

Effect of *Pyrus communis* (Common Pear) Seeds on Selected Parameters of Liver Function in Rats Treated with Cadmium

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Abstract

The study investigated possible hepatoprotective potentials of *Pyrus communis* (PC) seeds in rats treated with cadmium. Twenty Wistar rats weighing 150 g - 180 g were divided into 4 groups (A, B, C and D) of 5 rats each. Group A received distilled water only. 5mg/kg bwt cadmium was given to group B orally while group C were pre-administered with 500mg/kg bwt PC extract orally. Group D received 500mg/kg bwt PC. After two weeks of treatment, serum and liver homogenate were evaluated for haptoglobin, (Hp) total protein (TP), albumin, alanine transaminase, (ALT) and aspartate transaminase, (AST). The results showed significant ($p < 0.05$) reduction in liver and serum Hp concentrations in rats treated with cadmium. There was also significant ($p < 0.05$) decrease in liver TP, ALT and AST levels and significant ($p < 0.05$) increase in serum TP, ALT and AST levels in rats treated with cadmium. However, treatment with PC restored Hp, TP, ALT and AST levels back to near normal. We concluded that 5mg/kg bwt cadmium caused liver damage and depressed Hp synthesis, and PC protected the liver.

Keywords: Haptoglobin; Cadmium; Liver damage; *Pyrus communis*; Hepatoprotective.

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1. Introduction

Incidence of liver dysfunction as a result of exposure to environmental toxicants is increasing globally. Cadmium (Cd) causes liver injury by binding to vital intracellular and membranous components like structural proteins, enzymes, nucleic acids, and interferes with their functions [1]. Sources of heavy metals are found in mining and industrial wastes; food and water sources [2], absorption through skin contact [3], vehicle emissions, batteries, fertilizers [4], etc. One of the functions of the liver is the synthesis of the protein, haptoglobin, which is an acute phase reactant [5]. Haptoglobin functions as an antioxidant due to its ability to bind hemoglobin [6] forming haptoglobin-hemoglobin complex and thereby preventing the oxidative tissue damage that may be mediated by free hemoglobin [7]. Formation of the haptoglobin-hemoglobin complex prevents the hemoglobin driven generation of hydroxyl radical and lipid peroxide in inflammation, connective tissue diseases, surgery and burns, malignancy, chemical irritants, ischemic necrosis etc. Normal haptoglobin levels ranges from 45 to 200 milligrams of haptoglobin per deciliter of blood (mg/dL) [8]. Profound changes in serum haptoglobin level occur in a great variety of disease states. Haptoglobin synthesis undergoes two pathophysiological phenomena. Increase in haptoglobin production is seen during an inflammatory reaction and a decrease synthesis occurs during severe hepatocellular deficiency due to a failure in biosynthesis or in haemolytic conditions [9]. Common Pears (*pyrus communis*) are native to coastal and mildly temperate region of Western Europe and North Africa [10]. The fruit *Pyrus communis* is a pyriform pome with persistent or deciduous calyx, 4-12 cm long, greenish colored, dry and gritty. The fruit can be eaten raw, in jams or jellies, or in fruit salads. Sometimes, they are dried or candied [11]. Pear fruits contain many essential nutrients like carbohydrate, protein, ash and moisture, it also provides with some essential element like calcium, phosphorus, sodium and magnesium moderate enough to meet with the recommended daily allowances [12]. The present study was conducted to investigate haptoglobin expression in cadmium-induced liver toxicity with a view to investigating the possible protective potentials of *Pyrus communis*.

2. Materials and Methods

2.1. Plant Material and Extraction

Fresh fruits of *Pyrus communis* were purchased at a local market in Osogbo, Osun State, Nigeria. The fruits were cut open and the seeds removed with hand. The seeds were sliced, air dried for six weeks, and ground into fine powder. 350g of the powdered seeds was weighed and soaked in 3.5 litres of 70% methanol with constant agitation at room temperature for 10 days. The mixture was filtered using a muslin cloth and the filtrate was concentrated using Buchi Rotary Vacuum Evaporator followed by complete evaporation to dryness using Water Bath (SM-8A) to give a dark brown crude extract which was then oven-dried to give a thick paste.

2.2. Phytochemical Tests

Phytochemical tests were carried out on the extract using the standard procedures as described by Trease and Evans [13,14].

