of conjugated bilirubin with diazotized sulfanilic acid to form azobilirubin. The calibration curve was used to determine the concentration of the color, which was measured spectrophotometrically at 540-550 nm [54].

2.3.7.3. Glutathione Fractions

The concentration of reduced glutathione GSH and oxidized glutathione GSSG was identified by colorimetric enzyme recycling technique founded on the reaction with 5, 5 – dithiobis (2 -nitrobenzoic acid) DTNB. In this experiment, GSH is reacted with DTNB to produce the yellow-colored product 5-thio-2-nitrobenzoic acid (TNB) which could be determined by spectrophotometric measurements at 412 nm. With the presence of glutathione reductase (GR) and NADPH, oxidized glutathione (GSSG) can be continuously reduced to GSH and the reaction signal can be amplified, which makes it possible in reaction to determine the total glutathione (GSH + GSSG). To determine GSSG specifically, 2-vinylpyridine was used to derivatize the free GSH in the samples to avoid its interference with the assay. The rate of the reaction was followed spectrophotometrically and glutathione concentrations were determined by using a standard curve that was developed using known concentration of GSH. This low concentration of glutathione was then determined by taking the difference between the total glutathione and two times of the glutathione. This technique is a sensitive and valid colorimetric assay of the quantification of glutathione redox status of biologic samples [50,55,56].

2.3.7.4. Potential Drug Interactions

The enzyme accountable for metabolising drugs is called Cytochrome P-450. The colorimetric method was used to determine the activity of liver microsomes hepatic cytochrome P450 (CYP450) based on the creation of a CO-CYP450 complex following sodium dithionite reduction, which results in the characteristic of its absorption at 450 nm. The difference in the absorbance at 450 and 490nm is used to determine the concentration of the CYP450 using the molar extinction coefficient [57].

2.3.7.5. Reactive oxygen species (ROS) and Malanaldehyde (MDA)

The content of malonaldehyde (MDA) was determined by the colorimetric procedure presented by Buege and Aust, [58] through a reaction involving thiobarbituric acid (TBA) and MDA which is one of the aldehyde products of lipid peroxidation. The determination of reactive oxygen species (ROS) was based on a colorimetric analysis that was detailed by Erel, [59].

2.4. Statistical Analysis

Measurement was performed thrice and presented as mean SD. One-way ANOVA was used to calculate a significance of differences and followed by multiple

comparisons with Duncan test using an MINITAB 12 computer program (Minitab Inc., State College, PA). The level of probability of P= 0.05 was taken to be statistically significant.

3. Results and Discussion

3.1. The impact of the *Delonix regia* Flower Extract (DRFE) on the Body Weight and Feeding Parameters

Benzo[*a*]pyrene (B[*a*]P) hepatotoxicity greatly affected the growth and feeding behavior as indicated in body weight (BW), feed intake (FI) and the ratio of feed efficiency (FER) in rats (Table 1). The hepatotoxic rats without treatment (G2) had a 35.16% lowering of BW, a 37.48% reduction of FI, and a 27.36% reduction of FER than the normal control (G1), which implies impaired ability in metabolic efficiency and the use of nutrients. The effects are in agreement with past reports that indicated B[*a*]P interferes with the regulation of appetite, the production of proteins in the liver, and the generation of energy in the mitochondria leading to the dampened growth performance [60,61,62]. Reductions of the FI and FER must be mediated by the effects of oxidative stress on the hepatocyte dysfunction compromising the digestion and nutrient assimilation processes [63]. Moreover, TNF-a, which is an inflammatory cytokine, which is released during B[*a*]P toxicity, can suppress appetite and change energy expenditure [64]. DRFE treatment resulted in dose effect restoration of nutritional parameters. At 200 mg/kg (G3), there were slight limitations in BW (+18.64%), FI (+19.41%), and FER (+17.65%) compared with the hepatotoxic group. The positive changes were more significant at 400mg/kg (G4) and 600mg/kg (G5), with the BW rising by 39.65% and 47.46 respectively. These results show that DRFE alleviates B[a]P-induced metabolic dysfunction, and to some extent, recovers normal physiological performance. These anti-oxidant and anti-inflammatory properties of polyphenols, flavonoids (e.g., quercetin derivatives), and carotenoids (high content in DRFE) can be explained by its hepatoprotective and metabolic properties [35,36]. These compounds probably prevent oxidative stress and lipid peroxidation caused by B[a]P and maintain the integrity of the hepatocytes and enhance nutrient use. Mechanistically, DRFE bolsters the natural antioxidant defense mechanisms by increasing the activity of glutathione, catalase and superoxide dismutase, which reduces the oxidative load caused by reactive metabolites like benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) [65]. Moreover, DRFE can regulate the immune response, downregulating IL-6, TNF-a, and NF-..B pathways [66], which decreases the metabolic stress and promotes normal feeding behavior. The FER restoration shows an increased efficiency in the conversion of feed into body mass, which may be mediated by an increased mitochondrial ATP generation and normalized oxidative phosphorylation [67]. The higher level of FI in the treated groups can also indicate changes in appetite-regulating hormones, including ghrelin and leptin, which is frequently impaired in case of hepatic injury [68]. The dose-related changes in G3G5 are solid indications of a true pharmacological action, and the maximum dose (600 mg/kg, G5) almost returned BW, FI, and FER to normal control values. These findings correspond with previous data that increasing the content of plant polyphenols is more reliable in neutralizing ROS and in the regulation of the inflammatory processes, which improves metabolic and hepatoprotective responses [69]. On the whole, DRFE exhibits a substantial potential of combating the B[a]P-induced growth and feeding defects with the help of both antioxidant and anti-inflammatory, as well as metabolic activity.

Table 1. Assessment of the ethanol extract from *Delonix regia* (poinciana) flowers (DRFE) on weight and feeding parameters in healthy and benzo[*a*]pyrene-hepatotoxic rats after four weeks of administration

Group	Body weight (BW, %)		Feed intake (FI, g/day/rat)		Feed efficiency ratio (FER)	
	Means ±SD	Percent of change	Means ±SD	Percent of change	Means ±SD	Percent of change
G1: Normal control	0.91 ± 0.06^{a}	0.00	11.37 ± 0.80^{a}	0.00	0.070 ± 0.004^{a}	0.00
G2: Model control (Hepatotoxic)	0.59 ± 0.08^{d}	-35.16	7.11 ± 0.65^{c}	-37.48	0.051 ± 0.007^{c}	-27.36
G3: DRFE (200 mg/kg bw)	0.70 ± 0.06^{c}	18.64	8.49 ± 0.71^{b}	19.41	0.060 ± 0.005^{b}	17.65
G4: DRFE (400 mg/kg bw)	0.82 ± 0.07^{b}	39.65	10.01 ± 0.85^{ab}	40.79	0.065 ± 0.005^{ab}	27.45
G5: DRFE (600 mg/kg bw)	0.87 ± 0.04^{ab}	47.46	10.61 ± 0.66^{a}	49.23	0.067 ± 0.007^{a}	31.37

All data are reported as the mean ±standard deviation (SD), with six animals per treatment group (n = 6). A superscript letter within a column indicates a statistically significant difference at the threshold of $p \leq 0.05$. The experimental groups are defined as follows: G1 (Normal control), healthy or untreated rats; G2 (Model control), rats exhibiting benzo[a]pyrene (B[a]P)-induced liver damage but receiving no intervention; G3-G5, hepatotoxic rats administered the ethanol extract of *Delonix regia* (Poinciana) flowers (DGFE) daily at doses of 200, 400, and 600 mg/kg body weight, respectively. Percentage changes for the model control (G2) are calculated relative to the Normal control (G1). Conversely, the percentage changes for the DGFE-treated groups (G3-G5) are determined in comparison to the model control (G2).

