

Possible Protective Roles of Poinciana (*Delonix regia*) Seeds Against Carbon Tetrachloride-induced Biochemical and Histological Disorders in Rat Liver

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Abstract The present study aims to investigate the possible protective roles of poinciana (*Delonix regia*) seeds powder (DRSP) against carbon tetrachloride-induced biochemical and histological disorders in rat liver. Also, minerals, bioactive compounds content and antioxidant activity of the DRSP will be in the scope of this investigation. Proximate chemical composition of DRSP indicated that carbohydrates was the largest compound (50.49 ± 0.91 g.100g⁻¹) followed by crude fat (16.91 ± 1.06 g.100g⁻¹), crude fiber (13.73 ± 3.27 g.100g⁻¹), total protein (9.04 ± 0.43 g.100g⁻¹) and ash (1.25 ± 0.19 g.100g⁻¹). Also, DRSP is rich in different estimated minerals and the trend of the abundance in decreasing order is as follows Na> Zn> Fe> Mn>Cu>K>Mg>P>Ca>Se. Bioactive compounds content in DRSP indicated that polysaccharides were the largest compound followed by polyphenols, saponin, oxalate, tannin, carotenoids, and kaempferol. DRSP exhibited good antioxidant activity which compares well with the standard antioxidants. On the other side, biological experiments indicated that Injected of normal rats carbon tetrachloride, CCl₄, (model control) exhibited significantly ($p \leq 0.05$) decreased in body weight (BW, -48.35%), feed intake (FI, -34.31%) and feed efficiency ratio (FER, -32.88%) compared to the normal group. However, intervention with DRSP (5, 10, 15 and 20 g/100g diet) in feeding rats for 4 weeks led to significantly ($p \leq 0.05$) decrease on these parameters of the hepatotoxic rats (model control) by different rates. Also, Intervention with DRSP in feeding rats led to significantly ($p \leq 0.05$) enhancing in the serum lipid profile parameters, minimizing the liver functions and positively manipulates the CCl₄-related histopathological changes in liver of the hepatotoxic rats. These findings provide a basis for the use of DRSP for the prevention and treatment of hepatotoxicity. Data also supports the benefits of diet modification and DRSP supplementation in alleviating disorders associated with liver chemical pollutants, including CCl₄.

Keywords: minerals, bioactive compounds, antioxidant activity, liver functions, serum lipid profile, serum total protein, albumin

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1. Introduction

Liver is one of the most important organs in all vertebrates, acting as the body's metabolic engine room. It is involved in several vital functions such as carbohydrate, protein and fat metabolism, processes many of the products (glucose, plasma proteins and urea) that are released into the blood stream, makes some of the clotting factors needed to stop bleeding from acute or injury, and secretes bile into the intestine to help absorb nutrient, stored several products (glycogen, fat and vitamins) in the parenchymal cells [1]. Also, liver helps the body to fight against infection, and plays a very important and principal part in the xenobiotic biotransformation and detoxification process [2,3,4,5,6]. For these and other functions, the liver

is characterized by an enormous functional reserve, which often masks the clinical impacts of early damage to this organ. However, as the disease progresses, especially bile flow disorder, the consequences of liver damage can easily become life-threatening [7].

Liver diseases are among the most serious disorders. They may classified as acute or chronic hep (atitis, hepatosis and cirrhosis). The liver diseases are mainly caused by ubiquitous toxic chemicals including certain antibiotics, chemotherapeutics, peroxidized oils, aflatoxin, carbon tetrachloride, polycyclic aromatic hydrocarbons, pesticides, etc. [2,8,9]. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damage in liver [9]. Also, liver disease has many causes including parasites and viruses, immune system abnormality, genetics, cancer and other growths, chronic alcohol abuse, and fat accumulation in the liver

(nonalcoholic fatty liver disease [10]. Thus, liver diseases have become one of the major health problems facing various peoples of the world, especially in developing countries. It accounts for approximately 2 million deaths per year worldwide, one million due to complications of cirrhosis and one million due to viral hepatitis and hepatocellular carcinoma [11]. Additionally, number of serious complications can develop in liver disease including portal hypertension, varices, ascites, hepatic encephalopathy, infection and liver cancer. For these and other complications, liver disease has represented the most challenging health care problems worldwide which prompted various universities and research centers to innovate a lot of chemotherapy treatments. Over many decades, chemotherapy has been used to treat liver disease patients but this has been associated with many side effects and large financial cost which often leads to patient non-compliance [12]. Hence, there was a need to explore alternative treatments especially from natural sources that are cost-effective and have limited side effects. With this context, our several studies have been used different plant parts, contains huge bioactive compounds and exhibited different biological activities, in the preventive/curative studies of the liver disease [4,6,9] [13,14,15,16,17,18,19,20]. All these studies and others gave encouraging results, which led to the continuation of this direction of research, and our choice in this study fell on the seeds of poinciana (*Delonix regia*).



Figure 1. Poinciana (*Delonix regia*) tree in Mansoura University campus, Mansoura City, Dakhliya Governorate, Egypt . A, shoot system, A, flowering plant, C, seed pods on tree, D, seed pods, E, seed pods with seeds, F, seeds.

Poinciana (*Delonix regia*) is a leguminous plant of the family *Fabaceae*. The plant is widely grown as an ornamental or agroforestry tree all over the world including Egypt (<https://tropical.theferns.info/viewtropical.php?id=Delonix+regia>). Morphological description of *Delonix regia* is shown in Figure 1. It is erect unarmed tree. Leaves are abruptly bipinnate, leaflets are many but small and stipules are small. Flowers are large, showy, in terminal corymbs, bracts small. Calyx tube is very short, 5 lobes, valvate and subequal. Petals are 5, orbicular, imbricate, clawed, subequal. Margins are fimbriate. Stamens are 10 free, declinate, longexserted. Filaments are villous below and anthers are uniform. Ovary is subsessile, many ovuled. Style filiform and stigma truncate, ciliolate. Seed pods are elongate, flat, woody and dehiscent. Seeds are transverse and oblong [21]. *Delonix regia* has various medicinal and traditional

properties and various parts of this plant is used to treat different disorders. Leaves is traditionally used in treating gastric problems, rheumatic pains of joints. Further the leaves are reported for its antimicrobial, anti-inflammatory and anti-diabetic activities. Flowers were used in diarrhea and for curing chronic fever [22]. Also, it was noted to possess antibacterial activity [23]. Leaves and flowers extracts have an effective protective effects against the deteriorating effects on the liver caused by CCl_4 (17; 69). Furthermore, the bark was used as traditional fever remedy [24]. For seeds, *Delonix regia* produces large quantities of seed pods and seeds during the fruiting season, which at present are not utilized [25]. There has not been any documented tonnage of seeds produced annually, possibly because there has not been any reported commercial value. These seeds rot and are wasted because they are not put into use. Therefore, this study was conducted with the aim of exploring some aspects related to the potential hepatoprotective activity of *Delonix regia* seeds powder (DRSP) against carbon tetrachloride intoxication in rat liver. Also, to explain the previous possible roles, the proximate composition, bioactive compounds content and antioxidant activity of these seeds powder will be in the scope of this investigation.

2. Materials and Methods

2.1. Materials

2.1.1. *Delonix Regia* Seeds

Dried and matured fruit pods used for seeds preparation were obtained from of *Delonix regia* trees spread at Mansoura University, Mansoura, Egypt after the grateful facilities provided to the authors. Taxonomic confirmation of *Delonix regia* pods were carried out by Agricultural Plant Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt.