2.3. Acute Toxicity Testing

Toxicity study was carried out using OECD guide lines NO 423, Annex 3. Three female rats of the same age group and weight were dosed up to 5000 mg/kg body weight per oral. The animals were observed for the 1 hour, hourly for 4 hours and finally every 24 hours for 15 days.

2.4. Experimental Animals and Design

Twenty Wistar rats weighing 150 g - 180 g were used for the study. The animals were acclimatized for two weeks at the Animal House of the College of Health Sciences, Osun State University Osogbo, Nigeria where they had free access to standard rat pellets and clean water. The rats were divided randomly into four groups (A, B, C and D) of five rats each. Group A rats served as the control and received distilled water only. Rats in group B were given 5mg/kg body weight cadmium orally while group C rats were pre-administered with 500mg/kg bwt PC extract orally before 5mg/kg body weight cadmium was administered. Rats in group D were treated with 500mg/kg body weight *Pyrus communis* extract. All rats in each group were treated every day for the period of two weeks. All the rats in each group were sacrificed at the end of two weeks of treatment by cervical decapitation. Blood samples were collected prior sacrifice and sera separated were centrifuged at 3000rpm for 5minutes using bench centrifuge (MODEL 800D, MICROFIELD INSTRUMENT, ENGLAND). The clear sera were kept in the freezer at -4°C till assay. The animals were dissected immediately after sacrifice and liver tissues were harvested, cleaned of fat and connective tissue. 0.5 g of liver was cut, homogenized in 5ml of potassium phosphate buffer (liver / total buffer is 1:10) and centrifuged at 6000rpm for 10 minutes at 4°C. The supernatant obtained was used for biochemical assays.

2.5. Biochemical Evaluations

2.5.1. Assay of Serum and Liver Haptoglobin

Sera and liver haptoglobin levels were estimated using the principle of peroxidase activity of haptoglobin-methaemoglobin complexes as previously described by Owen and his colleagues [15] with modifications. The concentration of haptoglobin obtained from a calibration curve was expressed in terms of bound methaemoglobin.

2.5.2. Estimation of ALT and AST Activities

Serum and liver alkaline transaminase (ALT) and aspartate transaminase (AST) activities were determined by the colorimetric methods of [16] using Randox diagnostic kit.

2.5.3. Estimation of Total Protein and Albumin Concentrations

Serum and liver total protein levels was determined using Biuret reaction method [17] while albumin concentrations in the serum and liver was estimated as described by [18] using Randox diagnostic kits.

2.6. Statistical Analysis

Data obtained were analyzed using One Way Analysis of Variance (SPSS version 20.0), and results were considered to be statistically significant when p values less than 0.05.

2.7. Histological Study

Liver tissues harvested after sacrifice were fixed in 10% formalin. The tissues were transferred into an automatic processor where they went through a process of dehydration. The tissues were then cleared in xylene and embedded in paraffin wax. Serial sections of 5 micron thick were obtained using a rotary microtome. The tissue sections were deparaffinised, hydrated and stained using the routine hematoxylin and eosin staining method (H&E). The stained sections were examined with a Leica DM750 microscope interfaced with Leica ICC 50 camera.

2.8. Ethical Consideration

The study was performed according to rules and regulation of the Departmental Ethical Committee on the use of animals for research work.

