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EFFECTS OF AQUEOUS EXTRACT OF *Petroselinum crispum* ON LIVER AND HEMATOLOGICAL PARAMETERS IN CARBON TETRACHLORIDE (CCl₄) - INDUCED HEPATOTOXICITY IN MALE WISTAR RATS

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ABSTRACT

In recent years, focus on plant research has increased to show the potential of some medicinal plants in discovery of novel compounds which could be used in treatment of some diseases. Petroselinum crispum is widely used traditionally as a food additive and herbal remedy for many ailments. This study is aimed to assess the effects of Petroselinum crispum on some liver enzymes and some haematological parameters in Carbon tetrachloride (CCL₄) - induced hepatotoxicity in male wistar rats. A total of 25 wistar rats were used, which were divided into 5 groups of 5 rats each. Group 1: (control); Group 2: received IP injection of 120mg/kg mixture of CCL₄ and olive oil (1:1), Groups 3, 4 and 5 were orally administered 250, 500 and 1000mg/kg extract + IP injection of 120mg/kg mixture of CCL₄ and olive oil for 21 days respectively. On the 22nd day, the animals were sacrifice using chloroform anesthesia and the effects of the aqueous extract were assessed by quantifying liver enzymes such as serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and total serum protein (TP). The haematological parameters were assessed by analysis of parked cell volume (PCV), red blood cell count (RBC), white blood cells (WBC), haemoglobin (Hb) concentrations and platelet count (PLT) and followed by histopathological studies of the liver tissues. The three doses of the plant extract used in this study, showed some level of protective effect on CCL₄-induced hepatotoxicity as evident by the significant reduction (P < 0.05) in serum levels of AST, ALT, and ALP along with the improved histopathological liver sections compared to CCL₄ -treated animals. However the extract is more effective in the 500mg/kg compared to the 250mg/kg and 1000mg/kg. Also the results obtained for all the haematological parameters used in the study showed no significant difference among all the groups (P > 0.05).

Keywords: *Petroselinum crispum, Hepatotoxicity, Carbon tetrachloride (CCL₄), Parsley, Wistar rats*

INTRODUCTION

Liver is one of the most crucial and indispensable organ in the body with multifunctional capabilities. It is involved in the metabolism of nutrients such as lipids, proteins, and carbohydrates, as well as in the excretion of waste metabolites. In addition, liver is also the first destination of toxins from the intestinal tract, and thus, the liver is involved in the It also detoxifies drugs, alcohol, chemicals, heavy metals, infectious organisms, as well as toxin by-products from the blood (Abhilash *et al.*, 2014). The liver also metabolizes substances

breakdown and elimination of toxins such as drugs and other foreign chemical substances (Okaiyeto *et al.*, 2018). It serves as a storage compartment for numerous substances such as glycogen, vitamins, minerals, and iron. Whenever there is depletion in the level of blood sugar in the body and energy is needed, the liver breaks down stored glycogen into glucose that is then utilized by the body. in the bloodstream before they are distributed to the different parts of the body where they are needed (Nayak *et al.*, 2011).

Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion of reduced glutathione levels. In addition, serum levels of many biochemical markers like transaminases, alkaline phosphatase, bilirubin, triglycerides and cholesterol are elevated in liver disease (Subramaniam *et al.*, 2015). The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. (Friedman *et al.*, 2003).

Carbon tetrachloride (CCl₄), is a solvent, and a potent hepatotoxic agent that is widely used in animal models for induction of liver damage (Das and Holt, 2011). Its mechanism of action start at the hepatocytes where the activation of cytochrome P450 enzyme produces trichloromethyl and trichloromethyl peroxide free radicals. These free radicals tightly bind to the phospholipid molecules of the cell membranes, endoplasmic reticulum, and mitochondria, causing lipid peroxidation, oxidative stress and the release of byproducts that block the intracellular proteins and DNA (Ingawale *et al.*, 2014). The reactive aldehydes that is produced as byproducts of lipid peroxidation subsequently damage the structure and function of the cellular and intercellular membranes, resulting in hepatotoxicity and carcinogenicity (El Aaraq *et al.*, 2019).

Petroselinum crispum known as Parsley in English, Baqdnis in Arabic and Ganyen Faski in Hausa, is a herb that originated in the central Mediterranean region of southern Italy, Algeria, Morocco and Tunisia and now cultivated almost every in the world (Meenakshi, 2019). It is commonly used as a garnish in soups, salads, meats, vegetables and sauces. Traditionally, the leaf, seed and root are being used in herbal medicine as enema, orally as tea to control high blood pressure and tonic to strengthen the bladder (Awe and Banjoko, 2013). *Petroselinum crispum* leaves are rich source of bioactive compounds such as, furanocoumarins, essential oils, flavonoids, carotenoids, vitamins, minerals and fatty acids (Dadan *et al.*, 2018). These compounds have a wide spectrum of healing properties such as hepatoprotective, neuroprotective, analgesic, anti-diabetic etc (Chauhan and Aishwaya, 2018). Several studies have highlighted the role of these phytochemicals that may act as antioxidants to ameliorate the liver damage caused by CCl₄ to normal tissue (Adeyemi *et al.*, 2014).

The study of hematological status is one of the important ways for diagnosis of the root cause of diseases. Blood is the most important tissue, in which changes in metabolic processes are reflected, therefore, abnormal alteration in blood parameters are the reliable indicator of toxic effects of drugs, chemicals and diseases (Lodia and Kansala, 2012). Therefore, evaluation of various hematological parameters can be used to determine the extent of deleterious effect of extracts on the blood of an animal and it can also be used to explain blood-related functions of a plant extract or its products (Yakubu *et al.*, 2007).

Liver performs numerous and vital roles in maintaining homeostasis, it is the primary site of haematopoiesis in foetal life. It stores iron, folic acid and vitamin B12, secretes clotting factors and inhibitors (Rappai *et al.*, 2019). Hence, liver diseases cause wide range of abnormalities in haematological parameters such as generalized suppression of blood cell production and produces abnormal blood cell precursors that cannot mature into functional cells (Rappai *et al.*, 2019). Therefore, liver enzymes and hematological parameters are indicators of liver function, the present study is thus intended to provide additional scientific literature on the effects of aqueous extract of *P. crispum* on liver function and hematological parameters in Carbon tetrachloride (CCL₄) induced hepatotoxicity in adult male Wistar rats.