2.1.2. Chemicals and Kits

Carbon tetrachloride (CCl_4), as 10% liquid solution, was obtained from ElGhohorya Company for Trading Drugs, Chemicals and Medical Suppliers, Cairo Egypt. Kit's assays for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), malondialdehyde (MDA) were purchased from BIODIAGNOSTIC, Dokki, Giza, Egypt. Albumin (Alb), total protein (TP), triglycerides (TGs), cholesterol (Cho), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined using kits purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. TNF- α was assayed by kit was provided by Nawah Scientific, Almokattam, Cairo, Egypt. GSH and GSSG were assayed by the kits provided by MyBioSource, Inc., San Diego, CA, USA. Casein was obtained from Morgan Chemical Co., Cairo, Egypt. All other chemicals and solvents used were of analytical grade were purchased from ElGhohorya Company for Trading Drugs, Chemicals and Medical Suppliers, Cairo Egypt.

2.1.3. Machines

Throughout this study absorbance for different assays

were measured using UV-160A; Shimadzu Corporation, Kyoto, Japan. Also, minerals determined using of atomic absorption spectrophotometer (Perkin – Elmer, Model 2380, Waltham, MA, USA).

2.2. Methods

2.2.1. Preparation of *Delonix Regia* Seeds Powder (DRSP)

Seeds of *Delonix regia* were extracted from the fruits/pods manually using special sharp knives for this purpose. The collected seeds were manually sorted to exclude damaged and deformed ones, as well as foreign bodies. Seeds were dried in oven at 60°C for one hour and ground into a fine powder in high mixer speed (Moulinex Egypt, Al-Araby Co., Egypt). The material that passed through an 80 mesh sieve was retained for use.

2.2.2. Proximate Composition of DRSP

DRSP samples were analyzed for proximate composition including moisture (using oven method at 105°C for 4 h), protein (T.N. $\times$ 6.25, using Kjeldahl method through oxidation, distillation, and titration, semiautomatic apparatus, Velp company, Italy), fat (soxhelt semiautomatic apparatus Velp company, Italy, petroleum ether solvent), ash (dry ashing method, muffle furnace at 600°C up to material becoming ash), and fibers contents were determined using the official methods of analysis of the Association of Official Analytical Chemists [26]. Carbohydrates calculated by difference using the following formula: Carbohydrates (%) = 100 – (% protein + % fat + % Ash + % fiber).

2.2.3. Minerals

Minerals content of DRSP samples were prepared and determined according to the method mentioned by Singh et al., [27]. In brief, 0.5 g of defatted sample i.e. left behind for lipid estimation were transferred into a digested glass tube and 6 ml of tri-acids mixture (containing nitric acid: perchloric acid: sulfuric acid in the ratio of 20 : 4 : 1 v/v respectively) were added to each tube. The tubes content were digested gradually as follow, 30 min at 70°C; 30 min at 180 °C and 30 min at 220°C. After digestion i.e. until the mixture becomes colorless, the mixture was cooled, dissolved in distilled water, and the volume was increased to 50 ml in volumetric beaker. The mixture samples were filtration in ashless filter paper and aliquots were analyzed for minerals (Na, Zn, Fe, Mn, Cu, K, Mg, P, Ca, Se) using of atomic absorption spectrophotometer.

2.2.4. Bioactive Compounds Determination in DRSP

Total phenolics in DRSP were determined using Folin-Ciocalteu reagent according to Singleton and Rossi, [28] and Wolfe et al., [29]. Gallic acid and equivalents are used to express the results (GAE). The total carotenoids were determined by using the method reported by Litchenthaler, [30]. Tannins were determined by the method of Van-Burden and Robinson, [31]. Gallic acid (GA) was used as a standard to draw the standard curve, from which the tannins content were estimated. Alkaloids were detected according to the method of Harbome, [32]. Saponin

content was determined according to the method of Fenwick and OakenfuI1, [33]. Gallic acid was used as a standard to establish the standard curve, from which the saponin content of sample was determined. Oxalate was determined such as described by Oke, [34]. Kaempferol was measured according to the method mentioned in Fouda et al., [35]. Total polysaccharides were measured according to the method of Vazirian et al., [36] and were expressed as mg of starch equivalents.

2.2.5. Antioxidant Activity Determination

Antioxidant activity (AA) of DRSP and standards (α -tocopherol and butalated hydroxytoluene, BHT) was determined according to the β -carotene bleaching assay following the procedure described by Marco, [37].

2.3. Biological Experiments

2.3.1. Animals

Animals used in this study, adult male albino rats (160 $\pm$ 8.56g per each) were obtained from were obtained from the Laboratory Animal Unit, College of Veterinary Medicine, Cairo University, Egypt.

2.3.2. Basal diet (BD)

The basic diet prepared according to the following formula as mentioned by Reeves et al., [38] as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamins and minerals mixture component were formulated according to Reeves et al., [38].

2.3.3. Induction of Hepatotoxicant in Rats

Thirty six male albino rats were administrated by intraperitoneal (IP) injection of carbon tetrachloride (CCl₄) in olive oil, 50% V/V (2 ml/kg bwt), twice a week for two weeks to induce chronic damage of the liver according to the method described by Jayasekhar et al., [39]. Liver intoxication was confirmed by taking a random sample of experimental animals (4 rats) and biochemical (liver functions) examined.

2.3.4. Experimental Design

All biological experiments were performed in a complying with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council [40]. Rats (n=36 rats) were housed individually in wire cages in a room maintained at 25 $\pm$ 2°C, relative humidity (55 $\pm$ 3%), a 12-hr lighting cycle and kept under normal healthy conditions. All rats were fed on basal diet (BD) for two-week before starting the experiment for acclimation. After two week period, the rats were divided into main groups. First group (6 rats), as a negative control group, fed on BD and injected with olive oil (5 ml/kg body weight) which was used as a vehicle for the treatment of animals in CCl₄ group. Second main group (30 rats) was injected with CCl₄ to induce liver impaired rats then classified into sex equal sub groups as follow: group (2), as a positive control group, fed on BD and groups (3-6) fed on BD containing 5, 10,

15 and 20% (w/w) *Delonix regia* seed powder (DRSP), respectively.

2.3.5. Biological Evaluation

During the experimental period (28 days), the diet consumed was recorded every day and body weight was recorded every week. The body weight gain (BWG,%), food intake (FI) and food efficiency ratio (FER) were determined according to Chapman et al., [41] using the following equations: BWG (%)=(Final weight – Initial weight)/ Initial weight x100 and FER= Grams gain in body weight (g/28 day)/ Grams feed intake (g/28 day).

2.3.6. Blood Sampling

At the end of the 4-week experimental period, blood samples were collected after 12 h of fasting from the abdominal aorta after rats were anesthetized with ether. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum such as explained by Stroeve and Makarova, [42]. Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20°C until analysis. Specimens of the liver organ were taken immediately after sacrificing rats and immersed in 10% neutral buffered formalin for the histological examination.

2.3.7. Hematological Analysis

2.3.7.1. Liver Functions

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were measured in serum using the modified kinetic method of Yound, [43] and Tietz, [44], respectively. Alkaline phosphatase (ALP) activity was determined using modified kinetic method of Vassault et al., [45].

2.3.7.2. Serum Total Protein and Albumin

Serum total protein and albumin was determined by a colorimetric method described by Zheng et al., [46] and Vanessa et al., [47], respectively.

2.3.7.3. Serum Lipid Profile

Triglycerides (TGs), Total cholesterol (Cho), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were determined in serum according to the methods of Fossati and Prencipe, [48], Richmond, [49], Lopes-Virella et al., [50] and Islam et al., [51].

2.3.7.4. Complete Blood Count (CBC)

CBC including [hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), and platelets] were performed by automatic measurement using an Avantor Performance Materials Inc. Business, Center Valley, USA (H32 VET 3-Part differential analyzer of hematology).

2.3.8. Histopathological Examination

Specimens of liver were taken immediately after sacrificing rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned (4-6 µm

thickness), stained with hematoxylin and eosin and examined microscopically [52].