3. Results

The methanol extract of the seeds of *Pyrus communis* gave positive test reactions for the presence of terpenoids, tannins, flavonoids, steroids, cardiac glycosides and alkaloids (Table 1). Rats when fed with methanol extract of *Pyrus communis* up to 5000 mg/kg bwt orally showed no signs gross behavioural changes and no mortality under observation for 15 days. Table 2 showed absorbance values used to plot the calibration while figure 1 was the calibration curve of absorbance reading against amount of serum and corresponding haptoglobin concentrations. Table 3 showed effects of cadmium and extract of *Pyrus communis* on the synthesis of haptoglobin in the liver and its level in the serum. There was significant ($P < 0.05$) reductions both in the production of haptoglobin in the liver and its expression in the sera of rats treated with cadmium. Whereas, the synthesis and expression of haptoglobin were significantly ($P < 0.05$) increased in rats pre-administered with extract of *Pyrus communis* as well as in rats treated with extract alone. The point of inflexion in the calibration curve obtained corresponded to methaemoglobin-binding capacity of 50 mg/dl. However, since the test samples were diluted 1 in 5, the point amounted to haptoglobin content of 250mg/dl (as methaemoglobin). Aspartate transaminase, AST and alanine transaminase, ALT activities were significantly ($p < 0.05$) raised in the sera of rats treated with cadmium alone (Table 4). The sera total protein level in these rats was also significantly ($p < 0.05$) increased when compared to total protein level in control group. The increase in total protein level observed in rats treated with extract of *Pyrus communis* was not significant. Serum albumin levels in all the groups were not significantly different. Whereas, ALT and AST activities were significantly ($p < 0.05$) depressed in the liver of rats treated with cadmium only (Table 5). Total protein level was also significantly ($p < 0.05$) reduced in the liver of rats treated with cadmium alone. In rats treated with extract of *Pyrus communis*, liver AST and ALT activities were significantly ($p < 0.05$) higher. Likewise, liver total protein level was significantly ($P < 0.05$) reduced in rats treated with cadmium but increased significantly ($P < 0.05$) in rats treated with extract of *Pyrus communis*. Liver albumin levels were not significantly different in all the groups. Figures 2, 3, 4 and 5

were photomicrographs of liver of rats in groups A, B, C and D respectively. Figures 2 and 5 showed normal liver histoarchitecture while there was diffuse cellular injury with histoarchitectural distortion and features of cytotoxicity in figure 3. There is diffuse cellular injury with focal infiltrate (black arrow). Figure 4 showed features of recovering liver histoarchitecture.

Table 1: Phytochemical Properties of *Pyrus communis*

Chemical Compounds	Results
Terpenoids	+
Tannins	+
Saponnins	-
Flavonoids	+
Steroids	+
Cardiac Glycosides	+
Alkaloids	+
Phlobatamins	-

+ Means active compound present

- Means active compound absent

Table 2: Absorbance Values Used to Plot Calibration Curve

S/N	Amount of Serum in each Tube (ml)	Absorbance Readings
1.	0	0.032
2.	0.10	0.091
3.	0.20	0.143
4.	0.30	0.213
5.	0.40	0.262
6.	0.50	0.320
7.	0.60	0.343
8.	0.70	0.352
9.	0.80	0.348
10.	0.90	0.347
11.	1.00	0.350

Table 3: Haptoglobin Concentration (mg/dl) in Sera and Liver of Rats and Effects of Cadmium and *Pyrus communis* Extract

Haptoglobin Concentration	Control	Cadmium	Cadmium + Extract	Extract
Serum	178.70±49.16	35.38±19.83**	101.5±36.00**	133.3±96.20*
Liver	12.78±5.02	3.64±3.01**	9.71±3.38**	17.95±7.55*

Values are expressed as mean ± S.D (n=5). * Significantly different from control group p<0.05. +Significantly different from extract group.

Table 4: Effects of Cadmium and Extract of *Pyrus communis* on Aspartate aminotransferases (AST) Activity (U/I), Alanine aminotransferases (ALT) Activity (U/I), Total Protein Concentration (g/dl) and Albumin Concentration (g/dl) in Sera of Rats

	Control	Cadmium only	Cadmium + Extract	Extract
AST	35.40±2.70	49.60±2.07**	45.80±2.77**	35.20±4.08
ALT	40.80±4.82	55.40±1.67**	51.20±5.26**	39.20±1.48
Total Protein	6.46±0.36	8.04±0.58*	7.58±0.52	7.58±0.28
Albumin	3.28±0.24	3.60±0.60	3.34±0.27	3.42±0.62

Values are expressed as mean ± S.D (n=5). * Significantly different from control group p<0.05. +Significantly different from extract group.