MATERIALS AND METHODS

Plant Collection, Identification and Extraction

Fresh leaves *Petroselinum crispum* were collected from Yankaba market in Kano state in the northwestern part of Nigeria. The fresh leaves were identified by a plant taxonomist from the department of Botanical sciences, Bayero University Kano with accession number (BUKHAN 404). The aerial parts were separated and dried under shade at room temperature and powdered finely. The powdered (500g) herb leaves were placed in a 2000 ml (2L) round-bottom quick fit conical flask, and 2 liters of distilled water was added; the mixture was left for 24 hours, and filtered through qualitative Whatman filter paper. The resulting filtrate was then evaporated via water bath under reduced pressure to dryness. The extract was then kept in a refrigerator throughout the experiment and 250,500 and 1000mg/kg of the extract was dissolved in a distilled water and administered to the rats daily (Gulcin *et al.*, 2006).

Phytochemical Screening

Phytochemical analysis of the aerial parts of *Petroselinum crispum* was conducted according to the methods of Trease and Evans, 1989; and AOAC, 1990 for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, saponins, sterols and glycosides.

Experimental animals

A total of thirty seven (37) adult male Wistar rats were used for the study out of which 12 were used for LD₅₀ while 25 rats were used for the main experiment. After the acclimatization period of two weeks, the animals were randomly assigned into five (5) experimental groups of five (5) rats each. Food and water were given to the animals *ad libitum*. The study was performed in the animal house of the department of Human Physiology Bayero University Kano. The animals of **Group 1** received 1ml of distilled water orally, **Group 2** were injected 120mg/kg/bw mixture of CCL₄ and olive oil intraperitoneally (IP) every 48 hours, **Group 3** received 250mg/kg/bw of the extract daily orally and IP injection of 120mg/kg mixture of CCL₄ and olive oil every 48 hours for 21 days, **Group 4** received 500mg/kg/bw of the extract daily orally and IP injection of 120mg/kg mixture of CCL₄ and olive oil every 48 hours for 21 days and **Group 5** received 1000mg/kg of the extract daily and IP injection of 120mg/kg/bw mixture of CCL₄ and olive oil every 48 hours for 21 days (Jafar *et al.*, 2015).

Acute toxicity (LD50) study of the Parsley leaves extract

The method of Lorke (1983) was used to determine the LD₅₀ of *Petroselinum Crispum*. A total of 12 Wistar rats were used. This method involved two phases. The aqueous extract of parsley was administered orally using cannula.

Procedure

In phase one, three groups each containing three albino rats, were orally administered single dose of aqueous extract of parsley at doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight respectively and were observed for 24hours. In the second phase, another three groups each contains one albino rat were orally administered single dose of aqueous extract of parsley at doses of 1600mg/kg, 2900mg/kg, and 5000mg/kg respectively. They were observed for death and behavioral changes within 24hours. LD₅₀ value was then determined using $LD_{50} = \sqrt{(D_0 \times D_{100})}$. Where D₀ = Highest dose that gave no mortality and D₁₀₀ = Lowest dose that produced mortality

Determination of Biochemical markers

Blood samples (5ml) from all the groups were collected via cardiac puncture using a 5ml

syringe which was divided into 2 equal halves for both biochemical and hematological parameters, 1 half was then put in plain sample bottles and kept at room temperature for 1 hour and thereafter, centrifuged at 4000 rpm for 15 min. The serums obtained were used for liver function analysis. The serum level of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP) and Total protein (TP) were all estimated using mindray BA-88A Semi-automatic biochemistry analyzer.

Determination of hematological parameters

Cell blood counter (Mindray BC-10 Automatic Hematology Analyzer) was used for measuring of Red blood corpuscles (RBCs) count (10⁶/μL), Hemoglobin (Hb) content (g/L), Packed cell volume (%) Platelets (PLT) count (10³/μL), and White blood cells (WBC) count (10³/μL).

Histopathological Studies

After sacrifice, livers from all the groups were removed, cut into small pieces and then fixed in 10% formaldehyde solution overnight followed by dehydration with ascending grade of alcohol, cleared with toluene, infiltrated with molten paraffin wax 4-5 μm (thick), sections were cut using microtome. The sections were then stained with basic staining technique hematoxyline (H) for 40 sec and counterstained with acidic stain eosin (E) for 20 sec. After proper staining the slides were observed with leica DM 750 microscope with (magnification of 100X and 400X) and photographed with leica ICC50 HD Camera to identify the damages like necrosis, portal inflammation, vascular congestion, fatty infiltration, vacuolar degeneration, leukocyte infiltration, loss of structure of hepatic nodules and so forth (Auwioro, 2010; Dutta *et al.*, 2018).

Statistical Analysis

The data was presented as mean ± standard error of mean (S.E.M). One-way analysis of variance (ANOVA) was used to compare the means and then followed by turkey's post- hoc test to show multiple comparisons between groups. P < 0.05 was considered statistically significant. All the analysis was carried out using SPSS (Statistical Package for Social Science) Version 25.

RESULTS

Phytochemical Results

The preliminary phytochemical screening of *Petroselinum Crispum* leaves revealed the presence of glycosides, flavonoids, alkaloids, tannins, saponins, and steroids (Table 1).

Table 1: Phytochemical screening of aqueous extracts of *P. crispum*

Phytochemical screened	Present	Absent
Flavonoids	+	
Alkaloids	+	
Tannins	+	
Saponins	+	
Steroids	+	
Glycosides	+	

Acute toxicity (LD₅₀) of Aqueous Extract of *P. crispum*

There was no death recorded throughout the study at all the doses of the aqueous extract of

Petroselinium crispum in both phases of the study, and the LD₅₀ was found to be above 5000mg/kg declaring the extract as relatively nontoxic.