2.4. Statistical Analysis

All data were statistically analyzed using a computerized Costat program by one way ANOVA. Results were given as means $\pm$ standard deviation (SD). Differences between treatments at $P \leq 0.05$ were considered significant [53].

3. Results and Discussion

3.1. Proximate Composition, Minerals, Antinutritional Compounds Content and Antioxidant Activity of *Delonix Regia* Seed Powder (DRSP)

3.1.1. Proximate Composition

Table 1. Proximate composition of *Delonix regia* seed powder (DRSP, g.100g⁻¹)

Component	Range	Amount (n=3)
Moisture	8.03 – 10.43	9.56 $\pm$ 0.76
Total protein	8.17- 9.97	9.04 $\pm$ 0.43
Crude fat	14.56 – 17.54	16.91 $\pm$ 1.06
Ash	1.14 – 1.53	1.25 $\pm$ 0.19
Crude fiber	11.05 – 17.09	13.73 $\pm$ 3.27
Carbohydrate	49.03 – 51.61	50.49 $\pm$ 0.91

Proximate composition of *Delonix regia* seeds powder (DRSP) is presented in Table 1. From such data it could be noticed that carbohydrates was the largest compound (50.49 $\pm$ 0.91 g.100g⁻¹) followed by crude fat (16.91 $\pm$ 1.06 g.100g⁻¹), crude fiber (13.73 $\pm$ 3.27 g.100g⁻¹), total protein (9.04 $\pm$ 0.43 g.100g⁻¹) and ash (1.25 $\pm$ 0.19 g.100g⁻¹). Such data are in partially accordance with Oyedele et al., [54] who found that moisture, crude fibre, ash, crude fat, crude protein and carbohydrate in *Delonix regia* seeds were 10.12 $\pm$ 0.59, 14.6 $\pm$ 0.44, 1.03 $\pm$ 0.02, 17.16 $\pm$ 0.15, 8.75 $\pm$ 0.04 and 48.34 %, respectively. Also, these results did not agree with what was reported in several studies [24,25]. All of these previous studies, along with others, concluded that the variation observed in the chemical composition of the *Delonix regia* seeds could be due to one or combination of the following factors including, species, geographical location, soil conditions and around residents' activities. In general, the low moisture content recorded in *Delonix regia* seeds is an indicator that the seeds maynot support the growth of microorganisms and thus keep it without spoilage i.e. microbial growth for a long period of time. Such observation was confirmed by Azeez et al., [55] and Fagbohun et al., [56]. Also, the high fat content of the *Delonix regia* seed indicates that it could be a potential source of oil for different purpose such human nutrition, animal feeding and biodiesel but such a matter requires many future studies. Furthermore, the ash and protein contents of *Delonix regia* seed show that they are better sources of dietary minerals and protein especially when compared to the seeds of some other trees [57]. The same behavior was recorded for carbohydrates

content of the seeds. In this direction, Coimbra, and Jorge, [58] reported that *Delonix regia* seeds are rich in carbohydrate because it meets recommended dietary values of 40 % for children and adults.

3.1.2. Minerals Content

Data in Table 2 indicated the minerals content of *Delonix regia* seed powder (DRSP). Such data indicated that DRSP is rich in different estimated elements. The trend of the abundance of mineral concentration in the DRSP in decreasing order is as follows Na> Zn> Fe> Mn>Cu>K>Mg>P>Ca>Se. Data of the present study are in accordance with that obtained by Oyedele et al., [54]. and partially accordance with Amata and Nwagu, [24]. In general, there is a general public interest in the availability of both macro and micro elements in the foods consumed daily. Such elements are necessary for normal physiological function, the deficiency of which causes serious metabolic abnormalities and the increase of which leads to toxicity [59]. Their presence of determined elements in DRSP shows it is a good source for essential minerals in particular the micro/trace elements. Trace elements are biologically very significant to the human through prevention and/or fighting several diseases including anemia, immunodeficiency, cancer, atherosclerosis and heart diseases [60,61]. Zn is vital in several biological roles includes the cell growth, division and maturation, cell membrane stabilization, and in DNA and RNA synthesis [62,63]. Fe plays the main role as an integral part of hemoglobin in red blood cells i.e. the transfer of oxygen from the lungs to the tissues of all organs in the body, necessary for DNA synthesis, and plays important function in the human immune system [64]. Mn has an important role in the metabolism of lipids and lipoproteins and it participates in the pathogenesis of atherosclerosis and heart diseases [65]. Cu has an important part in the process of erythropoiesis, maturation, signal-mediated activity of immune cells, contributes to iron resorption in the digestive tract, catalyzes hemoglobin biosynthesis, and helping to incorporate heme iron [66]. Se has attracted attention because of its antioxidant properties which fight cell damage that may worsen brain and nervous system diseases like Parkinson's, Alzheimer's, and multiple sclerosis. Also, this cell damage, called oxidative stress, is linked to cancer, heart disease, and the decline of mental skills [59] [67]. On the other side, DRSP recorded low levels of macro elements including Ca, Mg and P subsequently may not be a good source of minerals for bone formation. Such observation was reported by Coimbra and Jorge, [58] and Zhou and Han, [68]. In line with this context, the following can be noted in the present study Na/K ratio for DRSP is 128.05. According to the study of Zhou and Han, [68] when the ratio of Na/K is higher than one in diet, such diets have been linked with increased risk of hypertension and heart disease-related mortality. To address this, providing the required Na/K ratio for DRSP must to be supplemented with other nutrients rich in K. Also, DRSP analyzed for minerals have Ca/P ratio of 0.4. According to the study of Cockell et al, [69] when the ratio of Ca/P is higher than one in diet, such diet is considered a good source of minerals for bone formation but with a ratio less than 0.5 is considered a poor source. To address this, providing the required Ca/P

ratio for DRSP must to be complemented with other food items rich in P.

Table 2. Minerals content of *Delonix regia* seed powder (DRSP, mg.100g⁻¹ dry weight)

Mineral	Range	Mean $\pm$ SD (n=3)
K	9.56 - 13.09	11.67 $\pm$ 0.98
Na	1499 – 1582	1520 $\pm$ 19.00
Mg	4.89 - 6.67	5.74 $\pm$ 0.59
Zn	1001 – 1032	1008 $\pm$ 15.00
Fe	972 – 995	988 $\pm$ 10.00
Cu	107.50 - 112.35	109 $\pm$ 2.98
Mn	204.12 – 218.27	212 $\pm$ 7.04
Ca	2.02 – 2.53	2.21 $\pm$ 0.09
Se	0.19 – 0.44	0.32 $\pm$ 0.18
P	4.08 – 5.89	5.03 $\pm$ 0.67

3.1.3. Antinutrients/Bioactive Compounds Contents

Antinutrients/bioactive compounds contents in *Delonix regia* seed powder (DRSP). were shown in Table 3. Such data indicated that polysaccharides were the largest compound followed by polyphenols, saponin, oxalate, tannin, carotenoids, and kaempferol. Data of the present study are in accordance with that obtained by Oyedele et al., [54]. for oxalate, tannins and saponin but not go along with Amata and Nwagu., [24] with regard to oxalate content. In general, polysaccharides play significant roles in food processing applications through using as thickening and gelling agents, and emulsion stabilizers [70,71]. Also, they exhibited different biological activities such anticoagulant, anti- inflammatory, anti-obesity, anti-osteoporosis, antioxidant and antimicrobial activities [72,73,74,75,76,77]. Furthermore, polysaccharides absorb cholesterol which are then eliminated from the digestive system i.e. hypocholesterolemic and hypolipidemic agents [78,79]. Oxalates are natural compounds found in vegetables, fruits, nuts, and grains. Some examples of foods that are higher in oxalates include green leafy vegetables, soy, almonds, potatoes, tea, rhubarb, cereal grains and beets than DRSP. They are also naturally created in the human body as a waste product. Oxalate has been implicated in the formation of kidney stones and a decrease in calcium absorption [80]. Thus, consumption of seeds of low oxalate content such as DRSP may not induce any of these implications. For the other bioactive compounds found in DRSP, Phenolics and carotenoids, they are playing several important biological roles including antioxidant and scavenging activities and inhibiting the low density lipoprotein oxidation [15] [77] [81,84].

