Phytochemical Screening and Hepatoprotective Activity of the Aerial Parts of *Lotus polyphyllos* E.D. Clarke Family Fabaceae Growing in Egypt

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Abstract: Qualitative phytochemical analysis of the total 70% ethanolic extract of the aerial parts of *Lotus polyphyllos* E.D. Clarke belonging to family Fabaceae growing in Egypt and the fractions thereof was performed via qualitative phytochemical tests and thin layer chromatography. They were also studied for their hepatoprotective activity against CCl_4 intoxicated rats. Sterols, triterpenes, diterpenes, cardiac glycosides, cyanogens, saponins, flavonoids, condensed tannins and coumarins were present and differentially distributed in the fractions. On the other hand steam volatile substances, proteins, amino acids, anthraquinones and alkaloids were absent. It was found that the total extract at a dose of 300 mg/kg body weight exhibited the highest hepatoprotective activity compared with standard silymarin by significant protection against the elevation in the level of the biochemical parameters (AST, ALT and ALP). The hepatoprotective activity was also supported by histological and histochemical studies of liver tissues.

[Samir M. Osman, Mahmoud A. Koheil, Soad M. Abdel Khalek, Amr M. Saad Eldeen and Ahmed S. Alazzouni. **Phytochemical Screening and Hepatoprotective Activity of the Aerial Parts of *Lotus polyphyllos* E.D. Clarke Family Fabaceae Growing in Egypt.** *Life Sci J* 2013;10(3):2408-2417] (ISSN: 1097-8135). http://www.lifesciencesite.com. 350

Key words: *Lotus polyphyllos* E.D. Clarke, Fabaceae, Phytochemical screening, Hepatoprotective activity

1.Introduction

Family Leguminosae is one of the largest families of the flowering plants. It is classified into about 600 genera with 1200 species (Trease, *et al.*, 2002). It was recently divided into 3 families: Fabaceae, Caesalpiniaceae and Mimosaceae. Family Fabaceae is the largest one with about two-third of all the genera and species belonging to Leguminosae (Gledhill, 2008). Genus Lotus, belonging to family Fabaceae, contains approximately 100 species distributed throughout the world, especially around the Mediterranean region (El-Youssef, *et al.*, 2008). The Genus is represented in Egypt by 20 species including *Lotus polyphyllos* E.D. Clarke (Abdel Majeed, *et al.*, 2010).

Previous phytochemical investigations revealed that the various species of genus Lotus were rich in carbohydrates, triterpenoids and their saponins, steroids, coumarins, tannins, proteins, nucleic acid, carotenoids and nitrogenous compounds (Ali, *et al.*, 2001). Cyanogenic glycosides and flavonoids were considered as the major constituents. The aerial parts were found to be rich in flavones and flavonol derivatives (Abdel-Kader, *et al.*, 2007).

Traditionally, plants of genus Lotus were used as contraceptives, for prophylaxis and treatment of sexually transmitted disorders and peptic ulcers(Abdel-Kader, *et al.*, 2007). They were also evaluated for their possible anti-canceragainst MCF7, HeLa, and HepG2 cell lines (Tselepi, *et al.*, 2011), estrogenic, anti-platelet aggregation (El-Youssef, *et al.*, 2008), anti-inflammatory (El-Youssef, *et al.*, 2008; Pereira, *et al.*, 2011), anti-bacterial against Gram-positive and Gram-negative bacteria (Dalmarco, *et al.*, 2010)and against proteolytic rumen bacteria (Min, *et al.*, 2005) and antihistaminic activities (Bak, *et al.*, 2005).

The current study was designed for phytochemical screening and investigation of the*in-vivo* hepatoprotective of the total 70% ethanolic extract of *Lotus polyphyllos* E.D. Clarke and the fractions thereof.