Table 2. Acute toxicity (LD₅₀) of Aqueous Extract of *P. crispum*

		PHASE I		
Dose(mg/kg bwt)	No. of rats	No. of deaths	Survival	Mortality ratio
10	3	0	3	0/3
100	3	0	3	0/3
1000	3	0	3	0/3
		PHASE II		
1600	1	0	1	0/1
2900	1	0	1	0/1
5000	1	0	1	0/1

Effects of aqueous extract of *Petroselinium crispum* on serum levels of some liver enzymes

Table 3 present the levels of liver enzymes of the experimental groups. There was a significant difference ($P < 0.05$) between the control (105.94±3.65) and all the experimental groups (291.26±12.81, 186.26±9.59 and 363.40±6.30) and also a significant decreased between the experimental groups and the CCL₄ group (410.85±11.53) ($P < 0.05$) for AST. Also there was a significant increased ($P < 0.05$) between the control (119.92±4.53) and all the experimental groups 3, 4 and 5 (295.76±15.45, 211.28±10.83 and 388.60±2.96) respectively and a significant decreased between the CCL₄ group (415.84±11.87) and groups 3 and 4

(295.76±15.45 and 211.28±10.83) for the ALT respectively. Similarly, there was a significant increased ($P < 0.05$) between the control (62.14±4.10) and all the treatment groups (105.84±3.21, 94.22±4.92 and 121.78±4.00) also a significant decreased between CCL₄ group (142.42±6.14) and all the groups for ALP. Contrary to the other parameters, there was no significant difference among all the groups ($p > 0.05$) for the Total protein (TP).

Effects of aqueous extract of *Petroselinium crispum* on hematological parameters

Table 4 shows hematological parameters of male Wistar rats. There was no significant difference ($P > 0.05$) between the control group and the treatment groups for the PCV, Hb, RBCs, WBC and PLT

RESULTS**Table 3.** Effects of Aqueous Extract of *Petroselinium Crispum* (Parsley) on Serum Levels of AST, ALT, ALP and TP in CCL₄-Induced Hepatotoxicity in Male Wistar Rats. Values are presented as mean ± S.E.M.

Biochemical parameters	Group 1 Control	Group 2 CCL ₄	Group 3 CCL ₄ +250mg/kg parsley	Group 4 CCL ₄ +500mg/k g parsley	Group 5 CCL ₄ +1000mg /kg parsley	P Value
AST(U/L)	105.94±3.65	410.85±11.53 ^a	291.26±12.81 ^{ab}	186.26±9.59 ^{ab}	363.40±6.30 ^{ab}	0.001
ALT(U/L)	119.92±4.53	415.84±11.87 ^a	295.76±15.45 ^{ab}	211.28±10.83 ^{ab}	388.60±2.96 ^a	0.001
ALP(U/L)	62.14±4.10	142.42±6.14 ^a	105.84±3.21 ^{ab}	94.22±4.92 ^{ab}	121.78±4.00 ^{ab}	0.001
TP(g/L)	65.74±3.71	37.44±3.86	47.45±7.47	43.54±9.57	39.58±13.22	0.165

Keys: AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, TP: Total protein, $P < 0.05$ considered statistically significant. a: means there is significant difference compared to control group; b: means there is significant difference compared to group 2 (CCL₄ only)

Table 4. Effects of aqueous extract of *Petroselinum crispum* on hematological parameters in CCL4-induced hepatotoxicity in male Wistar rats.

Hematological Parameters	Group 1 Control	Group 2 CCL ₄	Group 3 CCL ₄ +250mg/kg Parsley	Group 4 CCL ₄ +500mg/k g Parsley	Group 5 CCL ₄ +1000mg /kg Parsley	P Value
RBC (10 ⁶ /μL)	7.02±0.56	7.32±0.51	7.12±0.57	8.08±0.50	7.42±0.30	0.60
PCV (%)	47.5±0.34	47.1±0.18	47.7±0.25	48.02±0.21	47.6±0.26	0.16
Hb (g/dl)	17.21±1.86	16.8±3.19	16.58±2.14	18.16±1.89	19.24±0.41	0.89
WBC (10 ³ /μL)	11.70±3.25	18.66±4.64	11.98±1.17	10.20±2.29	19.46±4.00	0.08
PLT (10 ³ /μL)	715.80±33.34	887.20±39.03	731.20±74.77	760.00±88.17	590.00±96.76	0.10

P < 0.05 considered statistically significant. All data are presented as mean ± S.E.M.

Keys: RBC: Red blood cells, PCV: Parked cell volume, Hb: Hemoglobin, WBC: White blood cells, PLT: Platelets

Histopathological Result

Histopathological liver section of normal control group (group I) showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, portal triads and central vein. In the CCL₄-only intoxicated group (group II), necrosis of hepatic architecture

were observed in the liver section. The liver sections of the rats treated with CCL₄ and various doses of aqueous extract of petroselinium crispum, showed minimal necrosis and regeneration of hepatocytes compared to CCL₄-only intoxicated group. Result were shown below (Plate I-V).

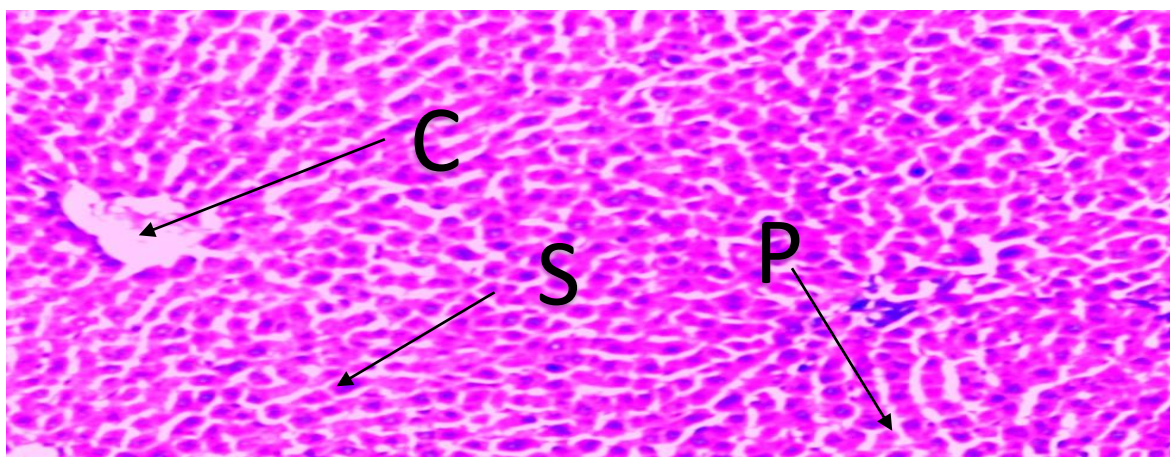


Plate I: Photomicrograph section of group 1 (control) hepatic tissue showing unremarkable hepatic tissue with defined Central vein (C), Portal triads (P), Sinusoidal spaces (S) and radiating cords of hepatocytes. (H & E X100)