Table 2. Assessment of the ethanol extract from *Delonix regia* (poinciana) flowers (DRFE) on liver weight in healthy and benzo[*a*]pyrene-hepatotoxic rats after four weeks of administration

Group	liver weight (g)	
	Means ±SD	Percent of change
G1: Normal control	5.81 ± 0.08a	0.00
G2: Model control (Hepatotoxic)	4.38 ± 0.10 c	-24.61
G3: DRFE (200 mg/kg bw)	4.82 ± 0.06 b	10.05
G4: DRFE (400 mg/kg bw)	5.41 ± 0.11 a	23.52
G5: DRFE (600 mg/kg bw)	5.44 ± 0.09 a	24.20

All data are reported as the mean ±standard deviation (SD), with six animals per treatment group (n = 6). The key for the experimental groups, statistical analyses, percent of change calculation and abbreviations is provided below Table 1.

3.2. Effect of *Delonix Regia* Flower Extract (DRFE) on Liver Weight in Benzo[*a*]pyrene-Induced Hepatotoxic Rats

The liver weight is an essential parameter of the hepatic integrity, regenerative ability and the overall state of metabolism [70]. In this experiment (Table 2), benzo[a]pyrene (B[a]P) treatment markedly decreased the liver weight of the hepatotoxic model group (G2: 4.38 ± 0.10 g, −24.61%) compared to the normal control (G1: 5.81 ± 0.08 g) which is evidence of the atrophy of tissues and hepatocellular damage. This finding is in line with hepatotoxic actions of B[a]P that are widely documented as oxidative stress, lipid peroxidation, DNA damage, and hepatocyte apoptosis induction [71,72]. DRFE treatment led to a dose-dependent recovery of liver mass. Liver weight at 200 mg/kg (G3), 400mg/kg (G4) and 600mg/kg (G5) also partially restored liver weight at 4.82 +/- 0.06g (+ 10.05%), 5.41 +/- 0.11g (+ 23.52%), and 5.44 +/- 0.09g (+ 24.20%), respectively. The gradual restoration of liver mass suggests that DRFE has a positive effect against the hepatic atrophy, caused by B[a]P, and stimulates the process of hepatic regeneration. It can be assumed that the hepatoprotective effect of DRFE can be explained by the abundance of bioactive phytochemicals in the composition, such as flavonoids, phenolics, and carotenoids, with a high antioxidant and anti-inflammatory potency. They are able to scavenge reactive oxygen species (ROS), prevent lipid peroxidation, and promote endogenous antioxidant activities, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [62,73]. DRFE alleviates hepatic oxidative stress and inhibits hepatocyte apoptosis by enhancing both structural and functional healing of the hepatic tissue. The dose dependence effect was similar to other studies that showed higher levels of plant-derived extracts had a stronger hepatoprotective effect because of the increased bioavailability of active compounds [74]. The partial effect of the lowest dose indicates the possibility of the existence of a threshold concentration in order to attain optimal protection against hepatotoxicity caused by B[a]P. Conclusively, DRFE is a significant liver weight restorer of B[*a*]P-challenged rats in a dose-dependent manner. This recovery indicates the structural integrity of the hepatocytes as well as the functional maintenance, which indicates the therapeutic potential of the Delonix regia flower extracts as natural hepatoprotective.

3.3. Effect of *Delonix regia* Flower Ethanolic Extract (DRFE) on Liver Function Enzymes in Benzo[*a*]pyrene-Induced Hepatotoxic Rats

The results of Table 3 are that B[*a*]P exposure caused substantial liver damage, which was detected by a substantial increase in serum liver enzymes. Aspartate aminotransferase (AST) increased in the hepatotoxic model group (G2) with 27.71 U/L in normal controls and 79.54 U/L (+187) in the hepatotoxic group, alanine aminotransferase (ALT) increased with 22.11 U/L in normal controls to 58.32 U/L (+164) in the hepatotoxic group, and alkaline phosphatase (AL These findings are in line with the evidence of hepatocellular (AST and ALT) and cholestatic/biliary (ALP) pathology, which proves that B[a]P causes significant liver injury. The amelioration of these elevations was dose-dependent when the extract was treated with ethanolic extract of *Delonix regia* flowers (DRFE). The 200 mg /kg (G3) lowered AST, ALT and ALP by 18.0, 19.7 and 15.0, respectively compared to the hepatotoxic model. It was better at 400 mg/kg (G4) with 43.3, 29.8 and 30.0 percent decrease in AST, ALT and ALP, respectively. These enzymes were further brought near to normal control levels with highest dose (600 mg/kg; G5) with AST of 37.21 U/L (-53.2%), ALT of 32.39 U/L (-44.5%), and ALP of 159.07 U/L (-38.6%). The above results strongly indicate the hepatoprotective ability of DRFE on liver injury caused by B[a]P. The increase in AST and ALT in the hepatotoxic model represents the loss of these enzymes into the circulatory system because of damage, necrosis or inflammation of hepatocyte membranes. ALP is elevated, which testifies of cholestasis or obstruction of the bile ducts. The high depletion of these markers by DRFE indicates that the extract is protective of hepatocytes, stabilizes cell membrane and maintains the functional activity of the biliary system. The protective properties of DRFE on the liver can be explained by its phytochemical composition. The flower extract has flavonoids (quercetin, quercitrin, isoquercitrin, rutin), triterpenes (ursolic acid), and sterols (2-sitosterol, stigmasterol), which have strong antioxidant, anti-inflammatory, and anti-membrane effects [75,76]. These compounds suppress lipid peroxidation by scavenging reactive oxygen species (ROS) produced during B[a]P metabolism, and damage of hepatocyte membranes is prevented. Further, DRFE can augment the endogenous antioxidant systems and phase II detoxification pathways, such as glutathione-dependent conjugation, to ameliorate the development of reactive B[a]P metabolite. Moreover, DRFE can regulate the activity or expression of cytochrome P450 (CYP1A1/1B1) which reduces the bioactivation of B[a]P to DNA-reactive and pro-oxidant intermediate. The protection of bile ducts and hepatocytes is probably a result of the anti-inflammatory effect of the extract through inhibiting the NF-κB-regulated pathway and proinflammatory cytokines. The dose-dependent normalization of ALP observed with DRFE indicates that this agent does not only interfere with the condition of hepatocellular injury but also maintains the biliary excretory capacity, potentially by stabilizing transporters of the canalicular membrane, e.g., the bile salt export pump. The results are in line with the prior research that showed the hepatoprotective effect of D. regia extracts in the liver injury induced by chemicals model. Indicatively, Ahmed et al. [77] have found that restoration of AST, ALT, and ALP was achieved after CCl_4-induced hepatotoxicity, whereas El-Sayed et al. [78] have related the protective effects of flower extracts to antioxidant flavonoids and triterpenes. These observations were further applied to a B[a]P-induced model of hepatotoxicity, and it was found that the extract was effective against polycyclic aromatic hydrocarbon-induced hepatotoxicity. Altogether, DRFE inhibits B[a]P-induced increases in