2.Material and Methods

Plant material

The aerial parts of *Lotus polyphyllos* E.D. Clarke were collected from the Northern Coast, Borg Elarab, Alexandria, Egypt, in July 2011. The plant identity was confirmed by its taxonomical features by a taxonomist, Flora and Phytotaxonomy Research Department, Horticultural Researches Institute, Agricultural Research Centre, Dokki, Cairo, Egypt. A voucher herbarium specimen (# LP 11243) has been deposited at the Department of Pharmacognosy, Faculty of Pharmacy, October 6 University.

Preparation and Extraction of Plant material

Freshly collected plant material was washed

3 times with running tap water and then with distilled water followed by shade drying and powdering using mixer grinder. 400 g of the powder were defatted by percolation using 6 liters of *n*-hexane. 360 g of the defatted powder were percolated using 8 liters of 70% ethanol, the percolate is then filtered through a Whatman no. 4 filter paper and the filtrate was evaporated to dryness by a vacuum dryer (Rotavapor).This extract was suspended in distilled water and exhaustively partitioned successively with *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol. The solvents were, separately, evaporated under reduced pressure to dryness. These fractions were refrigerated until use.

Qualitative Phytochemical Analysis of the Total Extract and the Fractionsthereof

Phytochemical screening was carried for the total extract of the aerial parts of *Lotus polyphyllos* E.D. Clarke and the fractions thereof for identification of steam volatile substances (El-Kamali, *et al.*, 2010), carbohydrates and/or glycosides, sterols and/or triterpenes, diterpenes, proteins and/or amino acids, alkaloids, saponins(Tiwari, *et al.*, 2011), fixed oils and/or fats(Himesh, *et al.*, 2011), anthraquinones(Sofowora, 1993), cardiac glycosides, flavonoids (Trease, *et al.*, 2002), coumarins (Kokate, 2003) and cyanogens(Harborne, 1972).Thin layer chromatography was performed following the standard procedures (Harborne, 1998; Wagner, *et al.*, 1996).

***In-vivo* hepatoprotective activity evaluation**

Experimental animals

Wistar strain male albino rats weighing between 130 – 150 grams and aged between 2.5 – 3 months were produced from the animal house of NODCAR that is located at El-Giza city, Egypt, and were used for this evaluation. Animals were acclimatized for seven days prior to the experiment. They were housed in well ventilated colony cages (six rats per cage) at room temperature (25 ± 2 °C)in hygienic conditions under natural light and dark schedule with free access to standard laboratory food and water. All the experiments were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Ethics Committee (Veeraraghavan, 2000).

Determination of acute toxicity (LD_{50})

LD_{50} of the total 70% ethanolic extract of the aerial parts of *Lotus polyphyllos* E.D. Clarke and the fractions thereof were determined according to Karber, 1931. Dose level of 1, 2, 3, 4 and 5 g/kg body weight were chosen as dose level that would be expected to allow the identification of dose producing evident toxicity. Animals were observed principally for changes in skin, eyes, mucous membrane and also for autonomic symptoms like sedation, lacrimation, piloerection, urinary incontinence and central symptoms (drowsiness, tremors and convulsions).

Experimental design

The design described in Srilakshmi, *et al.*, 2010, was followed. The rats were divided randomly into nine groups of six rats each. The hepatoprotective activity of the tested samples was tested using CCl_4 model.Group I (normal control) remained under normal conditions, where it received neither the tested plant extracts nor CCl_4 for the whole 8 days of the experiment. Group II (negative control) also remained under normal conditions for 6 days, but in the 7th day, it was subjected to a single intraperitoneal dose of 30% CCl_4/olive oil of 1mg/kg body weight. Group III (positive control) was subjected to oral dose of silymarin of 200mg/kg body weight once daily for 7 days. It was also subjected to a single intraperitoneal dose of 30% CCl_4/olive oil of 1mg/kg body weight at the 7th day. Groups IV, V, VI, VII, VIII and IX were received an oral dose of 300mg/kg of the total 70% ethanolic extract, *n*-hexane, methylene chloride, ethyl acetate, *n*-butanol and remaining aqueous fractions, respectively, once daily for 7 days. These groups were also subjected to a single intraperitoneal dose of 30% CCl_4/olive oil of 1mg/kg body weight at the 7th day. On the 8th day, blood was withdrawn from all groups by puncturing the retro orbital plexus and collected in sterile centrifuge tubes and allowed to clot for 45 minutes at room temperature (Raoand Mishra, 1998). Serum was isolated and used for estimation of various biochemical parameters.