Tannins are water-soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals [85]. Also, Lewu et al., [86] found that tannins complex proteins resulting in the reduction of protein digestibility and palatability. In the present study, tannin content in DRSP is lower than the critical value of 9.0 mg g⁻¹ that could induce tannin toxicity [87]. Kaempferol is a flavonoid phytoestrogen found in yellow fruits, broccoli, and grapes. It reduces the risk of chronic diseases, especially cancer, augments human body's antioxidant defense against free radicals,

and modulates apoptosis, angiogenesis, inflammation and metastasis [88]. Finally, saponins, triterpene glycosides, are bitter-tasting usually toxic plant-derived organic chemicals that have a foamy quality when agitated in water [89]. It has been reported that saponin possess chemopreventive roles such lowers blood cholesterol, inhibits the growth of cancerous cells and enhances immune system [86,90]. Thus, the presence of saponins in DRSP can provide a therapeutic effect, and the low content of saponin in these seeds shows that they may not pose any danger when consumed.

Table 3. Bioactive constituents of *Delonix regia* seed powder (DRSP)

Component	Range	Mean $\pm$ SD (n=3)
Polyphenols (mg gallic acid equivalent. g ⁻¹)	23.45 – 28.56	26.07 $\pm$ 3.04
Oxalate (mg .g ⁻¹)	2.33 – 2.79	2.69 $\pm$ 0.23
Carotenoids (mg .g ⁻¹)	0.05- 0.06	0.06 $\pm$ 0.01
Tannin (mg catechine equivalent. g ⁻¹)	1.27 - 1.46	1.39 $\pm$ 0.18
Polysaccharides (mg starch. g ⁻¹)	66.09 – 73.14	69.23 $\pm$ 4.92
Kaempferol (mg. g ⁻¹)	0.04 – 0.05	0.05 $\pm$ 0.00
Saponin (mg .g ⁻¹)	2.94 – 3.11	3.02 $\pm$ 0.16

3.1.4. Antioxidant Activity

Data in Table 4 indicated the antioxidant activity of *Delonix regia* seed powder (DRSP) and references/standards antioxidants was assayed by β -carotene bleaching (BCB). Such data indicated that DRSP recorded the antioxidant activity equal 62.56 $\pm$ 1.19% which compares well with the references/standards antioxidants used i.e. BHT (50 mg/ml), BHT (100 mg/ml) and α -tocopherol (50 mg/ml) by 70.73 $\pm$ 1.37, 65.81 $\pm$ 1.66 and 65.06 $\pm$ 1.03%, respectively. Several previous studies confirmed that the BCB method have been used successively to evaluate the antioxidant activity in different plant parts including seeds *in vitro* [20] [76,77] [81,83] [91-94]. Almost of these studies reported that polyphenols, tannins, carotenoids, and polysaccharides content, such as found in a highly content in DRSP, and antioxidant activity are highly correlated. Also, antioxidant activity of the leaves, bark, and flowers of *Delonix regia* have been evaluated by Shabir et al., [95] using different *in vitro* methods, viz. reducing power assay and DPPH assay, and reported that leaves extract exhibited more reducing power then bark and flowers. The antioxidant activities of the extracts were significantly correlated with their total phenolic content and total flavonoid contents for leaves, bark, and flowers. Antioxidants may have a positive effect on human health through protect the body against damage by free radicals. Such radicals attack cell's macromolecules including membrane lipids, proteins and DNA, lead to many health disorders/diseases including cancers, heart diseases, aging, inflammatory diseases, obesity, diabetes, anemia, etc. [83] [96-103]. At the end of this section, the present study made DRSP a complete package of healthy/functional food by being an excellent source of nutrients and nutraceutical compounds. Many of these compounds have shown important biological roles including antioxidant activity. All of these factors encourage the use of these seeds in many therapeutic nutritional applications, and in

this study they were limited to liver toxicity in experimental rats.

Table 4. Antioxidant activity of *Delonix regia* seed powder (DRSP)

Component	Amount
AA (%)	62.56 $\pm$ 1.19 ^c
AA [% of Butylated hydroxytoluene (50 mg/ml)]	70.73 $\pm$ 1.37
AA [% of Butylated hydroxytoluene (100 mg/ml)]	65.81 $\pm$ 1.66
AA [% of α -tocopherol (50 mg/ml)]	65.06 $\pm$ 1.03
BHT (50 mg/ml);;	88.89 $\pm$ 0.30 ^b
BHT (100 mg/ml)	95.06 $\pm$ 0.27 ^a
α -tocopherol (50 mg/ml)	96.12 $\pm$ 0.11 ^a

*Each value is the Mean $\pm$ SD of three replicates. Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$.

3.2. Biological Studies of *Delonix Regia* Seed Powder (DRSP)

3.2.1. Effect of *Delonix Regia* Seed Powder (DRSP) on Body Weight Gain (BWG), Feed Intake (FI) and Feed Efficiency Ratio (FER) of Hepatic Rats

Effect of *Delonix regia* seed powder (DRSP) on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of hepatic rats were shown in Table 5. Such data indicated that at the end of the experiment (8 weeks), the CCl₄-treated rats exhibited significantly ($p \leq 0.05$) decreased in BWG (-48.35%), FI (-34.31%) and FER (-32.88) compared to the normal group. However, intervention with DRSP (5, 10, 15 and 20 g/100g diet) in feeding rats for 4 weeks led to significantly ($p \leq 0.05$) decrease on the BWG, FI and FER of the hepatotoxic rats (model control) by the rates of -46.15, -24.18, -14.29 and -8.79%, -32.1, -24.37, -17.00 and -9.85%, and -27.4, -15.1, -10.9 and -8.22% of the normal group, respectively. The rate of decreasing in BWG and FI of the hepatotoxic rats were exhibited a dose- dependent manner. Such data are in agreement with several authors who reported that CCl₄ induced significantly decreasing in BWG, FI and FER in rats [4] [6] [13] [15,16]. Also, Hamzawy et al., [104] and Abd El-Rahman, [105] reported that hepatic rats reveal significant reduction of the body weight and feed intake. Furthermore, Morresion and Hark, [106] showed that liver disease can lead to malnutrition as the result of poor food intake, maldigestion, malabsorption and abnormalities in the metabolism and storage of nutrients. The same direction was reported by Dickerson and Lee, [107] who found that patients with acute or chronic liver disease are ill and commonly lose weight. Intervention with DRSP in the protocol feeding of rats led to an improvement in BWG, FI and FER by different rates. The reason for this can be attributed to the fact that DRSP contains many biologically active compounds (polysaccharides, polyphenols, saponins, oxalates, carotenoids and kaempferols), which have a significant and effective effect in this direction). In line with this trend, several studies have come to confirm that injected rats by CCl₄ caused decrease in BW, FI and FER which improved by consumption of plant parts contains bioactive compounds such as found in DRSP [6,14,17] [108-112]. Despite this,

the results of the current study, along with other studies, have shown that DRSP contains some anti-nutritional substances such as tannins [24,54]. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals [85]. Also, Lewu et al., [86] found that tannins complex proteins resulting in the reduction of protein digestibility and palatability. However, it must be noted that the content of tannins in DRSP as going in the present study is lower than the critical value of 9.0 mg g⁻¹ that could induce tannin toxicity/adverse effects [87].