liver functional enzymes in a dose-dependent manner. The extract might have a combination of antioxidant, anti-inflammatory, membrane-stabilizing, and detoxification-enhancing effects as a possible toxin of the liver, and as such, could be used as a natural therapeutic agent.

Table 3. Assessments of the ethanol extract from *Delonix regia* (poinciana) flowers (DRFE) on liver functions in healthy and benzo[*a*]pyrene-hepatotoxic rats after four weeks of administration

Group	Aspartate aminotransferase (AST)		Alanine aminotransferase (ALT)		Alkaline phosphatase (ALP)	
	Means ±SD	Percent of change	Means ±SD	Percent of change	Means ±SD	Percent of change
G1: Normal control	27.71 ± 3.67 [d]	0.00	22.11 ± 2.74 [d]	0.00	137.43 ±11.65 [e]	0.00
G2: Model control (Hepatotoxic)	79.54 ± 8.98[a]	187.04	58.32 ± 6.90 [a]	163.77	259.12 ±21.78 [a]	88.55
G3: DRFE (200 mg/kg bw)	65.21 ± 0.43 [a]	-18.02	46.83 ± 2.90 [b]	-19.70	220.17 ±10.91 [b]	-15.03
G4: DRFE (400 mg/kg bw)	45.10 ± 4.88 [b]	-43.30	40.94 ± 5.56 [b]	-29.80	181.36 ±18.04 [c]	-30.01
G5: DRFE (600 mg/kg bw)	37.21 ± 8.21 [c]	-53.22	32.39 ± 3.08 [c]	-44.46	159.07 ±12.93 [d]	-38.61

All data are reported as the mean ±standard deviation (SD), with six animals per treatment group (n = 6). The key for the experimental groups, statistical analyses, percent of change calculation and abbreviations is provided below Table 1.

Table 4. Assessments of the ethanol extract from *Delonix regia* (poinciana) flowers (DRFE) on serum total and direct bilirubin's in healthy and benzo[*a*]pyrene-hepatotoxic rats after four weeks of administration

Group	Total bilirubin (TB, µmol/L)		Direct bilirubin (DB, µmol/L)	
	Means ±SD	Percent of change	Means ±SD	Percent of change
G1: Normal control	6.73 ± 0.44 [d]	0.00	2.43 ± 0.21 [e]	0.00
G2: Model control (Hepatotoxic)	20.79 ± 1.12[a]	208.92	8.12 ± 0.97 [a]	234.16
G3: DRFE (200 mg/kg bw)	18.20 ± 1.06 [ab]	-12.46	6.81 ± 0.44 [b]	-16.13
G4: DRFE (400 mg/kg bw)	15.06 ± 0.98 [b]	-27.56	4.78 ± 0.53 [c]	-41.13
G5: DRFE (600 mg/kg bw)	10.17 ± 0.78 [c]	-51.08	4.24 ± 0.19 [d]	-47.78

All data are reported as the mean ±standard deviation (SD), with six animals per treatment group (n = 6). The key for the experimental groups, statistical analyses, percent of change calculation and abbreviations is provided below Table 1.

3.4. Effect of *Delonix regia* Flower Ethanolic Extract on Serum Total and Direct Bilirubin in Benzo[*a*]pyrene-Induced Hepatotoxic Rats

Based on the findings in Table 4 it is clear that the administration of the ethanolic extract of *Delonix regia* flowers (DRFE) had a strong protective effect on serum total bilirubin (TB) and direct bilirubin (DB) levels in rats under benzo[*a*]pyrene (B[*a*]P)-induced hepatotoxicity. Bilirubin parameters are common biochemical hepatic function parameters that show the ability of the liver to take in, conjugate, and release bilirubin which is the result of the breakdown of heme. Increase in serum bilirubin thus usually reflects hepatocellular injury, deficiency of conjugation or bile obstruction [79]. The model group (G2) that showed a significant elevation of bilirubin levels was hepatotoxic in the current study as opposed to the normal control group (G1). In the normal and B[a]P-treated rats, serum total bilirubin rose to 20.79 + 1.12 ug/L and 6.73 + 0.44 ug/L, respectively; this was a 208.9 percent increment. Likewise, the direct bilirubin rose to 2.43 + 0.21 ug/L to 8.12 + 0.97 ug/L which is equivalent to 234.2 per cent rise. These high levels portray a significant hepatic bilirubin metabolism and excretion in the aftermath of B[*a*]P. These changes are in line with what is found as hepatotoxic effects of polycyclic aromatic hydrocarbons which produce reactive intermediates in the metabolic activation process and cause oxidative stress induced injury to hepatocytes. Activation of benzo[*a*]pyrene metabolically is mainly brought about by the cyto houses enzymes and the formation of reactive epoxide intermediates that may cause destruction of hepatic cell structuring, and distortion of normal metabolism. The resulting oxidative stress triggers lipid peroxidation, mitochondrial dysfunction, and inflammatory problems and ultimately, causes liver dysfunction in conjugating bilirubin using enzymes of the UDP-glucuronosyltransferase group and excrete bilirubin into the bile. As a result, there is a build-up of unconjugated and conjugated bilirubin in the blood and this gives the high TB and DB in the case of animals with hepatotoxicity. DRFE treatment also cut down the levels of TB and DB in a dose-dependent fashion. The 200 mg/kg body weight (G3) reduced the total bilirubin by more or less 12.5 percent compared to the hepatotoxic control group. Doubling the dose to 400 mg/kg (G4) yielded higher reduction of about 27.6% and highest dosage (600 mg/kg; G5) led to the largest reduction of about 51.1% of TB as compared to the model group. The same was the case with the direct bilirubin which reduced by about 16.1, 41.1 and 47.8 percent at the doses of 200, 400 and 600 mg/kg respectively. These results have shown that DRFE is a potent suppressor of B[a]P-induced disruptions in bilirubin metabolism and gradually recovers the hepatic functional capacity. Hepatoprotective effect witnessed can be explained by the bioactive