Assessment of the hepatoprotective activity

The hepatoprotective activity was evaluated biochemically, histologically and histochemically. The biochemical parameters estimated were alanine aminotransferase (ALT, serum glutamate pyruate or SGPT), asparatate aminotransferase (AST, serum glutamate oxaloacetate transaminase or SGOT) and alkaline phosphatase (ALP). These were estimated using respected assay kits according to the methods described by the manufacturers. Rats were sacrified, quickly dissected and the liver tissues were washed by saline and fixed in 10% formalin. The specimens were possessed for paraffin embedding using the standard microtechnique and then sectioned to 6µm thickness (Galighor, *et al.*, 1976). For histological examinations, sections were stained with Haematoxylin and Eosin (H-E) (Drury and Wallington, 1980). In the histochemical study, sections were stained with Periodic Acid Schiff (PAS) to demonstrate the carbohydrate content (Phifer,*et al.*, 1973) and with Bromophenol blue to demonstrate total proteins (Maize, *et al.*, 1953). These were examined microscopically and photographed.

Statistical analysis

The statistical analysis was carried out by Analysis of Variance (ANOVA) followed by Turkey Kramer test. All the data were presented as means ± SEM. Values were considered statistically significant when the probability (P) < 0.05 (Winer, 1971).

3.Results and Discussion

Phytochemical screening is of a great importance in identification of new sources of medicinally valuable compounds that can be applied effectively in the natural medicine regimens. The results of the phytochemical and TLC screening for the total 70% ethanolic extract and the fractions thereof of the aerial parts of *Lotus polyphyllos* E.D. Clarke are given in Tables 1 & 2. The percentage of the total 70% ethanolic extract was found to be 10% while those of the *n*-hexane, methylene chloride, ethyl acetate, *n*-butanol and remaining aqueous fractions were found to be 3%, 7%, 7%, 8% and 70%, respectively. The results revealed the presence of carbohydrates and/or glycosides in the total extract and all of the fractions thereof. Fixed oils and/or fats were found to be present in the total extract and the *n*-hexane fraction only.

Sterols and/or triterpenes were found to be concentrated in the total extract, *n*-hexane and methylene chloride fractions and absent from other fractions. They have been reported to possess cytotoxic (Rios,*et al.*, 2001; Li,*et al.*, 2002; Rosas,*et al.*, 2007) and anti-inflammatory (Akihisa, *et al.*, 2007) activities.

The presence of diterpenes and cyanogens was confirmed in the total extract and the methylene chloride fraction and absent in all other fractions. Cardiac glycosides and condensed tannins were confirmed to be present in the total extract and the ethyl acetate fraction and absent from all other fractions. Tannins play important roles being strong antimicrobial (Akiyama, *et al.*, 2001; Funatogawa, *et al.*, 2004; Min,*et al.*, 2008), antiviral (Lin, *et al.*, 2004), antioxidant (Dangles, *et al.*, 2000; Gulcin, *et al.*, 2010; Sulaiman, *et al.*, 2011), antitumor (Lee, *et al.*, 1995; Sakagami, *et al.*, 2000) and hepatoprotective (Lin, *et al.*, 1998) agents.