Table 5: Effect of Cadmium and Extract of *Pyrus communis* on Aspartate aminotransferase (AST) Activity (U/l), Alanine aminotransferase (ALT) Activity (U/l), Total Protein Concentration (g/dl) and Albumin Concentration (g/dl) in Liver of Rats

	Control	Cadmium Only	Cadmium + Extract	Extract
AST	39.80±3.45	36.20±2.34* ⁺	37.80±2.39* ⁺	42.80±6.95*
ALT	50.40±1.14	40.60±3.05* ⁺	45.80±2.77* ⁺	56.80±2.58*
Total Protein	7.00±0.25	4.78±0.33* ⁺	5.80±0.44* ⁺	7.34±0.45
Albumin	3.60±0.29	3.20±0.15	3.42±0.31	3.76±0.28

Values are expressed as mean ± S.D (n=5). * Significantly different from control group p<0.05. ⁺Significantly different from extract group.

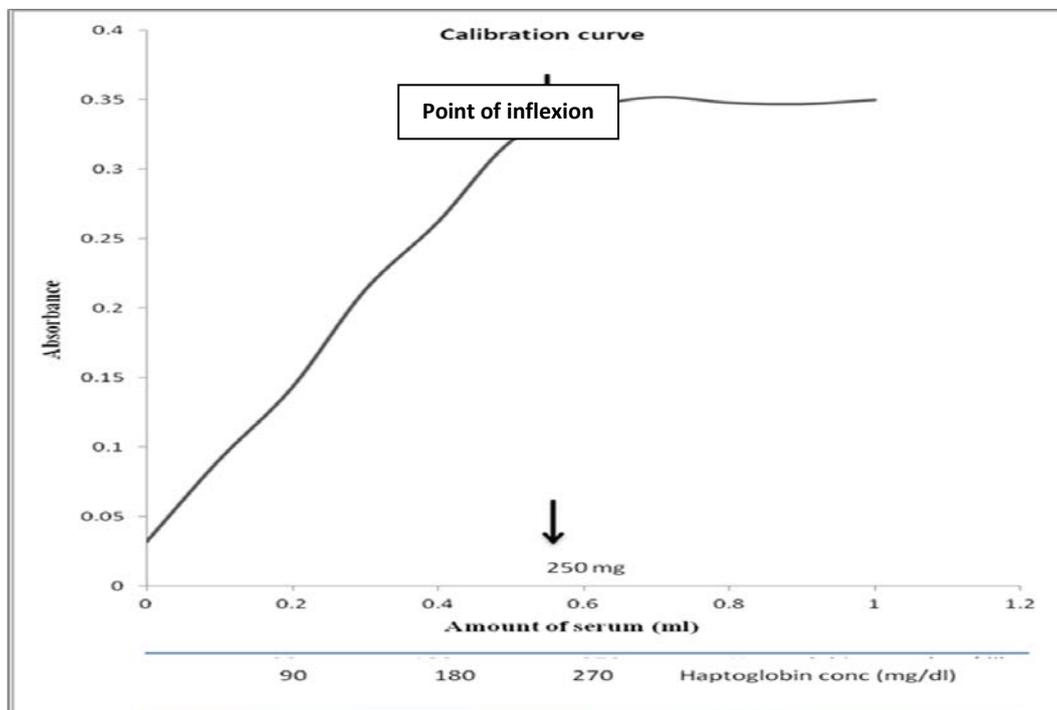


Figure 1: Calibration Curve of Absorbance Reading against Amount of Serum and Corresponding Haptoglobin Concentrations