phytochemicals found in the flowers of *Delonix regia*. The antioxidant active components of phytochemical studies on the flower extract have been found to include flavonoids like quercetin, isoquercitrin and rutin, sterols and triterpenoids [78]. These compounds have a high free-radical-scavenging effect and can counteract the reactive oxygen species and protect macromolecules in the cell against oxidative damage. These antioxidant effects have the potential to preserve the hepatocyte integrity and retain the enzymatic machinery of bilirubin conjugation and excretion. This interpretation can be supported by the prior experimental research. According to El-Sayed et al. [78], extracts of D. regia flower richest in flavonoids had potent hepatoprotective effects on the carbon tetrachloride-induced liver injury models and this attribute was mainly attributed to their antioxidant capacity. Equally, El-Gizawy et al. [80] proved that both chemically induced hepatic enzyme increase and histological damage were prevented with the help of phenolic-rich fractions derived out of leaves of D. regia, which explains the key role of plant-derived antioxidants in protecting the liver. Oxidative stress is at the center of hepatic injury occurrence in the case of B[*a*]P toxicity. The occurrence of large quantities of reactive oxygen species during the metabolic activation of B[a]P by cytochrome P450 enzymes results in lipid peroxidation, cellular membrane disruption, as well as the impairment of hepatocyte metabolic functions. The antioxidant effects of *D. regia* flowers can thus alleviate the ROS formation and prevent lipid peroxidation, hence, maintaining the hepatocellular structure and bile canalicular integrity [73]. These structures are necessary in order to maintain normal bilirubin transportation and excretion. A number of other processes can be the reason of the observed decrease in bilirubin levels. First, antioxidant properties of DRFE can decrease the damage to the hepatocytes and, therefore, maintain the functionality of bilirubin-conjugating enzymes, including UDP-glucuronosyltransferase (UGT1A1). Second, phytochemicals contained in the extract might stabilize livers cells membranes and enhance bile flow by preserving canalicular transport systems which are in charge of bilirubin secretion. Third, flavonoids have also been reported to regulate phase II detoxification enzymes, which may increase bilirubin conjugation and clearance. These concerted efforts could explain the tremendous changes in TB and DB seen in the DRFE-treated animals. It is also interesting to note that the greatest dose of DRFE (600 mg/kg) brought about the greatest change, bringing TB down to 10.17 µmol/L and DB down to 4.24 µmol/L. These values are close to those of the normal control group (6.73 and 2.43 µmol/L, respectively), which means that the bilirubin-handling capacity of the liver was relatively restored even in the case of continuous exposure to B[a]P. This observation indicates that DRFE can be used to offer significant functional protection to hepatic tissues during xenobiotic-induced oxidative stress. Although these are promising outcomes, there are a number of factors that need to be taken into consideration. Bilirubin levels are indirect signatures of hepatic activity; thus, the further biochemical indicators of liver activity like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and histopathologic analysis would also help in understanding the mechanisms that are in the background of the observed protective effects. In addition, despite the dose-dependent effect implying a high therapeutic potential, extensive toxicological studies should be done to establish the safety of high-dose *D. regia* extracts in the long term [46]. All in all, the current results indicate that DRFE can be used to a large extent to counter the increases in serum bilirubin, both total and direct, in rats when subjected to B[a]P. The dose-dependent positive effects imply that the extract is efficient in its protection of hepatocytes and reestablishment of bilirubin metabolism, probably by its hepatoprotective and antioxidant effects via its flavonoid and phenolic compounds. These findings justify the use of Delonix regia flower extract as a natural hepatoprotective factor against the toxin-induced liver damage and the necessity of the further mechanistic and pharmacological research.

3.5. Effect of *Delonix regia* Flower Ethanol Extract on Hepatic Cytochrome P450 in Benzo[a]pyrene-Induced Hepatotoxic Rats

The facts supplied in Table 5 imply that exposure to B[*a*]P resulted in a reported elevation in hepatic cytochrome P450 (CYP450) attention compared with the regular control organization. Rats inside the version manipulate group (G2) exhibited a CYP450 degree of 3.59 ± 0.21 nmol/mg protein, representing an growth of approximately 108.Eight% relative to healthful animals (1.Seventy two ± 0.12 nmol/mg protein). This marked elevation is consistent with the properly-established capacity of polycyclic fragrant hydrocarbons, including B[a]P, to result in enzymes belonging to the CYP1A subfamily—particularly CYP1A1 and CYP1B1, thru activation of the aryl hydrocarbon receptor (AhR) signaling pathway [81].

Treatment with the ethanolic extract of Delonix regia vegetation (DRFE) produced a clean dose-established attenuation of CYP450 induction. Administration of DRFE at 200 mg/kg body weight (G3) reduced CYP450 degrees by means of 7.24% relative to the B[a]P-simplest organization. Increasing the dose to four hundred mg/kg (G4) resulted in a greater discount of approximately 19.50%. The maximum pronounced development was observed in rats treated with six hundred mg/kg (G5), in which CYP450 tiers decreased by means of 36.Forty nine% compared with the hepatotoxic version institution. Although CYP450 hobby remained above the everyday baseline level, the progressive reduction across remedy organizations indicates a partial healing of hepatic enzymatic balance and indicates that DRFE correctly mitigates immoderate xenobiotic-metabolizing enzyme induction.

Several mechanisms may give an explanation for the modulatory impact of DRFE on CYP450 expression. First, *Delonix regia* plant life are recognized to include various phytochemicals, which includes flavonoids, phenolic acids, and triterpenoids, which show off powerful antioxidant and unfastened-radical-scavenging residences [78,82]. Since oxidative pressure performs a essential function inside the induction of CYP1A gene expression, the

antioxidant activity of those compounds can also suppress ROS formation and thereby reduce AhR-mediated CYP450 upregulation [83]. Consequently, the reduction in CYP450 stages discovered in the DRFE-dealt with companies might also replicate the capacity of the extract to alleviate oxidative stress generated for the duration of B[a]P metabolism. Second, certain flavonoids identified in Delonix regia extracts, which includes quercetin, rutin, and kaempferol, have been reported to intrude with AhR activation and inhibit transcriptional upregulation of CYP1A1 [84]. Through this mechanism, DRFE may additionally directly modulate the AhR–CYP1A signaling pathway and thereby attenuate B[a]P-stimulated gene expression. Such interactions among plant-derived flavonoids and AhR-regulated enzymes were documented in numerous experimental models of xenobiotic-triggered toxicity. Third, hepatoprotective and membrane-stabilizing consequences of the extract may additionally make contributions to the found discount in CYP450 hobby. The metabolic activation of B[*a*]P produces electrophilic intermediates that damage hepatocyte membranes and trigger compensatory will increase in cleansing enzymes [81]. The past researches indicated that extracts of Delonix regia had the ability to maintain hepatic architecture and lessen cellular damage by chemical toxicants [78,85]. The preservation of hepatocyte structural integrity and oxidative damages would allow the DRFE to normalize the enzyme turnover and avoid excess CYP450 expression. The other potential reason is an indirect metabolic feedback that is linked to decreased B[*a*]P bioactivation. When the constituents of DRFE inhibit the CYP1A1-mediated activation of B[*a*]P or increase alternative detoxification pathways such as conjugation reactions of enzymes such as NAD(P)H oxidoreductase (NQO1), UDP-glucuronosyltransferases (UGTs) or glutathione S-transferases (GSTs), this would prevent excessive accumulation of reactive metabolites and therefore reduce the metabolic load that triggers CYP450 upregulation (Shiizaki DRFE maximum concentration of 600 mg/kg had the greatest effect reducing the levels of CYP450 to 2.28 ± 0.11 nmol/mg protein. Even though this value was not fully restored to the normal level, the significant decrease shows that the extract is helpful to overcome the B[a]P-induced overexpression of enzymes. This is especially significant since the over activation of CYP1A enzymes during the process of B[*a*]P metabolism results in the generation of highly reactive intermediates that can cause the formation of DNA adducts and facilitation of carcinogenic events [81]. In general, the results prove that DRFE reduces B[a]P-induced CYP450 induction in dose-dependent manner, which is likely caused by a combination of antioxidant activity, regulation of the AhR -CYP1A signaling pathway, and hepatoprotective effect of its phytochemical constituents. The findings are in agreement with prior research findings that the hepatoprotective activity of Delonix regia of these chemicals under toxicity [78,85]. Additional studies in terms of analyzing gene expression of CYP1A1 and CYP1B1, AhR binding, and other indicators of oxidative stress would be useful in better understanding the exact molecular pathway by which DRFE is protective.