The tests for flavonoids showed their distribution in the total extract and all of the fractions thereof except for the *n*-hexane fraction. Over the last few years, several experimental studies have revealed biological and pharmacological properties of phenolic compounds, especially their antimicrobial (Barberan, *et al.*, 1990; Rauha, *et al.*, 2000; Zhu, *et al.*, 2004) and antiviral (Chiang, *et al.*, 2002; Du, *et al.*, 2003; Li, *et al.*, 2004). It is a well documented fact that the most medicinal plants that are enriched with phenolic compounds have excellent antioxidant and cytotoxic properties (Owen, *et al.*, 2000; Cai, *et al.*, 2004; Rao, *et al.*, 2007). Phenolics are active in protecting against liver damage (Yoshikawa, *et al.*, 2002; Oh, *et al.*, 2004; Jain, *et al.*, 2008) as well as helpful as anti-inflammatory in action (Azuma, *et al.*, 1986; Ferrandiz, *et al.*, 1991; Kupeli, *et al.*, 2007).

Saponins were found to be distributed in the total extract and all of the fractions thereof except for the *n*-hexane and methylene chloride fractions. They have been reported to possess antibacterial (Mandal, *et al.*, 2005; Soetan, *et al.*, 2006; Hu, *et al.*, 2012) and antiviral (Amoros, *et al.*, 1987; Simoes, *et al.*, 1999; Roner, *et al.*, 2007) activities. They also possess antioxidant (Huong, *et al.*, 1998) and hepatoprotective (Lee, *et al.*, 2008; Firdous, *et al.*, 2008; Huang, *et al.*, 2012) effects. Recent studies have indicated that saponins offer preferential chemical prevention strategy in lowering the risk of human cancer (Yan, *et al.*, 2009; Podolak, *et al.*, 2010). Saponins are widely well known to have expectorant activities (Guo, *et al.*, 2009).

The presence of coumarins was confirmed in the total extract, methylene chloride and ethyl acetate fractions and absent from all other fractions. The results also revealed the absence of steam volatile substance, proteins and/or amino acids, anthraquinones and alkaloids.

Determination of LD_{50} revealed that the total 70% ethanolic extract of the aerial parts of *Lotus polyphyllos* E.D. Clarke and the fractions thereof were safe at dose levels reaching 5g/kg body weight. Therefore, *Lotus polyphyllos* E.D. Clarke was considered as a safe edible plant and 300mg/kg body weight for each of its total extract and the fractions thereof was considered to be safe and convenient dose. Liver injuries induced by carbon tetrachloride was found to be the best characterized system of xenobiotic-induced hepatotoxicity besides that the changes associated with CCl_4-induced liver damage were similar to those of the acute viral hepatitis. Silymarin was used as standard hepatoprotective compound since it was reported to have a protective effect on the plasma membrane of the hepatocytes (Srilakshmi, *et al.*, 2010).

Effects of the total 70% ethanolic extract of the aerial parts of *Lotus polyphyllos* E.D. Clarke and the fractions thereof on the serum biochemical parameters of CCl_4 intoxicated rats were recorded in Table 3. Rats belongs to Group II (the negative control) that were intoxicated with CCl_4 significantly altered the biochemical parameters when compared with the normal control rats (P< 0.001), where there was elevation in the levels of AST, ALT and ALP. The hepatotoxicity of CCl_4 is supposed to be due to its active metabolite, trichloromethyl radical (Srivastava, *et al.*, 1990; Johnston and Kroening, 1998). This radical binds to the macromolecules inducing lipid peroxidative degradation of biomembranes of the

endoplasmic reticulum which is one of the principal causes of hepatotoxicity of CCl_4 (Cotran, *et al.*, 1994). Rats that received 200mg/kg body weight of the standard silymarin (belongs to Group III, the positive control) were protected considerably against the elevation in the levels of the biochemical parameters when compared with the CCl_4 group ($P< 0.001$). Pretreatment with the total 70% ethanolic extract at dose level of 300mg/kg body weight showed the highest hepatoprotective activity among the other fractions by significant protection against the elevation in the levels of the biochemical parameters (AST, ALT and ALP) when compared with the CCl_4 group ($P< 0.001$) and insignificantly when compared with the silymarin group ($P > 0.05$).