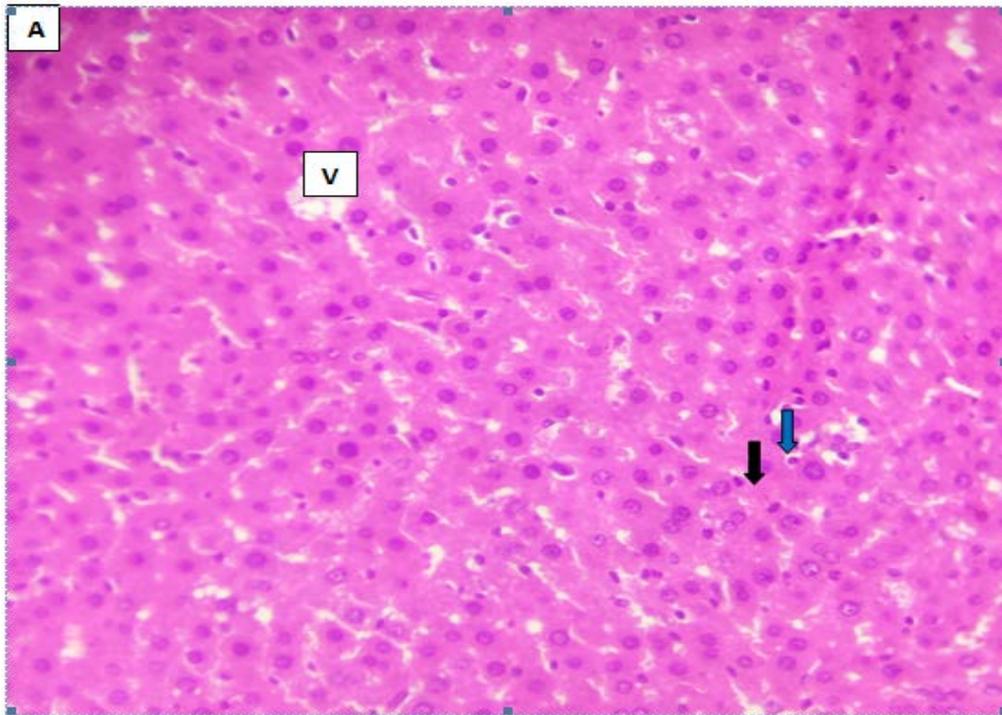


Figure 2: Photomicrograph of normal liver tissue A (Control) showing a normally radiating hepatocyte plate. V is a central vein. There sinusoidal lining cells have flattened condensed nuclei (black arrow) that distinguished them from the normal hepatocytes (blue arrow). Stain H&E. Mag. X400

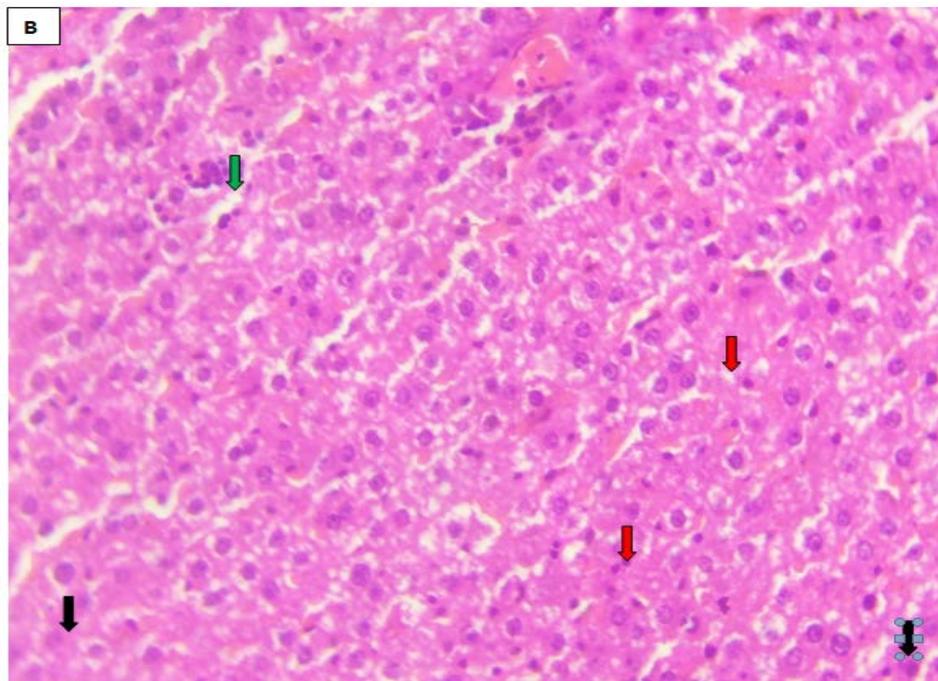


Figure 3: Photomicrograph of the liver tissue of group B (Cadmium only). There is a general histo-architectural distortion of normal radiating hepatocyte plate. The nuclei of the hepatocytes in B showed features of cytotoxicity like swollen nucleus (black arrow) and fragmented nucleolus (red arrow). Numerous black stained dot (Kupffer cells) (green arrow) are seen in the B. Stain H&E. Mag. X400.