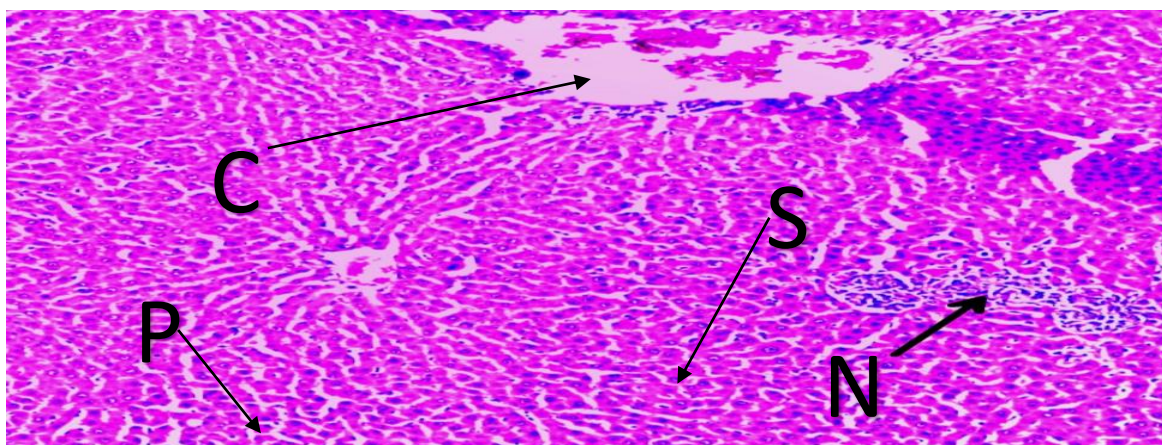


Plate II. Photomicrograph section of group 2 hepatic tissue showing unremarkable Central vein (C) with persistent multifocal hepatic necrosis confined to the vicinity of central vein, Sinusoidal spaces (S), focal hepatocytes necrosis (N) and inflammatory cells. (H & E X100)

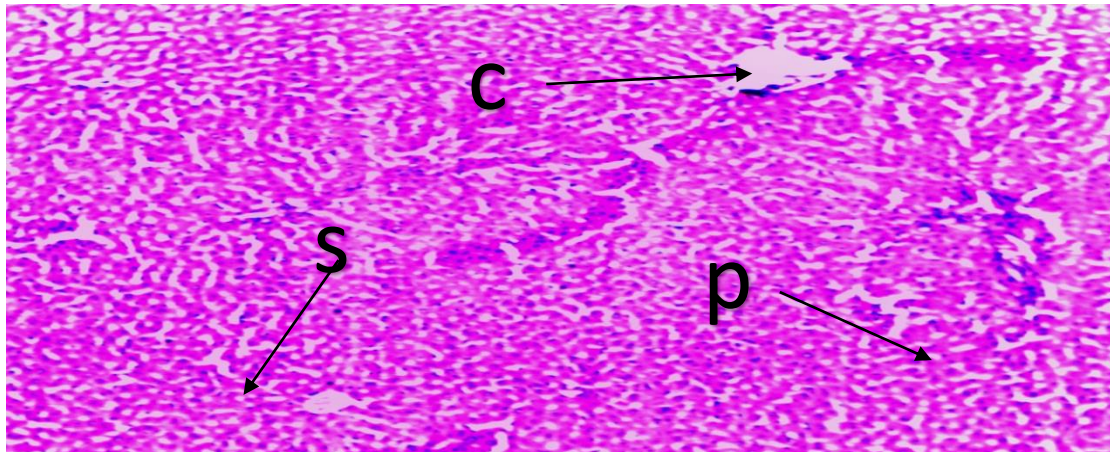


Plate III: Photomicrograph section of group 3 Wistar rats hepatic tissue showing unremarkable Central vein (C) with mild congestion, Sinusoidal spaces (S) and Portal triads (P) with preserve histoarchitecture of the liver tissue. (H & E X100)

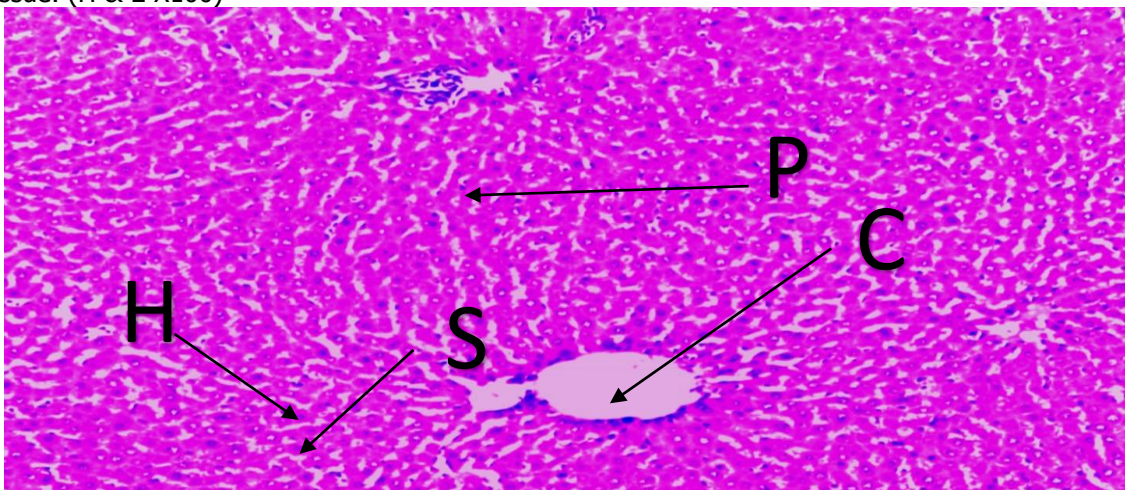


Plate IV: Photomicrograph section of group 4 Wistar rats hepatic tissue showing preserve histoarchitecture of hepatic tissue with defined Central vein (C) , Sinusoidal spaces (S) and Portal triads (P) and Hepatocytes (H). (H & E X100)