Since results of biochemical studies of blood samples of CCl_4 intoxicated rats showed significant increase in the levels of serum AST, ALT and ALP, reflecting the liver injury caused by CCl_4 and the blood samples from the rats pretreated with the total 70% ethanolic extract showed significant protection against the elevation in the levels of these serum markers, indicating the protection of the hepatic cells, the total 70% ethanolic extract of the aerial parts of *Lotus polyphyllos* E.D. Clarke could afford significant dose-dependent protection against CCl_4 induced hepatocellular injury. The potent and significant hepatoprotective activity of the total 70% ethanolic extract of the aerial parts of *Lotus polyphyllos* E.D Clarke may be attributed to its high content of phenolic compounds (Yoshikawa, *et al.*, 2002; Oh, *et al.*, 2004; Jain, *et al.*, 2008) and saponins (Lee, *et al.*, 2008; Firdous, *et al.*, 2008; Huang, *et al.*, 2012).

Table 1. Results of qualitative phytochemical analysis of the total extract and the fractions thereof of the aerial parts of *Lotus polyphyllos* E.D. Clarke by phytochemical tests.

Phytochemical test	Total extract and the fractions thereof					
	Total 70% ethanolic extract	*n*-Hexane fraction	Methylene chloride fraction	Ethyl acetate fraction	*n*-Butanol fraction	Remaining aqueous fraction
1. Test for detection of steam volatile substance						
1.1. Hydrodistillation test	-					
2. Tests for detection of carbohydrates and/or glycosides						
2.1. Molisch's test	++	+	+	++	+	++
2.2. Fehling's test	++	-	-	+	-	+
3.Tests for detection of proteins and/or amino acids						
3.1. Biuret test	-	-	-	-	-	-
3.2. Xanthoproteic test	-	-	-	-	-	-
4. Tests for detection of fixed oils and/or fats						
4.1. Spot test	++	++	+	-	-	-
4.2. Saponification test	++	++	+	-	-	-
5. Tests for detection of sterols and/or triterpenes						
5.1. Salkowski's test	+++	++	+++	-	-	-
5.2. Libermann's test	+++	++	+++	-	-	-
6. Test detection of for diterpenes						
6.1. Cu acetate test	++	-	++	-	-	-
7. Tests for detection of anthraquinones						
7.1. Borntrager's test	-	-	-	-	-	-
7.2.Modified Borntrager's	-	-	-	-	-	-
8. Tests for detection of cardiac glycosides						
8.1. Baljet test	+	-	-	+	-	-
8.2. Keller Killiani test	+	ND	ND	ND	ND	ND
9. Test for detection of cyanogens						
9.1. Picric acid paper test	+	-	+	-	-	-
10. Tests for detection of alkaloids						
10.1. Mayer's test	-	-	-	-	-	-
10.2. Dragendorff's test	-	-	-	-	-	-
11. Test for detection of saponins						
11.1. Froth test	+	-	-	+	+	+
12. Tests for detection of flavonoids						
Amyl alcohol test	++	-	++	++	+	++
NaOH test	++	-	++	++	+	++
Shinoda's test	++	-	++	++	+	++
13. Tests for detection of tannins						
Ferric chloride test	++ (Green)	-	-	++ (Green)	-	-
13.2. Gelatin test	+	-	-	+	-	-
14. Test for detection of coumarins						
14.1. NaCl test	++	-	+	+	-	-

Strongly present: +++, Present: ++, Weakly present: +, Absent: -, ND: Not Determined.

Table 2. Results of qualitative phytochemical analysis of the total extract and the fractions thereof of the aerial parts of *Lotus polyphyllos* E.D. Clarke by thin layer chromatography.