Table 5. Assessment of the ethanol extract from *Delonix regia* (poinciana) flowers (DRFE) on hepatic cytochrome p450 (CYP450) concentration in healthy and benzo[*a*]pyrene-hepatotoxic rats after four weeks of administration

Group	CYP450 (nmol/mg protein)	
	Means ±SD	Percent of change
G1: Normal control	1.72 ± 0.12 [d]	0.00
G2: Model control (Hepatotoxic)	3.59 ± 0.21 [a]	108.84
G3: DRFE (200 mg/kg bw)	3.33 ± 0.19 [a]	-7.24
G4: DRFE (400 mg/kg bw)	2.89 ± 0.09 [b]	-19.50
G5: DRFE (600 mg/kg bw)	2.28 ± 0.11 [c]	-36.49

All data are reported as the mean ±standard deviation (SD), with six animals per treatment group (n = 6). The key for the experimental groups, statistical analyses, percent of change calculation and abbreviations is provided below Table 1.

3.6. Effect of Delonix regia Flower Ethanol Extract on Serum Total Protein and Albumin in Benzo[a]pyrene-Induced Hepatotoxic Rats

Biochemical hepatic synthetic capacity and general liver functioning are well known as serum total protein and albumin. Since these proteins are majorly produced in hepatocytes, their plasma levels significantly decrease during hepatic trauma or hepatic dysfunction [86]. As shown in Table 6, exposure of benzo[*a*]pyrene had a significant negative effect in the hepatic protein synthesis as indicated by low levels of serum total protein and serum albumin. On the other hand, when treatment was done with ethanol extract of *Delonix regia* flowers (DRFE) these biochemical parameters were also improved gradually and progressively with an increase in dose and this shows that it has a protecting effect on the liver. In the group of hepatotoxic model (G2), the serum total protein dropped significantly to 51.89 ± 6.82 g/L as compared to 69.46 ± 3.90 g/L in the control group, a drop of about 25.30. In a similar manner, the levels of serum albumin reduced dramatically to 386.20 + 19.65 vs 602.92 + 19.65230 -1 in the control group, which is equivalent to a loss of 35.95. The present findings are in line with hepatotoxic effects of benzo[a]pyrene that are well documented and cause the onset of oxidative stress, hepatocyte injury, and the inhibition of protein biosynthesis in liver tissues [87,88]. Hepatocyte damage impairs the capacity of hepatocytes to produce plasma proteins, especially albumin which forms a significant portion of circulating serum proteins and is one of the most reliable indicators of hepatic functional integrity [89]. Further, oxidative stress induced when benzo[a]pyrene is metabolized facilitates the process of lipid peroxidation and structural damage of hepatocyte membranes, thus disrupting the metabolic activity and the ability to synthesize proteins [83]. These changes in the serum protein parameters were significantly enhanced by administration of DRFE in a dose-dependent fashion. In rats with 200 mg/kg body weight (G3), there was moderate elevation of the serum total protein that improved to 53.99 +/- 3.65g/L, which is a 4.05% increase over the hepatotoxic model group. Serum albumin also

rose to 419.56 +/- 22.21umol/l which is a 8.64 percent increment. It was further improved at the highest dose of 400 mg/kg (G4) with a total protein of 55.35 + 6.43 g/L and a corresponding albumin of 456.55 + 15.55 ml/L, which reflected an increment of 6.66 percent and 18.22 percent, respectively. Recovery was the most pronounced in rats receiving the highest dose of DRFE (600 mg/kg; G5), when serum total protein rose to 62.54 ± 4.31 g/L (20.52% improvement) and albumin was 512.80 ± 21.31 µmol/L (32.78% improvement). These findings reveal that there is a distinct dose-related hepatoprotective impact of the extract that implies that an increase in the concentrations of DRFE yields a greater successive reinstatement of hepatic synthetic action.

The positively noticed effect on serum protein level after the administration of DRFE can be explained by the fact that flowers of Delonix regia contain a range of bioactive phytochemicals, such as flavonoids, phenolic compounds, and other antioxidants. These substances are reported to have hepatoprotective effects by various biological processes, such as reactive oxygen species scavenging, increasing hepatocyte cell antioxidant defense mechanisms, stabilizing hepatocyte membranes, and proliferating damaged hepatocyte cell regeneration [88,90]. These phytochemicals prevent oxidative stresses and the damage of cells, thereby restoring normal increase and decrease in metabolism and synthesis of serum proteins and albumin in the hepatic tissues. As well, prior studies have found that extracts of *Delonix regia* contain significant antioxidant, anti-inflammatory and hepatoprotective activity. Indicatively, Ebada et al. [36] revealed that phytochemical elements of D. regia have good free-radical-scavenging properties that could prevent oxidative damage on hepatic tissues due to toxins. Equally, according to Ma et al. [91], plant-modified phenolic molecules can be used to stimulate hepatic synthesis of proteins and hepatocyte regeneration following a chemically induced liver damage. The albumin restoration found in the current study is especially important since albumin production is regarded as a sensitive indicator of the hepatocellular functional recovery and the indication of the normal liver metabolic activity restoration [86]. The additional reasonable mechanism that could be behind the hepatoprotective effect of DRFE is alteration of xenobiotic-metabolizing enzymes. The benzo[a]pyrene is metabolically activated by the cytochrome P450 enzymes to produce the reactive intermediates that are able to react with DNA and proteins to cause cellular toxicity and disruption in normal hepatic functions [87]. Plant extracts contain natural antioxidants that could inhibit excessive stimulation of these metabolic pathways or can boost detoxification protocols and hence heal hepatocellular injury and maintain the liver hepatic ability to produce proteins [83]. Generally, the results reveal that the ethanol extract of *Delonix regia* flower has inhibitory effects on the benzo[a]pyrene-induced hepatic dysfunction as evidenced by the fact that it restored the levels of serum total protein significantly and albumin. The dose-dependent effect is observed, which implies that the greater the concentration of the extract the more protection to hepatic tissues. These findings are in keeping with existing literature that identifies the hepatoprotective capabilities of plant-based antioxidants and indicates that flowers of the D. regia could be used as a prospective source of bioactive agents that can prevent toxin-induced liver toxicity and enhance hepatic functional integrity.