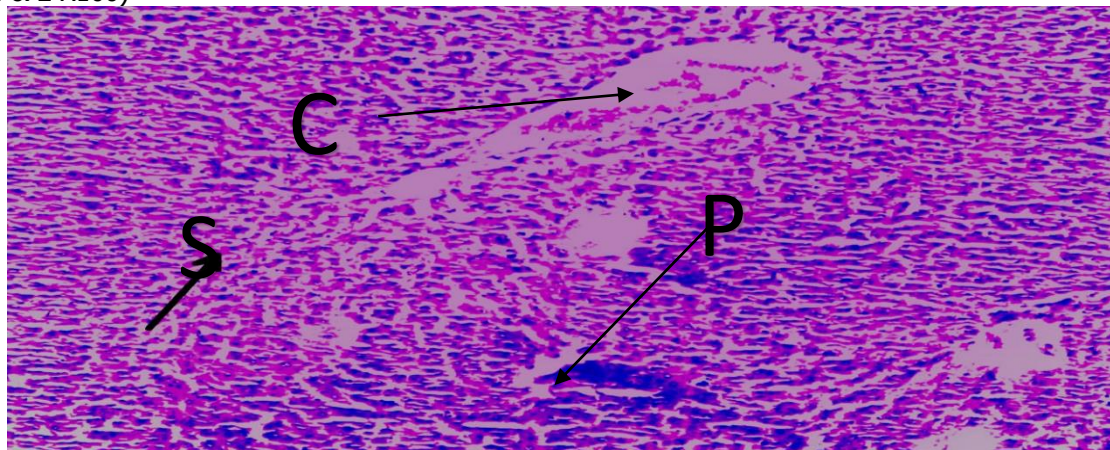


Plate V: Photomicrograph section of group 5 Wistar rats hepatic tissue showing distorted histoarchitecture of hepatic tissue with cytoplasmic vacuolation (V), dilated sinusoidal spaces (S), necrosis around the central vein (C) and mild to moderate inflammatory reactions. (H & E X100)

DISCUSSION

The results of the phytochemical screening of aqueous extract of *Petroselinum crispum* revealed the presence of glycosides, flavonoids, alkaloids, tannins, saponins, and steroid which is in accordance with the report of Al-Howiriny *et al.* (2003). Flavonoids being among the compounds found in *Petroselinum crispum* are secondary plant metabolites responsible for colour and aroma of flowers. Flavonoids interfere with multiple signal transduction pathways involved in liver diseases leading to inhibition of oxidative stress, apoptosis, and angiogenesis (Ravishankar *et al.*, 2013).

The results indicated that the administration of rats with CCL₄ was able to induce hepatotoxicity because the activity levels of ALT, AST and ALP liver enzymes were significantly increased in CCL₄ group when compared with the control group. The results of this study are in agreement with that of Leelaprakash *et al.* (2011), who reported statistically significant increase in plasma activities of ALT, AST and ALP in CCL₄ induced animals. However, there was no statically significant difference found between the control group and all the experimental groups in the serum level of TP which is in accordance with the reports of Ayman *et al.*, (2015).

In this study, it was observed that the aqueous extract of *Petroselinum crispum* was able to reduce to some extent the toxic effect of CCL₄ more effectively at dose of 500mg/kg administered in group 4 compared to the 250mg/kg and 1000mg/kg for groups 3 and 5 respectively. Parameters such as AST, ALT and ALP significantly decreased in the groups that were administered CCL₄ and *Petroselinum crispum* together as compared to the group which CCL₄ was administered alone. Frank *et al.* (2012) reported that CCL₄ produces oxidative damages in the liver resulting in increased in AST, ALT, ALP levels and decrease in total protein (TP). Therefore, the significant reduction in serum levels of AST, ALT and ALP in the experimental groups compared to the CCL₄ group is an indication of possible stabilization of plasma membrane as well as possible repair of hepatic tissue damage caused by CCL₄ which is similar to the report of Ozturk *et al.* (2012).

The elevation in the plasma levels of cytoplasmic and mitochondrial enzymes accurately reflects liver injury induced with CCL₄. Such increase in the levels of certain enzymes (ALT, AST) under the influence of CCL₄ have been attributed to the disruption or damage of the structural integrity of the liver (Al-Howiriny *et al.*, 2003). The Significant decreases in the level of ALT and AST compared to the CCL₄ group by the extract suggests its protective effects on the structural integrity of the hepatocyte cell

membrane. The increased level of the plasma alkaline phosphatase (ALP) is another measure of liver damage occurring due to the *de novo* synthesis by the liver cells as revealed by Recknagel *et al.* (1973). In the present study it was observed that on treatment with the *Petroselinum crispum* extract, there was a significant decrease in plasma alkaline phosphatase level indicating the ability of the extract to gradually reduce the damage caused by CCL₄. This effect might be due to either a decrease in *de novo* synthesis or by a feedback mechanism (Al-Howiriny *et al.*, 2003). However, administration of 250mg/kg and 500 mg/kg of the extract was more effective in lowering the enzymes level compared to 1000mg/kg. It was earlier suggested that the extract at higher doses above 1000mg/kg could be toxic (Awe and Banjoko, 2013).

There was no significant difference found in the blood parameters (PCV, Hb, RBCs, WBCs and PLT) in this study which is similar to the findings of Awe & Bonjoko, (2013) and Al-Awaida *et al.* (2014) who conducted a study on leaf ethanol extract of *Petroselinum crispum* in male Wistar rats, they reported no significant differences in the erythrocytic parameters (PCV, RBC and Hb) at all the doses studied in all experimental groups when compared to the control. Similarly they reported no significant difference in WBC and PLT at all doses which is similar to the present study. The reason for the absence of significant difference in the blood parameters might be due to the presence of different compounds found in the *Petroselinum crispum* which might counter the effects of one another.

Contrary to the findings of Ahmed *et al.* (2020) whose study on fresh leaves of *Petroselinum crispum* added as food supplement on Potassium Bromate- fed Wistar rats for (30 and 60 days) was found to significantly increased the blood parameters (P < 0.05) in the experimental groups compared to control group. The difference might be attributed to differences in route of administration, duration of administration, concentration of the extract (20mg/kg parsley and 20mg/kg parsley with 600mg/kg potassium bromate) environmental factors, constituents of the toxic substances present in the agents used and other interventions that differs between the two studies.

A study by Al-Daraji *et al.* (2012) on the Influence of *Petroselinum crispum* as Feed Additive on Hematological Traits of Local Iraqi Geese also reported a significant increase in PCV, Hb concentration and WBC when compare to the control which is in contradiction with the findings of the present study in which no significant differences in all the parameters.