Thin layer chromatography for detection of	Solvent system	Extract / fraction	Total bands	Spray reagent
Sterols / triterpenes	Ethyl acetate: methanol: water (77:15:8)	T	7	Vanillin / sulphuric acid
		H	2	
		MC	5	
	Benzene: ethyl acetate (2:1)	T	1	
		H	7	
		MC	1	
Cardiac glycosides	ethyl acetate: methanol: ethanol: water (81:11:4:8)	T	2	Chloramine trichloroacetic acid.
		E	2	
Cyanogens	Chloroform: methanol (5:1)	T	1	2% alcoholic α-naphthol followed by sulphuric acid
		MC	1	
Saponins	chloroform: glacial acetic acid: methanol: water (64:32:12:8).	T	6	Vanillin / sulphuric acid
		E	4	
		B	4	
		A	6	
Phenolics	Ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:26)	T	10	5% Aluminum chloride
		MC	6	
		E	7	
		B	4	
		A	7	
	Chloroform: ethyl acetate (60:40)	T	11	
		MC	13	
		E	6	
		B	1	
		A	1	
	Chloroform: methanol (9:1)	T	8	
		MC	7	
		E	8	
		B	1	
		A	9	

T: total 70% ethanolic extract, H: *n*-hexane fraction, MC: methylene chloride fraction,
E: ethyl acetate fraction, B: *n*-butanol fraction, A: remaining aqueous fraction.

Results of histological and histochemical studies provided supportive evidence for biochemical analysis. In normal control rats (belongs to Group I), liver sections showed normal hepatic cells with normal cellular architecture and normal hepatic lobules. Normal concentration and distribution of carbohydrate and protein contents were also detected (Figure 1).In negative control animals (belongs to CCl_4 intoxicated, Group II), the sections showed total loss of cellular architecture with enlarged nuclease, marked fatty changes and marked portal vein congestion. Marked carbohydrate depletion with abnormal distribution and mild depletion of protein with non-homogenous distribution were also shown (Figure 2).

Pretreatment of the animals with silymarin (belongs to positive control, Group III) resulted in mild degree of cellular degeneration with mild nuclear enlargement, mild cellular infiltration and moderate fatty changes. Marked protection against depletion of carbohydrate and protein caused by $CCl_4$with normal distribution except in the areas representing fatty changes were also observed (Figure 3).Pretreatment of the animals with the total 70% ethanolic extract of the aerial parts of *Lotus polyphyllos* E.D, Clarke (belongs to Group IV) showed a mild degree of cellular degeneration with mild nuclear enlargement, mild cellular infiltration with mildly congested portal vein and mild fatty changes.It showed also a significant protection against depletion of carbohydrate and protein caused by CCl_4 with normal distribution except in the areas representing fatty changes (Figure 4). The protection against depletion of carbohydrate and protein contents in the liver tissues with normal distribution indicated maintaining of liver function, storage and synthesis, as carbohydrate is one of the most important components stored in the liver and proteins are typically synthesized in its tissues (Anthea, *et al.*, 1993). Pretreatment with the other fractions showed moderate to mild protection against the toxic effects of CCl_4 on the liver cells (Figures 5-9).

Table 3. Effects of total 70% ethanolic extract of the aerial parts of Lotus polyphyllos E.D. Clarke and the fractions thereof on the serum biochemical parameters of carbon tetrachloride intoxicated rats (values are mean ± SEM).

Groups	Serum biochemical parameters		
	AST	ALT	ALP
I (normal control)	198 ± 35	79 ± 9	398 ± 18
II (CCl_4) (negative control)	2047 ± 25 [a]	1179 ± 28 [a]	1235 ± 32 [a]
III (Silymarin) (positive control)	475 ± 30 [a*]	226 ± 32 [*]	411 ± 3 [*]
IV (Total 70% ethanolic extract)	483 ± 13 [a*]	281 ± 29 [b*]	422 ± 38 [*]
V (*n*-Hexane fraction)	1153 ± 6 [a*]	1110 ± 31 [a]	675 ± 5 [a*]
VI (Methylene chloride fraction.)	3198 ± 34 [a*]	2688 ± 16 [a*]	933 ± 34 [a*]
VII (Ethyl acetate fraction)	1407 ± 26 [a*]	1381 ± 31 [a#]	878 ± 25 [a*]
VIII (*n*-Butanol fraction)	763 ± 35 [a*]	757 ± 31 [a*]	612 ± 16 [b*]
IX (Remaining aqueous fraction)	1762 ± 3 [a*]	1681 ± 26 [a*]	894 ± 13 [a*]

Superscripts a, b donate statistical significance in comparison to normal group P < 0.001, < 0.01 respectively.
Superscripts *, # donate statistical significance in comparison to CCl_4 group *P* < 0.001, < 0.01 respectively.