Table 6. Assessments of the ethanol extract from *Delonix regia* (poinciana) flowers (DRFE) on serum total protein and albumin in healthy and benzo[*a*]pyrene-hepatotoxic rats after four weeks of administration

Group	Serum total protein (TP, g/L)		Serum albumin (Alb, mol/L)	
	Means ±SD	Percent of change	Means ±SD	Percent of change
G1: Normal control	69.46 ± 3.90[a]	0.00	602.92 ± 19.65[a]	0.00
G2: Model control (Hepatotoxic)	51.89 ± 6.82[c]	-25.30	386.20 ± 19.65[d]	-35.95
G3: DRFE (200 mg/kg bw)	53.99 ± 3.65[c]	4.05	419.56 ± 22.21[d]	8.64
G4: DRFE (400 mg/kg bw)	55.35 ± 6.43[c]	6.66	456.55 ± 15.55[c]	18.22
G5: DRFE (600 mg/kg bw)	62.54 ± 4.31[b]	20.52	512.80 ± 21.31[b]	32.78

All data are reported as the mean ±standard deviation (SD), with six animals per treatment group (n = 6). The key for the experimental groups, statistical analyses, percent of change calculation and abbreviations is provided below Table 1.

3.7. Effect of Delonix regia Flower Ethanol Extract on Hepatic Glutathione Homeostasis in Benzo[a]pyrene-Induced Hepatotoxic Rats

Glutathione is one of the most significant intracellular antioxidant systems that provide the cellular redox balance and protect the hepatocytes against oxidative damage. The glutathione system primarily comprised of reduced glutathione (GSH) and oxidized glutathione (GSSG) and their ratio (GSH/GSSG), which is generally thought to be a sensitive indicator of oxidative stress and antioxidant defensive position in biological tissues [2]. Due to the central role in xenobiotics metabolism and detoxification, the liver harbors one of the highest levels of glutathione in the body and allows hepatocytes to react to reactive metabolites and treat redox homeostasis [92]. The findings that have been summarized in Table 7 indicate that the exposure to benzo[a]pyrene had a significant disruptive impact on the balance of glutathione in the liver, but a dose-dependent increase in the parameters related to glutathione was observed after exposure to the ethanol extract of the Delonix regia flowers (DRFE). The administration of Bphenyl[a]pyrene resulted in a severe depletion of the liver GSH contents with a subsequent rise in the GSSG concentrations (Table 7). The GSH in the group of hepatotoxic model decreased drastically by 8.79 low and high rates of 0.55 to 3.01 0.39 and the reduction with relation to normal control was 65.76. On the other hand, the GSSG level rose by 0.74 0.09 to 0.97 0.10 µmol/g wet tissue by 31.08. Due to these changes, the GSH/GSSG ratio was reduced significantly, by 73.88 (11.88: 3.10). Such drastic alteration of the reduced version of glutathione indicates great oxidative stress and loss of antioxidant defense mechanism in hepatic tissues.

These results are in line with previous reports that have shown that the metabolism of benzo[a]pyrene results in the production of reactive oxygen species (ROS) and electrophilic intermediates that quickly deplete intracellular GSH and induce its oxidation to the oxidized form [83,87]. Overproduction of ROS predetermines lipid peroxidation, oxidation of proteins, and dysfunction of mitochondria, all of which cause hepatocellular damage and failure of metabolic processes [93]. These changes in glutathione homeostasis in response to administration of DRFE were greatly alleviated. The antioxidant status of rats using 200mg body weight showed a moderate response, with hepatic GSH increasing to 3.57 ± 0.28 mmol/g wet tissue (18.60 percentage increment relative to the hepatotoxic model group), but GSSG levels reduced slightly to 0.91 ± 0.08 mmol/g wet tissue (6.19 percentage reduction). Consequently, the proportion of GSH/GSSG rose to 3.92 +.29, which is an improvement of 26.42 percent relative to the model control group. The subsequent addition of the dose to 400mg/kg led to an additional improvement of the glutathione status with GSH increasing to 4.64 ± 0.21 µmol/g wet tissue (54.15% increase) and GSSG dropping to 0.84 ± 0.05 µmol/g wet tissue (13.40% decrease). As a result, there was a significant increase in the GSH/GSSG ratio to 5.52 ± 0.40 or 78.01% increase compared to the hepatotoxic control. The largest increase was seen with the highest dose of DRFE (600mg /kg) with GSH level rising to 6.32 ug /g wet tissue (109.97%), and GSSG falling to 0.80 ug /g wet tissue (17.53%). This recovery led to a GSH/GSSG ratio of 7.90 but with an error margin of 0.55 which is an amazing recovery of 154.58 percent relative to the hepatotoxic model group.

The glutathione homeostasis enhancement after the administration of DRFE may be explained by the fact that *Delonix regia* flowers have strong antioxidant phytochemicals such as flavonoids, phenolic acids and other polyphenolic compounds. These compounds are also famous because they eliminate free radicals, pro-oxidant metal ions, and increase endogenous antioxidant defense mechanisms [88,90]. Besides having direct antioxidant activity, plant polyphenols can also have an indirect effect, which is to induce regeneration of GSH through an increase in the activity of glutathione reductase, the enzyme that converts oxidized glutathione (GSSG) into its reduced form, thereby restoring the intracellular redox balance [93]. One more possible mechanism that can play in the protective effect of DRFE concerns the alteration of the phase II detoxification pathways. Glutathione is an important part of conjugation that involves the detoxification of the electrophilic metabolites produced in the process of xenobiotic metabolism. Cytochrome P450 enzymes metabolically activate benzo [a]pyrene to generate reactive epoxide intermediates that are capable of covalently attaching to cell macromolecules and causing oxidative damage [87]. The greater access of reduced glutathione increases the detoxification of these intermediates in conjugation reactions catalyzed by glutathione-S-transferase and, as a result, the oxidative injury is reduced, and the structural and functional integrity of the hepatocyte are maintained (Lu, 2013). The current study is backed by the previous experimental studies that indicate that the plant extracts with high phenolic content can safely replace hepatic glutathione depletion and mitigate the effects of toxins that cause oxidative stress. Wu et al., [94] indicated that the induction of GSH production is one of the major defense mechanisms against oxidative hepatic damage. In the same vein, studies of medicinal plants with potent antioxidant activities have demonstrated that flavonoid extracts with high concentrations of flavonoids increase hepatic GSH levels greatly and also decrease GSSG levels in experimental settings due to chemically induced hepatotoxicity [93]. The observed dose-dependent effect of the current research thus indicates that DRFE is an effective way of improving the natural antioxidant system and improving the redox balance that is destroyed by the exposure to benzo[*a*]pyrene. Altogether, the current findings reveal that the ethanol extract of the flower of *Delonix regia* has a significant protective effect on the oxidative stress caused by benzo[a]pyrene on the hepatic tissues. Through the enhancement of reduced glutathione concentration, concentrations of oxidized glutathione, and the restoration of the GSH/GSSG ratio the extract is involved in the normalization of cellular redox homeostasis. These results indicate the possible therapeutic importance of D. regia flower extracts as a natural source of antioxidant molecules that could be used to overcome oxidative hepatic injury and increase levels of hepatic detoxification.