The differences might be due to the differences in terms of duration of the experiment, concentration of the extract (80g/d, 160g/d and 240g/d) fresh parsley leaves against (250mg/kg, 500mg/kg and 1000mg/kg) aqueous extract of *Petroselinum crispum* with IP injection of 120mg/kg mixture of CCL₄ with olive oil of the current study, environmental factors and other intervention factors. However, they also reported no significant increase in RBC count between the control and experimental groups which is in line with the current study. Similarly, a study by Abdel-Wahhab *et al.*, (2018) contradict the findings of the current study where he reported that oral administration of *Petroselinum crispum* with Isoniazid resulted in occurrence of physiological or hypochromic anemia which monitored from the significant decrease ($P < 0.05$) in hemoglobin content, RBCs count (erythrocytopenia) also, thrombocytopenia (decrease in platelets count) and leucopenia (reduction of TLC) were recorded in compare to control group. The differences may occur as a result of difference in concentration of the extract, duration, rout of administration and the constituent of the toxic agent used (250mg/kg aqueous parsley and 250mg/kg/d parsley with 50mg/kg/d isoniazid) orally for 8weeks against (250, 500 and 1000mg/kg) aqueous extract of *Petroselinum crispum* orally with 120mg/kg mixture of CCL₄ with olive oil IP injection for 3 weeks for the present study.

The histopathological examination of the liver tissue of group 1 (control) showed normal histoarchitecture of the hepatic tissue with well - marked central vein, portal triads, and regular cords of hepatocytes. While sections of group 2 (CCL₄ only) the liver morphological changes was evidenced by marked necrosis which are confined to the vicinity of the central vein, severe dilatation of sinusoidal area, degeneration of the hepatocytes, fatty deposits development and inflammatory cells which is in accordance with the studies of Musab *et al.* (2015) and Liu *et al.* (2013) while the liver sections of the rats in groups (3 & 4) treated with CCL₄ and various doses (250mg/kg & 500mg/kg) of aqueous extract of *Petroselinum crispum*, showed unremarkable central vein with preserved histoarchitecture, minimal necrosis and regeneration of hepatocytes compared to CCL₄-only intoxicated group, indicating the hepatoprotective effect of the tested extract. This findings are also in line with the report of (Awe & Bonjoko, 2013). However, the liver sections of rats in group 5 treated with CCL₄ and 1000mg/kg showed distorted histoarchitecture of hepatic tissue with cytoplasmic vacuolation, dilated sinusoidal space, degeneration

of hepatocytes, necrosis around the central vein and mild to moderate inflammatory reactions which is in agreement with the findings of (Awe and Bonjoko, 2013) who reported that at higher doses (≥ 1000 mg/kg) *Petroselinum crispum* could be toxic but not lethal to the liver tissue. Contrary to the report of Al-howiriny *et al.* (2002) who reported that even at doses above 1000mg/kg the ethanolic extract of *Petroselinum crispum* exhibited significant anti-inflammatory and anti-hepatotoxic activities. He reported that in animals pretreated with the plant extract and subsequently given CCL₄, there was relatively well preserved cytoarchitecture around periportal tract area.

Hepatic fibrosis, necrosis, fatty deposition, inflammation and degeneration of hepatocytes are present in various hepatic diseases. It is well known that constant fibrosis can lead to the development of hepatocellular carcinoma (Okita *et al.*, 2002). Interrupting and/ or reversing hepatic damage may well be a new approach for improving its progression to hepatocellular carcinoma. In this study, *Petroselinum crispum* a traditional herb used for treatment of various hepatic disorders had therapeutic effects on hepatic injury induced by CCL₄ exposure in rats. The beneficial effects of *Petroselinum crispum* in the treatment of chemically-induced liver injury were evident in liver pathology, as evidenced by the severity of the liver morphological changes and fibrosis, generalized improvement of some types of pathological lesions such as fatty liver and cellular degeneration, and reduced hepatic collagen fiber staining in group 3 and 4 rats. These *Petroselinum crispum* preventive effects may be mediated by inhibition of hepatic stellate cell activation. In accord with the preventive effects of *Petroselinum crispum* components during the development of chemically induced hepatic damage (Liu *et al.*, 2002), the present result shows that *Petroselinum crispum* is effective in the prevention of CCL₄-induced liver damage, which would be a highly significant therapeutic advantage. Fatty liver is simply the build-up of fat in the liver. It is known that fat accumulates in the liver with a multifactorial phenomenon thought are caused by a blockage of lipoprotein secretions; impaired synthesis or peroxidation of phospholipids, or both; the toxic effects of free alkyl radicals on cell membranes; and disturbances in methylation reactions (Junnila *et al.*, 2000). The free alkyl radicals of CCL₄ metabolite are thought to cause fatty infiltration in the liver. The action of free alkyl radicals on biomembranes was found to develop haloalkylation-dependent blocking at the exit of the lipoprotein micelles from the Golgi apparatus (Jer-Yuh *et al.*, 2006).

Hepatoprotective agents exert their action against CCL₄-induced liver injury by impairment of CCL₄-mediated lipid peroxidation, either through decreased production of free radical derivatives or due to the antioxidant activity of the protective agent itself (Ahmed *et al.*, 2000). Administration of different antioxidants together with CCL₄ may help to prevent the development of liver injury due to the free radical scavenging properties possessed by antioxidants that decreased the overload on the endogenous body antioxidants (Khalaf *et al.*, 2009). The results of the present study revealed that the aqueous extract of *Petroselinum crispum* possesses some hepatoprotective effect against experimental CCL₄-induced liver damage in animals which could result from the natural flavonoid content (puerarin) along with the antioxidant and anti-inflammatory activity of the plant extract. Zheng *et al.*, (1992) reported that *Petroselinum crispum* is rich in myristicin which showed a high activity as an inducer of the detoxifying enzyme glutathione S-transferase (GST) in the liver and small intestine mucosa of mice. Fejes *et al.* (1998) indicated that *Petroselinum crispum* contains flavonoids, essential oils, coumarins and vitamin C. The hepatoprotective role of *Petroselinum crispum* could result from the natural flavonoid contents along with the antioxidant and anti-inflammatory activity of the extract.

CONCLUSION

Based on the findings of this study, it was concluded that:

1. The results of the phytochemical screening of *Petroselinum crispum* revealed the presence of flavonoids, alkaloids, tannins, saponins, steroids and glycosides.
2. There was no death of the rats recorded in both the two phases of the LD₅₀ of the plant extract, and therefore, the LD₅₀ of the plant extract was found to be above 5000mg/kg.