3.2.2. Effect of *Delonix Regia* Seed Powder (DRSP) on Organs Weight (G) of Hepatic Rats

Data in Table 6 were shown the effect of a dietary intervention with *Delonix regia* seed powder (DRSP) on organs body of hepatotoxic rats. Such data indicated that

injection of rats with CCl₄ (model control) leads to decrease the liver, spleen and kidney weight than the normal group by the rate of -29, -12.5 and -14%, respectively. However, intervention with DRSP (5, 10, 15 and 20 g/100g diet) in feeding rats for 4 weeks led to significantly ($p \leq 0.05$) increase on the liver, spleen and kidney weight of the hepatotoxic rats (model control) by the rates of -10.8, 4.6, -3.0 and 4.6%, -8.10, -6.25, -2.50 and 0.0%, and -5.71, -2.14, -1.43 and -1.43% of the normal group, respectively. The rate of increasing in organs weight of the hepatotoxic rats was exhibited a partially dose- dependent manner. In similar studies, the results showed that consumption of some plant parts that contain bioactive compounds (polysaccharides, polyphenols, carotenoids and kaempferols) similar to those found in DRSP, has produced improving effects on the weight of organs in rats [108,112,113].

Table 5. Effect of DRSP on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of hepatic rats

Groups	BWG (%)		FI (g/day/rat)		FER	
	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change
G1 (-)	0.91 $\pm$ 0.07 ^a		11.98 $\pm$ 0.61 ^a		0.073 $\pm$ 0.003 ^a	
G2 (+)	0.47 $\pm$ 0.08 ^d	-48.35	7.87 $\pm$ 0.58 ^d	-34.31	0.049 $\pm$ 0.004 ^c	-32.88
G3 (5%)	0.49 $\pm$ 0.03 ^d	-46.15	8.14 $\pm$ 0.71 ^d	-32.1	0.053 $\pm$ 0.002 ^c	-27.4
G4 (10%)	0.69 $\pm$ 0.02 ^c	-24.18	9.06 $\pm$ 0.59 ^c	-24.37	0.062 $\pm$ 0.005 ^{bc}	-15.1
G5 (15%)	0.78 $\pm$ 0.06 ^b	-14.29	9.94 $\pm$ 0.66 ^{bc}	-17	0.065 $\pm$ 0.004 ^b	-10.9
G6 (20%)	0.83 $\pm$ 0.03 ^b	-8.79	10.80 $\pm$ 0.49 ^b	-9.85	0.067 $\pm$ 0.006 ^b	-8.22

Each value represents the mean value of three replicates $\pm$ SD. Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3, G4, G5 and G6, model group treated with 5, 10, 15 and 20 % of DRSP, respectively. DRSP, *Delonix regia* seed powder, BWG, body weight gain; FI, feed intake; FER, feed efficiency ratio.

Table 6. Effect of DRSP on organs weight (g) of hepatic rats

Groups	Liver		Spleen		Kidney	
	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change
G1 (-)	6.5 $\pm$ 0.6 ^a		0.80 $\pm$ 0.2 ^a		1.40 $\pm$ 0.1 ^a	
G2 (+)	4.6 $\pm$ 0.2 ^c	-29.0	0.70 $\pm$ 0.1 ^c	-12.5	1.20 $\pm$ 0.1 ^b	-14.0
G3 (5%)	5.8 $\pm$ 0.2 ^b	-10.8	0.74 $\pm$ 0.2 ^b	-8.10	1.32 $\pm$ 0.1 ^a	-5.71
G4 (10%)	6.8 $\pm$ 0.6 ^a	4.6	0.75 $\pm$ 0.1 ^{ab}	-6.25	1.37 $\pm$ 0.2 ^a	-2.14
G5 (15%)	6.3 $\pm$ 0.4 ^a	-3.0	0.78 $\pm$ 0.1 ^a	-2.5	1.38 $\pm$ 0.2 ^a	-1.43
G6 (20%)	6.8 $\pm$ 0.4 ^a	4.6	0.80 $\pm$ 0.1 ^a	0.0	138 $\pm$ 0.3 ^a	-1.43

Each value represents the mean value of three replicates $\pm$ SD. Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3, G4, G5 and G6, model group treated with 5, 10, 15 and 20 % of DRSP, respectively. DRSP, *Delonix regia* seed powder, RBCs, red blood cells; WBCs, white blood cells.

Table 7. Effect of *Delonix regia* seed powder (DRSP) on liver functions of hepatic rats

Groups	AST		ALT		ALP	
	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change
G1 (-)	30.23 $\pm$ 4.12 ^e		25.6 $\pm$ 1.7 ^d		125 $\pm$ 4.1 ^e	
G2 (+)	65.20 $\pm$ 1.4 ^a	115.68	56.5 $\pm$ 5.4 ^a	120.70	231.51 $\pm$ 0.57 ^a	85.2
G3 (5%)	61 $\pm$ 5.0 ^{ab}	101.79	52.67 $\pm$ 0.57 ^{ab}	105.70	171.21 $\pm$ 2.65 ^b	36.97
G4 (10%)	57.31 $\pm$ 4.3 ^b	89.58	47.15 $\pm$ 0.5 ^b	84.20	160.20 $\pm$ 2.1 ^{bc}	28.16
G5 (15%)	47.17 $\pm$ 5.3 ^c	56.00	39.21 $\pm$ 0.57 ^b	53.20	151.37 $\pm$ 3.7 ^c	21.10
G6 (20%)	40.22 $\pm$ 3.7 ^d	33.00	32.39 $\pm$ 0.57 ^c	26.50	141/91 $\pm$ 4.5 ^d	13.53

Each value represents the mean value of three replicates $\pm$ SD. Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3, G4, G5 and G6, model group treated with 5, 10, 15 and 20 % of DRSP, respectively. DRSP, *Delonix regia* seed powder, AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase.

3.2.3. Effect of *Delonix Regia* Seed Powder (DRSP) on Liver Functions of Hepatic Rats

Effect of *Delonix regia* seed powder (DRSP) on liver functions (AST, ALT and ALP) of hepatic rats were

shown in Table 7. Such data indicated that at the end of the experiment (8 weeks), the CCl₄-treated rats exhibited significantly ($p \leq 0.05$) increased in AST (115.68%), ALT (120.70%) and ALP (85.2%) compared to the normal group. However, intervention with DRSP (5, 10, 15 and

20 g/100g diet) in feeding rats for 4 weeks led to significantly ($p \leq 0.05$) decrease on the AST, ALT and ALP by 101.79, 89.58, 56.00 and 33.00%, 105.70, 84.20, 53.20 and 26.50%, and 36.97, 28.16, 21.10 and 13.53% of the normal group, respectively. The rate of decreasing in AST, ALT and ALP of the hepatotoxic rats were exhibited a dose- dependent manner. The present data showed that CCl_4 can significantly increase the liver enzymes ALT, AST and ALP, due to its cytotoxic effects, which resulted in destroying the structure and function of the liver cell membrane and intracellular organelles and the release of these enzymes in the blood circulation [113,114,115]. These harmful effects lie in the following mechanism: CCl_4 was transformed to trichloromethyl radicals ($\text{CCl}_3\cdot$) by liver cytochrome P_{450} which actively binds to O_2 forming trichloromethyl peroxy radicals ($\text{CCl}_3\text{OO}\cdot$). The last free radicals causes lipid peroxidation to all cell organelles membrane-bound fatty acids. Serial enzyme measurements, AST, ALT and ALP are often considered sensitive markers for determining the course of liver damage due to their presence of the cytoplasm facilitates blood flow after liver cell damage [113,116,117]. Data of the present study showed that DRSP significantly ($p \leq 0.05$) reducing AST, ALT and ALP levels which demonstrating that it can prevent live cells damage. This prophylactic/curative effects could be attributed to DRSP content of some important nutrients and bioactive constituents such polysaccharides, polyphenols, tannins, saponins, carotenoids and kaempferols). In similar studies, plant parts extracts contains such bioactive compounds exhibit protective activities against liver injury induced by toxic chemicals including CCl_4 [18] [113] [118,119,120]. Also, similar studies conducted using other parts of *Delonix regia*, such as flowers and leaves, have indicated that they have an effective protective effects against the deteriorating effects on the liver caused by CCl_4 (17; 69). All the results of these studies suggested that the protective effect of *Delonix regia* extracts is due to the presence of antioxidant phenolic compounds, that is, total phenolic content and total flavonoid content. Therefore, it is worth noting here that DRSP contain these same active compounds, along with many other compounds, which were the reason for showing protective activities against liver injury induced by toxic chemicals including CCl_4 . Also, the reason for these protective effects of DRSP may be related to the fact that it contains different classes of bioactive compounds, which have been shown in numerous studies to have the ability to reduce bilirubin levels through the extract of active regulators that increase the activity of enzymes, transporter synthesis, and steps related to bilirubin clearance pathway [115,119] [121,122,123].