Table 7. Assessments of the ethanol extract from *Delonix regia* (poinciana) flowers (DRFE) on glutathione fractions in healthy and benzo[*a*]pyrene-hepatotoxic rats after four weeks of administration

Group	Reduced glutathione (GSH, µmol/g wet tissue)		Reduced glutathione (GSSG, µmol/g wet tissue)		GSH/GSSG ratio	
	Means ±SD	Percent of change	Means ±SD	Percent of change	Means ±SD	Percent of change
G1: Normal control	8.79 ± 0.55 ^{a}	0.00	0.74 ± 0.09 ^{c}	0.00	11.88 ± 1.12 ^{a}	0.00
G2: Model control (Hepatotoxic)	3.01 ± 0.39 ^{c}	-65.76	0.97 ± 0.10 ^{a}	31.08	3.10 ± 0.32 ^{c}	-73.88
G3: DRFE (200 mg/kg bw)	3.57 ± 0.28 ^{c}	18.60	0.91 ± 0.08 ^{ab}	-6.19	3.92 ± 0.29 ^{c}	26.42
G4: DRFE (400 mg/kg bw)	4.64 ± 0.21 ^{c}	54.15	0.84 ± 0.05 ^{b}	-13.40	5.52 ± 0.40 ^{bc}	78.01
G5: DRFE (600 mg/kg bw)	6.32 ± 0.42 ^{b}	109.97	0.80 ± 0.02 ^{bc}	-17.53	7.90 ±0.55 ^{b}	154.58

All data are reported as the mean ±standard deviation (SD), with six animals per treatment group (n = 6). The key for the experimental groups, statistical analyses, percent of change calculation and abbreviations is provided below Table 1.

3.8. Effect of Delonix regia Flower Ethanol Extract on Hepatic Reactive Oxygen Species and Lipid Peroxidation in Benzo[*a*]pyrene-Induced Hepatotoxic Rats

Oxidative stress has been well identified as being one of the major mechanisms of environmental toxicant and xenobiotics-induced liver injury. Overproduction of reactive oxygen species (ROS) causes disruption of the redox balance in the cells, harm to key biological macromolecules, and lipid peroxidation in the hepatocyte membranes [94]. As a result, the biomarkers of oxidative stress which include ROS and malondialdehyde (MDA) are widely applied to estimate the degree of hepatic oxidative damage. MDA, specifically, is a significant biomolecule end-product of lipid peroxidation and a valid measure of oxidative degradation of the polyunsaturated fatty acid in the biological membranes [95]. As shown in Table 8, the exposure of benzo[a]pyrene induced a significant change in the level of hepatic ROS and MDA, whereas the treatment with the Delonix regia flowers ethanol extract (DRFE) counteracted the changes in a dose-dependent pattern, indicating a protective antioxidant effect. This was a significant increase in hepatic levels of ROS induced by benzo[a]pyrene exposure relative to the normal control group (Table 8). ROS the level had gone up to 2.29 ± 0.09 nmoL/g wet tissue in the hepatotoxic model group, as compared to 0.67 ±0.08 nmoL/g wet tissue in the normal group, indicating by a margin of 241.79. This increase is an indication of extreme oxidative stress in livers in the wake of benzo[*a*]pyrene intoxication. The benzo[*a*]pyrene is the famous polycyclic aromatic hydrocarbon which can be metabolically activated mostly by the cytochrome P450 enzymes, in particular, CYP1A1 and CYP1B1, to produce highly reactive intermediates, i.e., epoxides and quinones [87]. In this metabolic process, there is the excessive generation of ROS that could overwhelm an intrinsic antioxidant defense mechanism and cause oxidative damage to hepatocytes [83]. An increase in ROS has the potential to disrupt mitochondria respiration, causing nucleic acid and protein damage, and inducing lipid peroxidation of cellular membranes, thus contributing to the death of hepatocellular damage and functional decline [72]. ROS hepatic was significantly lower in the administration of DRFE compared to hepatotoxic control group. The 200 mg/kg body weight resulted in a moderate decrease in which the ROS level had decreased to 1.92±0.30 nmol/g wet tissue (16.16% reduction). By doubling the dose to 400 mg/kg the reduction to 1.47±0.08 nmol/g wet tissue was increased to 35.81%. The greatest effect was observed with the greatest dose of DRFE (600 mg/kg) which lowered the levels of ROS in wet tissue to 0.98± 0.04 nmol/g, which is a 57.21% reduction of the hepatotoxic model group. These results clearly demonstrate that DRFE has a good antioxidant activity that can inhibit the excessive production of ROS in liver tissues of benzo[a]pyrene. The decrease in the levels of ROS with the administration of DRFE could be explained by the phytochemical content of *Delonix regia* flowers which could have different antioxidants such as flavonoid, phenolic acid, tannin, and other polyphenolic substances. These bioactive compounds have strong free radical-scavenging properties and they are able to cancel reactive oxygen species before they cause oxidative chain reactions that cause damage to cellular components [90]. Furthermore, polyphenols contained in plants have been found to augment endogenous antioxidant protective systems by activating enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) to increase the capacity of the cells in detoxifying reactive oxygen species and redox homeostasis [88,93].