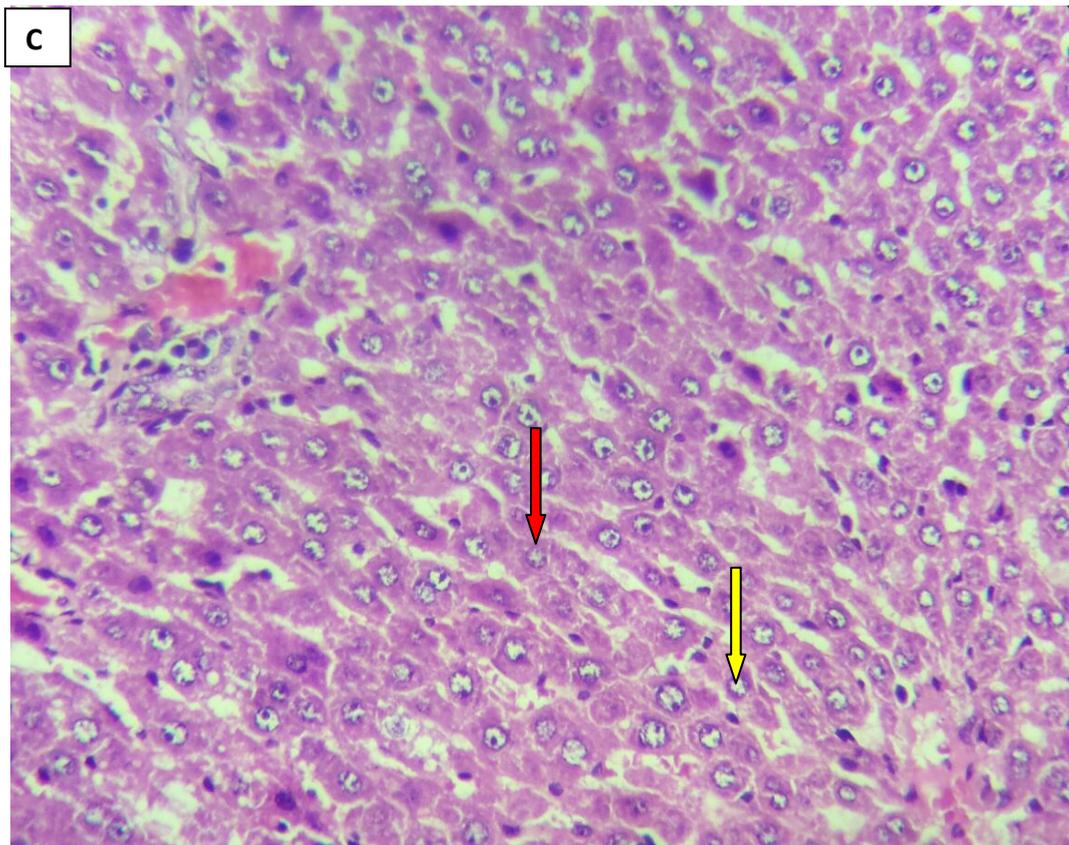


Figure 4: Photomicrograph of liver of rat pre-treated with extract of *Pyrus communis* showing recovering liver architecture. Some hepatocytes display clearing of nucleoplasm (yellow arrow) and fragmented nuclei (red arrow). Stain H&E. Mag. X400.

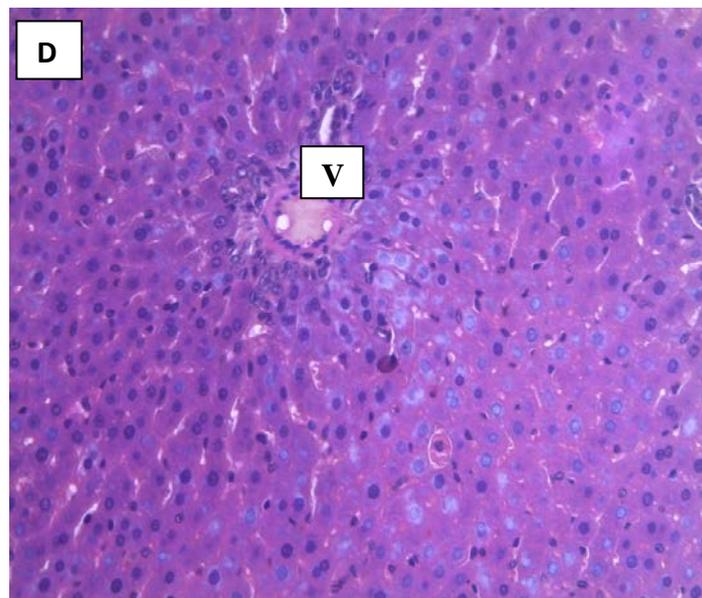


Figure 5: Photomicrograph of the liver of rat treated with extract of *Pyrus communis* only showing normally radiating hepatic plate. V is a portal tract with congested hepatic vein. Stain H&E. Mag. X400