3.2.4. Effect of DRSP on Serum Albumin and Total Protein of Hepatic Rats

Data in Table 8 were shown the effect of *Delonix regia* seed powder (DRSP) on serum albumin and total protein of hepatic rats. From such data it could be noticed that at the end of the experiment (8 weeks), the CCl_4 -treated rats exhibited significantly ($p \leq 0.05$) decreased in Alb (-30.9%) and TP (27.0%) compared to the normal group. However, intervention with DRSP (5, 10, 15 and 20 g/100g diet) in feeding rats for 4 weeks led to significantly ($p \leq 0.05$) decrease on the Alb and TP by -23.8, -21.4, -14.0 and -

7.0%, and -23.0, -13.6, -10.9 and -6.8% of the normal group, respectively. The rate of decreasing in Alb and TP of the hepatotoxic rats were exhibited a dose- dependent manner. In similar studies, CCl_4 -induced significant decrease in the serum albumin and total protein content as the consequence of liver injury [111,112,113] [118,120,124]. Also Koneri et al., [125] indicated that hypoalbuminaemia is most commonly in the case of advanced chronic liver diseases and a useful index of the severity of cellular dysfunction in such diseases. Data of the present study indicated that DRSP significantly ($p \leq 0.05$) increased serum Alb and TP levels which demonstrating that it can prevent or repair the liver cells damage. Such a role of the DRSP in treating the serum Alb level is of high importance, since human serum Alb is the major protein of blood plasma i.e. constitutes about 50%. In this way, Alb is the transport protein that binds to and carries around various ligands such as water, fatty acids, hormones, bilirubin, thyroxine hormone, pharmaceuticals, and cations. Thus, one of the main functions of Alb is to regulate the oncotic pressure of blood [126,127]. Oncotic (colloid osmotic) pressure is known as a type of osmotic pressure caused by plasma proteins, especially albumin in the plasma of blood vessels (or any other fluids in the body such as blood and lymph), which causes fluid withdrawal back to the capillary [128]. The colloids also displace the water molecules resulting in a relative water molecule deficit as the water molecules return to the circulation within the lower venous pressure end of the capillary [129]. This may have the opposite effect of both hydrostatic blood pressure, which pushes water and small molecules out of the blood into the interstitial spaces within the arterial end of the capillaries, and interstitial colloidal osmotic pressure. Oncotic pressure also strongly affects the physiological function of the circulatory system and pressure through the glomerular filter [130,131].

Table 8. Effect of DRSP on Albumin and total protein of hepatic rats

Groups	Albumin (Alb)		Total protein (TP)	
	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change
G1 (-)	4.2 $\pm$ 4.9 ^a		7.3 $\pm$ 0.16 ^a	
G2 (+)	2.9 $\pm$ 0.3 ^c	-30.9	5.3 $\pm$ 0.13 ^b	-27.0
G3 (5%)	3.2 $\pm$ 0.3 ^b	-23.8	5.6 $\pm$ 0.13 ^b	-23.0
G4 (10%)	3.3 $\pm$ 0.14 ^b	-21.4	6.3 $\pm$ 0.13 ^{ab}	-13.6
G5 (15%)	3.6 $\pm$ 0.14 ^{ab}	-14.0	6.5 $\pm$ 0.24 ^a	-10.9
G6 (20%)	3.9 $\pm$ 0.06 ^a	-7.0	6.8 $\pm$ 0.26 ^a	-6.8

Each value represents the mean value of three replicates $\pm$ SD. Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3, G4, G5 and G6, model group treated with 5, 10, 15 and 20 % of DRSP, respectively. DRSP, *Delonix regia* seed powder.

3.2.5. Effect of *Delonix Regia* Seed Powder (DRSP) on Serum Lipid Profile Parameters of Hepatic Rats

Effect of *Delonix regia* seed powder (DRSP) on serum lipid profile TG, Cho, LDL and VLDL) of hepatic rats were shown in Table 9. The data indicated that at the end of the experiment (8 weeks), the CCl_4 -treated rats exhibited significantly ($p \leq 0.05$) increased in TG (34.64%), Cho 29.13%) and LDL-C (33.65%) whereas, the HDL-C decreased (-48.68%) compared to the normal group. However, intervention with DRSP (5, 10, 15 and 20

g/100g diet) in feeding rats for 4 weeks led to significantly ($p \leq 0.05$) decreased in serum TGs, Cho and LDL-C as well as the HDL-C level was significantly ($p \leq 0.05$) declined. The rate of increasing in HDL and declined in TGs, Cho and LDL were exhibited a dose-dependent manner. Such data are according with that obtained by several authors who found that CCl_4 increased cholesterol synthesis and hyperlipidemia, which resulted increasing serum TG and TC levels, shifting the glucose metabolism towards lipogenesis. The prophylactic/curative effects of DRSP in enhanced the serum lipid profile parameters could be attributed to the involvement of hypolipidemic effect in the DRSP. The different biological effects (hypolipidemic, antioxidant and scavenging activities) of DRSP are probably due to the presence of bioactive constituents such polysaccharides, polyphenols, saponins, oxalates, carotenoids and kaempferols. Polyphenolic compounds such as found in DRSP cause decreasing cholesterol absorption by deactivating the coenzyme-A (HMG-CoA)

reductase hydroxymethylglutaryl [132]. Also, McAnlis et al., [133] found that phenolics having a high affinity for protein was bound to albumin and never incorporated into the LDL particle. Furthermore, the possible hypocholesterolemic effects of several dietary components, such as found in DRSP including alkaloids, polyphenols, carotenoids, polysaccharides, kampherol etc., have attracted much interest [113]. Such compounds exert their beneficial effects on cardiovascular health including improve the serum lipid profile parameters by antioxidant, scavenging and anti-inflammatory activities [20] [134-136]. On the other side, LDL oxidation and endothelial cell damage is believed to be involve in the early development of atherosclerosis [137]. Several authors reported that the presence of phenolics, polysaccharides and alkaloids such as found in DRSP significantly reduced LDL oxidation *in vitro* from various oxidases including 15-lipoxygenase, copper-ion and linoleic acid hydroperoxide [100] [133] [137-139].