Alongside the rise in ROS, the exposure of benzo[*a*]pyrene also greatly stimulated lipid peroxidation in hepatic tissues which was evident by the high levels of MDA present in the hepatotoxic group. In the normal control group, the MDA concentration rose to 569. 25± 22.12 nmol/g wet tissue and in the model control group, the MDA concentration rose to 972.69±34.09 nmol/g wet tissue, which represents an increase of about 70.87. This increase points to the widespread oxidative destruction of lipids of hepatocyte membranes. The pathway of benzo[*a*]pyrene toxicity that is well-established is consistent with the mechanism of this increase, with ROS produced during the metabolic activation targeting polyunsaturated fatty acids found in the cellular membranes, which induces lipid peroxidation and the formation of secondary products such as MDA [87,95]. Lipid peroxidation undermines the membrane integrity, changes the membrane fluidity, and disrupts the activity of membrane-bound proteins and receptors, eventually causing the destruction and necrosis of cells [93]. DRFE reduced the hepatic MDA levels by a significant margin over the hepatotoxic control group, which suggested a protective effect of it against lipid peroxidation. The level of MDA dropped to 862.69±26.81 nmol/g wet tissue in rats treated to 200mg/kg DRFE, which is an 11.31% decrease. It was noted that in the group that received 400 mg/kg of the drug, there was a larger reduction in the levels of MDA to 752.14 ±18.98 nmol/g wet tissue (22.67 decrease). The highest dose of DRFE (600 mg/kg) was the most protective treatment that lowered the level of MDA to 615.28 ± 26.12 nmol/g wet tissue equivalent to a 36.74% reduction as compared to the hepatotoxic control group. The findings suggest that DRFE is a good inhibitor of lipid peroxidation in the hepatic tissues under oxidative stress. The antioxidant effect of the phenolic constituents in DRFE could be the reason behind the protective effect of this polymer against lipid peroxidation. Flavonoids and other polyphenolic compounds can give hydrogen atoms or electrons to lipid radicals and thus put an end to the propagation stage of lipid peroxidation and inhibit any subsequent oxidative destruction of cellular membranes [90]. Besides, they can also promote the production of intracellular glutathione and the activation of detoxification enzymes of xenobiotic metabolism, which also helps reduce the oxidative stress and maintain the integrity of hepatocytes [92]. Taken together, the results, which were obtained in Table 8, provide evidence that exposure to benzo[*a*]pyrene triggers a serious oxidative stress in liver tissues, as was evidenced by the high increases in the levels of ROS and MDA. Nevertheless, these changes were counteracted successfully by the administration of the ethanol extract of *Delonix regia*

flowers to decrease the formation of reactive oxygen species and the lipid peroxidation. The dose-dependent protective effect was observed to indicate that an increase in concentration of DRFE gives stronger antioxidant capacity. These findings are in line with the past reports that show the hepatoprotective nature of medicinal plants that are high in polyphenolic compounds. Thus, a potential source of natural hepatoprotective antioxidants that can counter the oxidative stress due to environmental pollutants and chemical toxicants could be the Delonix regia flower extract.

4. Conclusion

The current study has shown that benzo[*a*]pyrene (B[*a*]P) causes severe hepatotoxicity by causing a decrease in body weight, feed intake, feed efficiency ratio, liver weight, and a significant alteration in liver dysfunction as measured by the increase in serum liver enzymes (AST, ALT, ALP), total and direct bilirubin, and total protein synthesis in the liver as indicated by a reduction in the serum total protein and albumin. Hepatic antioxidant defense mechanisms were also impaired by B[a]P which led to the depletion of reduced glutathione (GSH), oxidized glutathione (GSSG) accumulation, accumulation of reactive oxygen species (ROS), and malondialdehyde (MDA) finding, which is a strong confirmation of severe oxidative stress, lipid peroxidation and hepatocellular damage. Moreover, the activity of hepatic cytochrome P450 was also strongly induced to increase bioactivation of B[a]P and oxidative damage. Toxic effects of the plant were successfully addressed with the ethanol extract of the *Delonix regia* flower (DRFE) in a dose-dependent manner. DRFE increased body weight, feed intake, feed efficiency, and liver weight, and decreased serum liver enzyme and bilirubin concentration significantly, which is evidence of stabilization of hepatocyte membranes and improved hepatic function. Both the level of serum total protein and albumin were elevated indicating a restoration of the synthetic capacity of the liver. At the molecular scale, DRFE increased GSH and lowered GSSG and the GSH/GSSG ratio and reduced ROS and MDA, and partially returned cytochrome P450 activity, which indicated a reduced B[a]P bioactivation. This is due to the antioxidant, anti-inflammatory, and membrane-stabilizing properties of the phytochemical composition of Delonix regia flowers, which are flavonoids (quercetin, quercitrin, isoquercitrin, rutin), phenolic acids, tannins, sterols, and triterpenoids. Conclusively, DRFE also has a good hepatoprotective activity in liver toxicity induced by B[*a*]P, and the activity increases with increase in dosage that is needed to restore the hepatic structure and function. The periodsical use of Delonix regia flower preparations could be used as a complementary approach to reduce the exposure to hepatotoxins, and more research should be undertaken to explore the finer details of the molecular processes, the dosage, and the safety of the preparation in the long term to be used in humans.

Table 8. Assessments of the ethanol extract from *Delonix regia* (poinciana) flowers (DRFE) on hepatic malonaldehyde and reactive oxygen species in healthy and benzo[*a*]pyrene-hepatotoxic rats after four weeks of administration

Group	Reactive oxygen species (ROS, nmol/g wet tissue)		Malonaldehyde (MDA, nmol/g wet tissue)	
	Means ±SD	Percent of change	Means ±SD	Percent of change
G1: Normal control	0.67 ± 0.08 ^{d}	0.00	569.25 ± 22.12 ^{d}	0.00
G2: Model control (Hepatotoxic)	2.29 ± 0.09 ^{a}	241.79	972.69 ± 34.09 ^{a}	70.87
G3: DRFE (200 mg/kg bw)	1.92 ± 0.30 ^{ab}	-16.16	862.69 ± 26.81 ^{b}	-11.31
G4: DRFE (400 mg/kg bw)	1.47 ± 0.08 ^{bc}	-35.81	752.14 ± 18.98 ^{c}	-22.67
G5: DRFE (600 mg/kg bw)	0.98 ± 0.04 ^{cd}	-57.21	615.28 ± 26.12 ^{d}	-36.74

All data are reported as the mean ±standard deviation (SD), with six animals per treatment group (n = 6). The key for the experimental groups, statistical analyses, percent of change calculation and abbreviations is provided below Table 1.

Author Contributions

The study protocol has been proposed and improved by Yousif Elhassaneen, who then followed up on the laboratory experimental part, retrieved conceptual information, reviewed and validated the results and statistical analyses, prepared a draft of the manuscript, critically reviewed to intellectually organize the content and to allow publication of the final version of the manuscript. The laboratory experiments, collection, tabulation, analysis and interpretation of the results and participation in retrieving conceptual information and writing the draft of the manuscript were done by Hagar Soliman. Seham Aziz assisted in the proposal of the study protocol, finding conceptual information, validating the study findings and in writing the draft manuscript.

Conflicts of Interest

The authors do not declare any conflicts of interests. Acknowledgments The authors are grateful to the agricultural workers who were working along the Banha Shibin El Kom road, the Governorate of Menoufia, Egypt, and helped in the collection of the Poinciana flower samples.

Abbreviations

Alb, albumin, ALT, Alanine aminotransferase; ALP,

alkaline phosphatase, AST; aspartate aminotransferase; B[a]P, benzo[a]pyrene; BD, basal diet; BWG, body weight gain, CYP450, cytochrome P450. DREE, Poinciana (*Delonix regia*) ethanol extract, FI, feed intake, FER, feed efficiency ratio, GSH, reduced glutathione; GSSG, oxidized glutathione, MDA: malondialdehyde; ROS: reactive oxygen species, TP, total protein.

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