4. Discussion

This study was aimed at determining protective ability of *Pyrus communis* seeds on some liver function parameters in heavy metal induced liver injury. Phytochemical screening of *Pyrus communis* seeds extract showed the presence of terpenoids, tannins, flavonoids, steroids, cardiac glycosides and alkaloids. Phytochemicals are biologically active compounds that are found in plants in small amounts. Alkaloids have both anti-bacterial and anti-fungal properties and have been used to prepare some drugs. Flavonoids have protective effects including anti-inflammatory, antioxidant, anti-viral and anti-fungal properties [19]. Tannins have anti-bacterial and anti-inflammatory properties [20]. Tannin is a general name for a group of polymeric phenolic compounds. Phenolics function as antioxidants via radical scavenging by H-donation, prevention of chain inhibition by donating electrons or by binding of metal ion catalysts [21]. Tanins in reaction with proteins gives a typical tanning effect which is important in the treatment of inflamed or ulcerated tissues [22]. The anti-inflammatory and antioxidative properties in the phytochemicals present in *Pyrus communis* are responsible for protecting liver damaged by cadmium toxicity this study.

Haptoglobin is synthesized in the liver [23], and it is physiologically present in serum within the range of 45 to 200 milligrams of haptoglobin per deciliter of blood (mg/dL) [8]. A decreased level of haptoglobin in serum is associated with conditions of intravascular hemolysis such as hemolytic anemia, malaria, congenital ahaptoglobinemia and severe liver diseases e.g. cirrhosis, hepatitis where protein synthesis is affected [24]. The treatment of rats with cadmium in this study resulted in significant reduction in the concentrations of haptoglobin, a C-reactive protein, in both liver and serum. Cadmium is known to impair synthesis of protein in liver and causes oxidative damage [25]. Treatment with *Pyrus communis* significantly improved synthesis of haptoglobin and other proteins in both pre-treated rats and those that were administered with plant extract alone.

The significant reduction in total protein and albumin levels seen in rats treated with cadmium in this study and significant increase of same in the serum may be as a result of oxidative injury of ROS on the membrane components of liver cells and resulting escape of intracellular contents into the circulation. Cadmium induces production of ROS in liver cells which attack membrane and intracellular constituents like proteins, lipids and nucleic acids [25, 26, 27]. The significant decrease in liver AST and ALT activities and significant increase in serum activities of these liver marker enzymes in rats treated with cadmium is a an indication of loss of structure and functions of hepatocyte membrane and release of the enzymes into the circulation. Serum ALT activity was also observed to be higher than AST. Serum ALT: AST ratio becomes ≥ 1 (normally < 1) in severe liver diseases [28]. The general liver histo-architectural distortions and varying degree of cytotoxicities seen in rats administered with cadmium in this study might have been resulted into significant ($p < 0.05$) decrease in total protein and haptoglobin concentrations observed in the results.

The near normal levels of haptoglobin, total protein, liver and sera ALT and AST, and normal liver histoarchitecture of rats treated with extract of *Pyrus communis* seed in this study showed that the plant is hepatoprotective. This may be due to previously reported anti-inflammatory and antioxidant properties of the phytochemicals present in the extract of *Pyrus communis* seeds [29].

5. Conclusion

We concluded that 5mg/kg body weight of cadmium used in this study for the period of 2 weeks caused severe liver damage and depressed synthesis of proteins including haptoglobin, a C-reactive protein and serum antioxidant. Also, extract of *Pyrus communis* seeds protected against severe cadmium-induced liver damage and restored haptoglobin expression.

6. Recommendation

The plant is a rich source of bioactive compounds which can be explored in Nutraceuticals and also in the development of health supplements.

7. Limitation

The limitation of the study was the inability to elucidate the secondary metabolites in the plant extract using GCMS.

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