Table 9. Effect of DRSP on serum lipid profile (mg/dL) of hepatic rats

Groups	TGs		Cho		HDL-C		LDL-C	
	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change
G1 (-)	149.54 $\pm$ 3.81 ^d		173.63 $\pm$ 6.25 ^d		53.66 $\pm$ 2.43 ^a		150.27 $\pm$ 5.91 ^c	
G2 (+)	201.35 $\pm$ 2.97 ^a	34.64	224.21 $\pm$ 6.29 ^a	29.13	27.54 $\pm$ 3.30 ^c	-48.68	200.84 $\pm$ 6.22 ^a	33.65
G3 (5%)	196.43 $\pm$ 4.34 ^a	31.36	216.45 $\pm$ 4.81 ^a	24.66	29.90 $\pm$ 2.31 ^c	-44.28	189.35 $\pm$ 7.32 ^{ab}	26
G4 (10%)	188.23 $\pm$ 3.81	25.87	201.71 $\pm$ 5.81 ^b	16.17	33.83 ^c $\pm$ 1.93	-36.95	179.29 $\pm$ 4.50 ^b	19.31
G5 (15%)	178.91 $\pm$ 5.91 ^b	19.64	193.37 $\pm$ 7.01 ^c	11.37	39.37 ^b $\pm$ 1.80	-26.63	165.56 $\pm$ 5.73 ^b	10.18
G6 (20%)	165.50 $\pm$ 4.52 ^c	10.67	188.29 $\pm$ 3.08 ^c	8.44	41.44 $\pm$ 2.35 ^b	-22.77	161.62 $\pm$ 4.01 ^b	7.55

Each value represents the mean value of three replicates $\pm$ SD. Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3, G4, G5 and G6, model group treated with 5, 10, 15 and 20 % of DRSP, respectively. DRSP, *Delonix regia* seed powder. TG, triglycerides, Cho, cholesterol HDL-C, high-density lipoproteins cholesterol, LDL-C, low-density lipoproteins cholesterol.

Table 10. Effect of DRSP on red blood cells, white blood cells, hemoglobin and platelet of hepatic rats

Groups	Hb (g/dL)		Platelet ($10^3/\mu\text{L}$)		RBCs ($10^6/\mu\text{L}$)		WBCs ($10^3/\mu\text{L}$)	
	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change
G1 (-)	13.5 $\pm$ 0.2 ^a		324 $\pm$ 5 ^a		4.85 $\pm$ 0.21 ^a		5.6 $\pm$ 0.3 ^a	
G2 (+)	9.5 $\pm$ 0.2 ^c	-29.63	220 $\pm$ 3 ^e	-32.1	3.26 $\pm$ 0.32 ^c	-32.78	3.2 $\pm$ 0.1 ^c	-42.86
G3 (5%)	9.7 $\pm$ 0.3	-28.15	231 $\pm$ 4 ^d	-28.7	3.35 $\pm$ 0.17 ^c	-30.93	3.2 $\pm$ 0.2 ^c	-42.86
G4 (10%)	10.8 $\pm$ 0.2 ^c	-20	241 $\pm$ 5 ^c	-25.62	3.47 $\pm$ 0.22	-28.45	3.6 $\pm$ 0.1 ^c	-35.71
G5 (15%)	12.3 $\pm$ 0.3 ^b	-8.89	243 $\pm$ 4 ^c	-25	3.71 $\pm$ 0.17 ^{bc}	-23.51	3.9 $\pm$ 0.2 ^{bc}	-30.36
G6 (20%)	12.3 $\pm$ 0.4 ^b	-8.89	285 $\pm$ 6 ^b	-12	3.92 $\pm$ 0.14 ^b	-19.2	4.7 $\pm$ 0.1 ^b	-16.1

Each value represents the mean value of three replicates $\pm$ SD. Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. G1, normal group; G2, model group (hepatotoxic); G3, G4, G5 and G6, model group treated with 5, 10, 15 and 20 % of DRSP, respectively. DRSP, *Delonix regia* seed powder, Hb, hemoglobin, RBCs, Red blood cells; WBCs, White blood cells.

3.2.6. Effect of *Delonix Regia* Seed Powder (DRSP) on the Hematological Parameters of Hepatic Rats

Hematological data of rats injected by CCl_4 and interventional with DRSP were shown in Table 10. Such data indicated that Hb, RBCs, WBCs and platelets, were significantly ($p \leq 0.05$) decreased in the CCl_4 -treated group compared to the control rats. DRSP-treated groups

showed a significant ($p \leq 0.05$) increase in Hb, RBCs, WBCs and platelets related to the CCl_4 -injected group. The present research data confirmed that CCl_4 has harmful effects on all tested hematological parameters, with characteristic leukopenia. Such data are in accordance with several authors who found that depletion in the count of RBC's along with the Hb level was detected in rats injected with CCl_4 which could be attributed to disturb

hematopoiesis, destruction of erythrocytes, and reduction in the rate of their formation and/or enhanced removal from circulation due to CCl_4 toxicity [140,141]. Also, according to Ballinger, [142], depletion in erythrocytes count and Hb level leads to iron-deficiency anemia which is characterized by a microcytic hypochromic blood picture, also hyperactivity of bone marrow, which leads to production of red blood cells with impaired integrity that are easily destroyed in the circulation. This could be another reason for decreasing hematological values [143]. Data of the present study showed that the DRSP caused an improvement in all tested hematological parameters to a nearly normalized value. Such protective effect may be explained through several mechanisms since the DRSP is a complex mixture of several nutrients (vitamins and minerals) and phytochemicals (phenolics, alkaloids, carotenoids and polysaccharides [30]. For example, vitamins C and E are strong antioxidants which play an important role in detoxification process through removing the free radicals, protecting DNA from oxidative damage and reducing micronucleus frequencies in polychromatic erythrocytes of bone marrow [144,145]. Phytochemicals detected in DRSP exhibited several biological effects including antioxidant and scavenging activities and inhibiting the lipid peroxidations [19,20] [119] [146].

3.2.7. Effect of *Delonix Regia* Seed Powder (DRSP) on Rat Liver Histological Disorders Induced by Carbon Tetrachloride

Effect of *Delonix regia* seed powder (DRSP) on rat liver histological disorders induced by carbon tetrachloride was illustrated in Figure 2. Light microscopic examination of liver of rats from group 1 revealed the normal histological architecture of hepatic lobules with normal central vein and normal hepatocytes (Photo A). On the contrary, liver of rats from group 2 exhibited marked hepatocellular vacuolar degeneration (Photos B and C) and activation of Kupffer cells (Photo C). However, liver of rats from group 3 showed activation of Kupffer cells (Photo D) and slight vacuolar degeneration of some hepatocytes (Photo E). On the other hand, liver of rats from group 4 described no histopathological alterations except slight hydropic degeneration of some hepatocytes was observed in few examined sections (Photo F). Furthermore, liver of rats from group 5 revealed no histopathological alterations (Photos G and H). Otherwise, some examined sections from group 6 showed slight activation of Kupffer cells (Photo I) whereas, other sections revealed no histopathological alterations (Photo J). Oxidative stress is viewed as a common pathological mechanism leading to the onset and progression of liver damage [147]. The liver damage due to oxidative stress includes parenchymal cells, Kupffer cells, endothelial cells, and stellate cells. A variety of cytokines such as $\text{TNF-}\alpha$ may be secreted within Kupffer cells, thereby potentially augmenting inflammation and apoptosis. Also, lipid peroxidation resulting from oxidative stress stimulates cell proliferation and collagen synthesis in hepatic stellate cells [148]. Furthermore, Hajam et al., [149] reported that ROS cause damage to the hepatocyte membranes resulting in collagen accumulation in cells subsequently the development of liver fibrosis and cirrhosis. In contrast, in CCl_4 -intoxicated rats treated with DRSP, liver tissues

showed varying degrees of improvement in histopathological changes which exhibiting dose-dependent manner. The degenerative alterations were mitigated and the liver's histological architecture improved significantly. Some of these improvements in liver tissue architecture could be attributed to the biological activities of DRSP including, antioxidant and scavenging activities as well as inhibition of lipid oxidation. In similar study, Blessing et al., [150] found that aqueous extracts of seeds of *Delonix regia* has the capacity to diminish oxidative stress and subsequent cytotoxicity, thus protecting intact hepatocytes or cells that have not yet sustained irreversible damage. All of these findings indicated that DRSP effectively normalized liver function and partially repaired hepatic tissue damage.