REFERENCES

- Abdel-Wahhab, K. G., Abdel-Baset, M. A., Abd El-Reheem, M.M., Abdel-Wahhab, S. M. E. and Soliman, M. A. (2018). Rosemary and parsley extracts minimize Isoniazid®-induced haematological deterioration and enhance the oxygenation potential in adult male albino rats. *Journal of Innovations in Pharmaceutical and Biological Sciences*, 5 (2): 93-100.
- Abhilash, G., Maheswari, Y.J., Gopal, A. and Chanda, D. (2014). Review on some medicinal plants with hepato-protective activities. *Research Review Journal of*

3. There was a significant decreased in serum level of AST between the experimental groups (291.26±12.81, 186.26±9.59, 363.40±6.30) compared to the CCL₄ group (410.85±11.53).
4. The results also shows a statistical significant decreased in serum level of ALT in the treatment groups (295.76±15.45, 211.28±10.83, 388.60±2.96) compared to CCL₄ group (415.84±11.87).
5. The plant extract also shows a significant decreased in serum level of ALP in the experimental groups (105.84±3.21, 94.22±4.92, 121.78±4.00) compared to CCL₄ group (142.42±6.14).
6. There was no statistical significant difference found between the experimental groups and the control in all the haematological parameters studied in this study.
7. The aqueous extract of *Petroselinum crispum* was able to gradually stabilize and possibly restore to some extent the hepatic injury caused by CCL₄ as was observed in the histological slides with the extracts more effective in group 4 (500mg/kg) than group 3 and 5 (250mg/kg and 1000mg/kg) respectively.

Recommendations

- ❖ . Further studies are required in order to isolate the active compound responsible for the protective effects of *Petroselinum crispum* so that it may be used more precisely and efficiently to combat various liver disorders
- ❖ Caution should also be exercised in the use of Parsley in treatment of ailments to avoid overdosing due to the lack of standardized dosing in herbal medicine due to paucity of information on Pharmaco-genetics and Pharmacodynamics of herbal preparations.

Pharmacognosy and Phytochemistry; pp. 22:33.

- Adeyemi, D. O., Ukwanya, V. O., Obuotor, E. M. and Adewole, S. O. (2014). Anti-hepatotoxic activities of *Hibiscus sabdariffa* L. in animal model of streptozotocin diabetes-induced liver damage. *BMC Complementary Alternative Medicine*, 14: 14-277.
- Ahmed, S., Rahman, A., Alam, A., Saleem, M., Athar, M. and Sultana, S. (2000). Evaluation of the efficacy of Lawsonia alba in the alleviation of carbon tetrachloride-induced oxidative stress.

- Journal of Ethno pharmacology*; 69: 157-64.
- Ahmed, W.E., Ali, A. E. and Osman, I.M. (2020). Antioxidant Activities of Parsley (*Petroselinum crispum*) on the Induced Biochemical and Histopathological Changes of Potassium Bromate-Fed Rats. *Pakistan Journal of Nutrition*, 19: 80-85.
- Al-Awaida, W. M. A and Baker, J. A (2014). Protective role of aqueous medicinal herbal extracts against oxidative stress on Glucose-6-phosphate dehydrogenase activity and RBC fragility. *Journal of Life Sciences*; 11(2).
- Al-Daraji, H.J., Al-Mashadani, H.A., Al-Hassani, A.S., Mirza, H.A., Al-Hayani, W.K. (2012). The Influence of Parsley (*Petroselinum crispum*) as Feed Additive on Hematological Traits of Local Iraqi Geese. *International journal of scientific research*, 1 (1): 1-5.
- Al-Howiriny, T.A., Al-Sohaibani, M.O., El-Tahir, K.H. and Rafatullah, S. (2003). Preliminary evaluation of the anti-inflammatory and anti-hepatotoxic activities of 'Parsley' *Petroselinum Crispum* in rats. *Journal of natural remedies*; vol 3: 54-62.
- AOAC (1990), Official Methods of Analysis, 15th ed, Washington, DC: Association of Official Analytical Chemists; 429-434.
- Auwioro, O.G. (2010). Histochemistry and Tissue Pathology Principle and Techniques. 2nd edition.
- Awe, E.O and Banjoko, S.O. (2013). Biochemical and haematological assessment of toxic effects of the leaf ethanol extract of *Petroselinum crispum* (Mill) Nyman ex A.W. Hill (Parsley) in rats. *BMC Complementary and Alternative Medicine*; 13:75.
- Ayman, F.K., Haiam, O.E. and Hanaa, F.E. (2015). Protective effect of peppermint and parsley leaves oils against hepatotoxicity on experimental rats. *Annals of Agricultural Science*; 60 (2), 353-359.
- Chauhan, E.S. and Aishwaya, J. (2018). Nutraceuticals potential of *Petroselinum crispum*: A Review. *Journal of Complementary and Alternative Medicine*; 7, 1-6.
- Dadan, M., Rybak, K., Wiktor, A., Nowacka, M., Zubernik, J., Witrowa-Rajchert, D. (2018). Selected chemical composition changes in microwave-convective dried parsley leaves acted by ultrasound and steaming pre-treatments—An optimization approach. *Food Chemistry journal*; 239, 242-251.
- Das, P., and Holt, S. (2011). Liver disease and renal dysfunction. *Journal of Medicine*; 39, 492-496
- Dutta, S., Chakraborty, A.K., Dey, P., Kar, P., Guha, P. and Sen, S. (2018). Amelioration of CCl₄ induced liver injury in swiss albino mice by antioxidant rich leaf extract of *Croton bonplandianus*. *Baill Plos One*; 13(4): e0196411.
- El-Aarag, B., Khairy, A., Khalifa, S. A. M and Hesham R. El-Seedi, H. R. (2019). Protective Effects of Flavone from *Tamarix aphylla* against CCl₄-Induced Liver Injury in Mice Mediated by Suppression of Oxidative Stress, Apoptosis and Angiogenesis. *International Journal of Molecular Sciences*; 20, 5215.
- Fejes, S., Kery, A., Blazvoics, A., Lugasi, A., Lemberkovics, E., Petri, G. and Szoke, E. (1998). Investigation of the in vitro antioxidant effect of *Petroselinum crispum* (Mill). Nym ex A. W. Hill. *Acta Pharm Hung*, 68 (3): 150-156.
- Frank, P.R., Suresh, V., Arunachalam, G., Kanthlal, S. and Ziaudheen, V. (2012). Evaluation of hepatoprotective effect of *Adiantum incisum* forsk leaf extract against CCl₄ induced hepatotoxicity in rats. *International Research journal of Pharmacology*; 3, 230-234.
- Friedman, S.E., Grendell, J.H., Quaid, M. and Kenneth, R. (2003). Current diagnosis and treatment in gastroenterology. New York: Lang Medical Books/McGraw-Hill; P. 664-79
- Gulcin, I., Elmastas, M. and Aboul-Enein, H.Y. (2006). Determination of Antioxidant and Radical scavenging Activity of Basil (*Ocimum basilicum* L. Family Lamiaceae) Assayed by Different methodologies. *Phytotherapy Research*; 21: 354-361.
- Ingawale, D.K., Mandlik, S.K. and Naik, S.R. (2014). Models of hepatotoxicity and the underlying cellular, biochemical and immunological mechanism (s): a critical discussion. *Environmental toxicology and pharmacology*; 37(1): p. 118-133.
- Jafar, S., Mehri, L. and Parisa, R. (2015). Effects of Aqueous Extracts of *Petroselinumsativum* on Some Blood Serum Biochemical Factors and Hepatotoxicity on Ethylene Glycol-Induced Kidney Calculi in Rats. *2nd International Conference on Agriculture, Environment and Biological Science*; Aug 16-17.