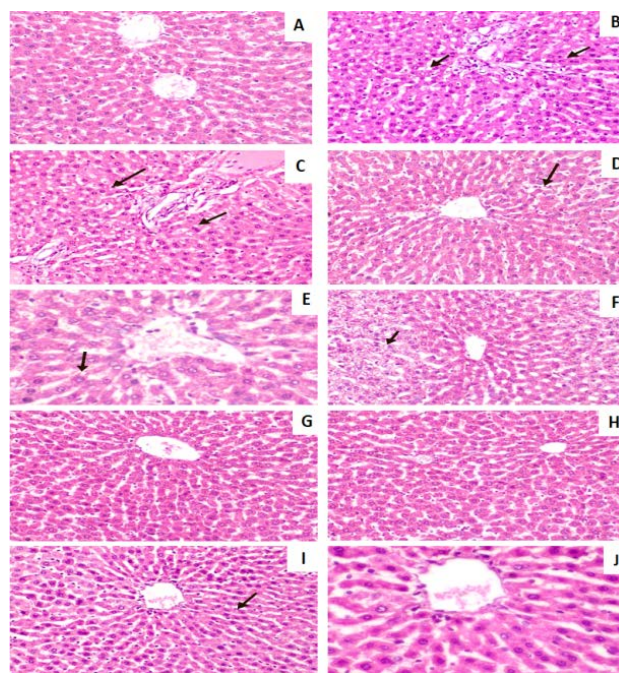


Photo A, photomicrograph of liver of rat from group 1 showing the normal histological architecture of hepatic lobule (H & E X200); **Photo B**, photomicrograph of liver of rat from group 2 showing marked hepatocellular vacuolar degeneration (H & E X200); **Photo C**, photomicrograph of liver of rat from group 2 showing marked hepatocellular vacuolar degeneration and activation of Kupffer cells (H & E X200); **Photo D**, photomicrograph of liver of rat from group 3 showing activation of Kupffer cells (H & E X200); **Photo E**, photomicrograph of liver of rat from group 3 showing slight vacuolar degeneration of some hepatocytes (H & E X400); **Photo F**, photomicrograph of liver of rat from group 4 showing slight hydropic degeneration of some hepatocytes (H & E X200); **Photo G**, photomicrograph of liver of rat from group 5 showing no histopathological alterations (H & E X200); **Photo H**, photomicrograph of liver of rat from group 5 showing no histopathological alterations (H & E X200); **Photo I**, photomicrograph of liver of rat from group 6 showing slight activation of Kupffer cells (H & E X200); **Photo J**, Photomicrograph of liver of rat from group 6 showing no histopathological alterations (H & E X400).

Figure 2. Effect of *Delonix regia* seed powder (DRSP) on rat liver histological disorders induced by carbon tetrachloride

4. Conclusion

Carbon tetrachloride (CCl_4) causes severe hepatic damage by inducing a state of oxidative stress.

Intervention with (*Delonix regia*) seeds powder (DRSP) are able to prevent or inhibit liver injuries induced by chemical toxin i.e. CCl_4 . As shown in Figure 3, the manipulation process includes improving the liver functions enzymes activities, serum lipid profile, serum total protein and albumin, complete blood count (the counts of white blood cells, red blood cells and platelets, the concentration of hemoglobin), and histological

changes thereby adversely affecting the injuries process to the benefit of the biological system. Such biological improvements induced by DRSP could be attributed to their rich content of bioactive compounds and related biological activities. We recommended that *Delonix regia* seeds powder by the tested concentrations to be included in our daily dishes, drinks and pharmaceutical preparations/formulae.

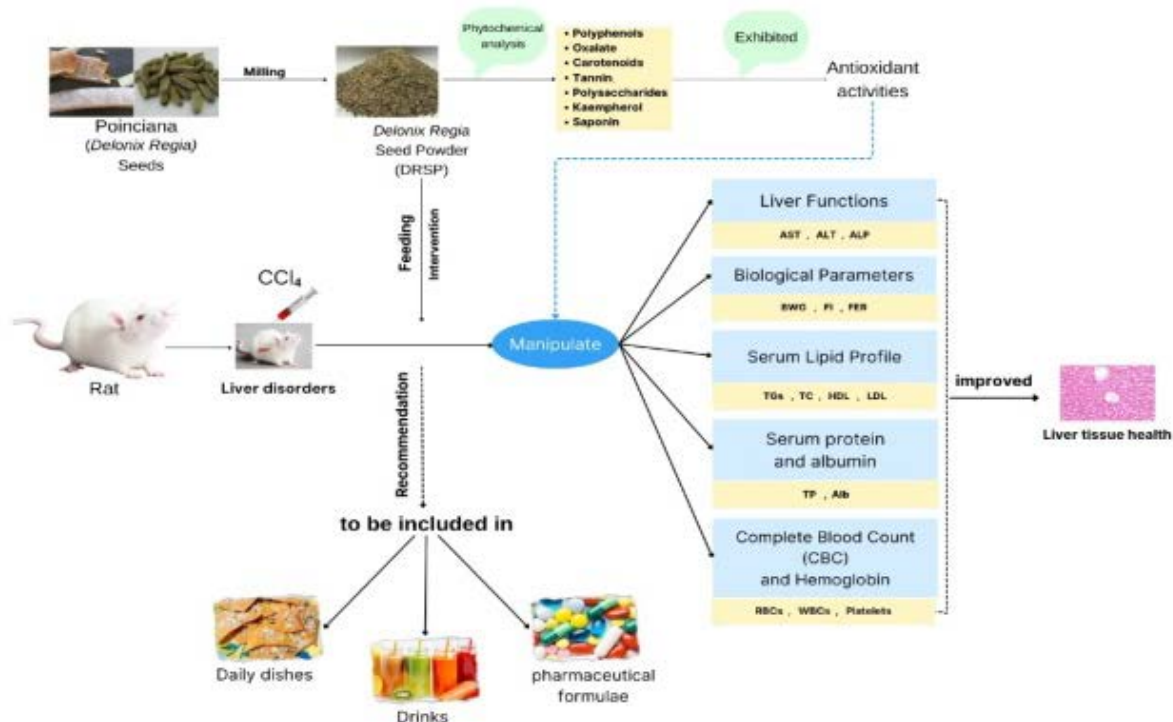


Figure 3. Graphical summary showing the effect of feeding intervention of *Delonix regia* seeds powder (DRSP) on carbon tetrachloride (CCl_4) causes severe hepatic damage in rats

Ethical Considerations

The ethical issues of this study was reviewed and approved by the Scientific Research Ethics Committee (Animal Care and Use), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval # 21- SREC- 06-2021).

Conflict of Interest

The authors declare that they have no conflict of interest in publishing this paper.

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Authors' contribution

Yousif Elhassaneen participated in preparing, developing and reviewing the study protocol, following up on the application of practical experiments, verifying the validity of the results and statistical analyses, preparing a draft of the paper, conducting a critical review to organize the content intellectually, and granting approval to publish the final version of the paper. Yasmine Abd-ElAziz conducted the practical experiments, collected, analyzed, tabulated and interpreted the data, and also participated in conceptual information retrieval and preparation of the draft of the paper. Seham Khader participated in retrieving conceptual information, following up on practical experiments, and preparing the paper draft. Mai Gharib made significant contributions to the concept and design of the work and preparation of the draft paper.

Abbreviations

AA, antioxidant activity, Alb, albumin, ALT, Alanine aminotransferase, ALP, alkaline phosphatase, AST,

aspartate aminotransferase, BD, basal diet, BCB, β -carotene bleaching, BHT, butylated hydroxytoluene, BWG, body weight gain, CCl_4 , carbon tetrachloride, Cho, cholesterol, DRSP, *Delonix regia* seed powder, FER, feed efficiency ratio Hb, hemoglobin, HDL-c, high density lipoprotein, RBCs, red blood cells, FI, feed intake, TC, total cholesterol, TG's, triglycerides, TP, total protein, WBCs, white blood cells.

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