- Jer-Yuh, L., Chang-Che, C., Wen-Hong, W., Jeng-Dong, H., Mon-Yuan, Y. and Chau-Jong, W. (2006). The protective effects of Hibiscus sabdariffa extract on CCl₄-induced liver fibrosis in rats. *Food and Chemical Toxicology*, 44: 336-43.
- Junnila, M., Rahko, T., Sukura, A. and Lindberg, L.A. (2000). Reduction of carbon tetrachloride-induced hepatotoxic effects by oral administration of betaine in male Han-Wistar rats: a morphometric histological study. *Journal of Veterinary Pathology*, 37 (3), 231-238.
- Khalaf, A. A., Mohy Eldin, M. M., Mohamed, S. M. and Ahmed, A. M. (2009). Comparative study on the protective effect of some antioxidants against CCl₄ hepatotoxicity in rats. *Egyptian Journal of Natural Toxins*, 6 (1): 59-82.
- Leelaprakash, G., Mohan Dass, S. and Sivajothi, V. (2011). Antioxidant and hepatoprotective activities of Vernonia cinerea extract against CCl₄ induced hepatotoxicity in albino rats. *International Journal of Pharmaceutical Sciences Review Resources*; 10, 30-34.
- Liu, C.L., Wang, J.M., Chu, C.Y., Cheng, M.T. and Tseng, T.H. (2002). In vivo protective effect of protocatechuic acid on tert-butyl hydroperoxide induced rat hepatotoxicity. *Journal of Food and Chemical Toxicology*; 40 (5), 635-641.
- Liu, Y., Meyer, C. and Xu, C. (2013). Animal models of chronic liver diseases. *American Journal of Physiology and Gastrointestine*; 304:449-468.
- Lodia, S., and Kansala, L. (2012). Antioxidant activity of rubia cordifolia against lead toxicity. *International Journal of Pharmacological Sciences and Research*. 3(7):2224-2232.
- Lorke, D. (1983). A New Approach to Practical Acute-Toxicity Testing. *Arch, Toxicology*; 53: 275- 289.
- Meenakshi, N. (2019). 23 wonderful parsley health benefits; reviewed by Sarah Pledgser.
- Musab, A. M., Imad, M.T., Abd-elwahab, H.M. and Hozeifa, M. H. (2015). Hepatoprotective effect of *adansonia digitata* l. (baobab) fruits pulp extract on ccl₄-induced hepatotoxicity in rats; *world journal of pharmaceutical research*; Vol, 4.
- Nayak, S.S., Jain, R., Sahoo, A.K. (2011). Hepatoprotective activity of *Glycosmis pentaphylla* against paracetamol-induced hepatotoxicity in Swiss albino mice. *Pharmaceutical Biology*; 49:111-7.
- Okaiyeto, K., Nwodo, U.U., Mabinya, L.V. and Okoh, A.I. (2018). A review on some medicinal plants with hepatoprotective effects; *Journal of Pharmacognosy Reviews*; 12:186-99.
- Okita, K., Sakaida, I. and Hino, K. (2002). Current strategies for chemoprevention of hepatocellular carcinoma. *Journal of Oncology* 62 (Suppl. 1), 24-28.
- Ozturk, M., Akdogan, M., Keskin, I., Kisioglu, A., Oztas, S. and Yildiz, K. (2012). Effect of Silybum marianum on acute hepatic damage caused by carbon tetrachloride in rats. *Biomedical Resources*; 23, 268-274.
- Rappai, M., Rao, P.S., and Ilanthodi, S. (2019). A Study of Variation in Haematological Parameters in Chronic Liver Disease. *Journal of Evolution of Medical and Dental Sciences*; doi: 10.14260/jemds/428.
- Ravishankar, D., Rajora, A.K., Greco, F., Osborn, H.M.I. (2013). Flavonoids as prospective compounds for anti-cancer therapy. *International Journal of Biochemistry and Cell Biology*. 45, 2821-2831.
- Recknagel, R.D., Glende E.A., Ugajio, G., Koch, K.R. and Srinivasan S. (1973). *Int. Symp. Hepatotoxic*. Academic Press: New York; 7.
- Subramaniam, S., Khan, H.B.H., Elumalai, N. and La.kshmi, S.Y.S. (2015). Hepatoprotective effect of ethanolic extract of whole plant of *Andrographis paniculata* against CCl₄-induced hepatotoxicity in rats. *Comprehensive Clinical Pathology*; 24:1-7.
- Treare, G.E. and Evan, W.C (1985). *Pharmacognosy* 17th edition, Bahiv Tinal, London, 149 pp.
- Yakubu, M. T., Akanji, M. A. & Oladiji, A. T. (2007). Heamatological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem. *Pharmacognosy Magazine*, 3(9), 34-38.
- Zheng, G.Q., Kenney, P.M., Zhang, J. and Lam, L.K. (1992). Inhibition of benzo (a)pyrene – induced tumorigenesis by myristicin, a volatile aroma constituent of parsley leaf oil. *Carcinogenesis*, 13 (10): 1921-1923.