Figure 1. Photomicrograph of liver tissues of normal control rats showing normal hepatic cells with normal cellular architecture and normal hepatic lobules (**a**). PAS staining showing normal concentration and distribution of carbohydrate (**b**). Bromophenol staining showing normal concentration and distribution of protein (**c**).

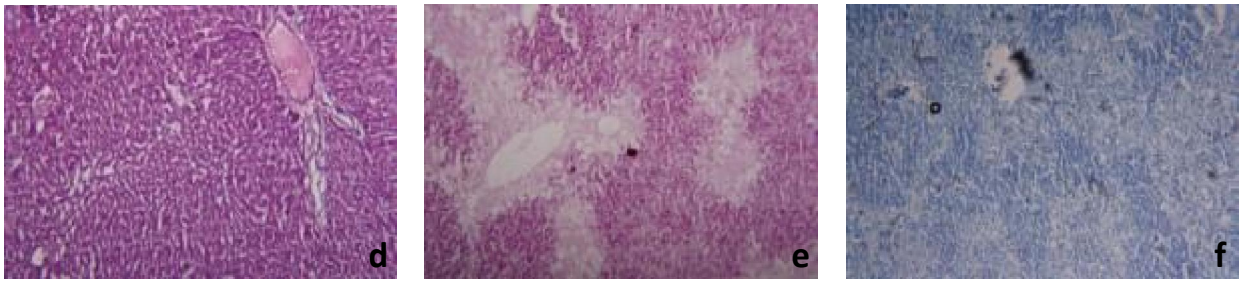

Figure 2. Photomicrograph of liver tissues of CCl_4 intoxicated rats showing total loss of cellular architecture with enlarged nuclease, marked fatty changes and marked portal vein congestion (**d**). PAS staining showing marked carbohydrate depletion with abnormal distribution (**e**). Bromophenol staining showing mild depletion of protein with non-homogenous distribution (**f**).

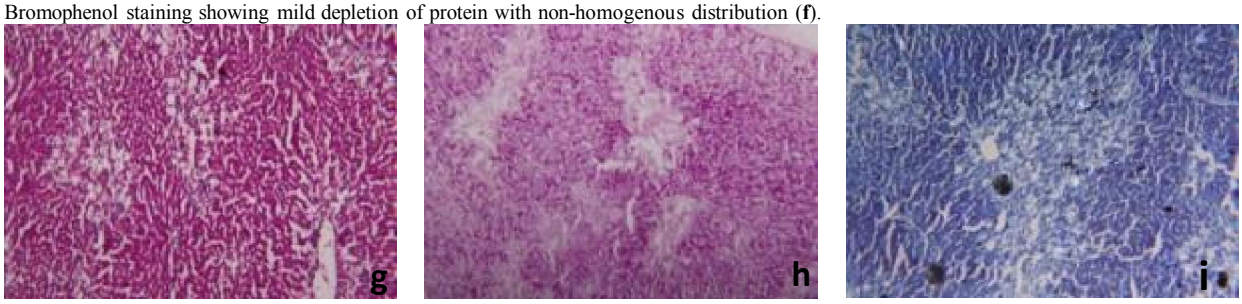

Figure 3. Photomicrograph of liver tissues of silymarin pretreated rats showing mild degree of cellular degeneration with mild nuclear enlargement, mild cellular infiltration and moderate fatty changes (**g**). PAS staining showing marked protection against depletion of carbohydrate caused by CCl_4 with normal distribution except in the areas representing fatty changes (**h**). Bromophenol staining showing marked protection against depletion of protein caused by CCl_4 with normal distribution except in the areas representing fatty changes (**i**).

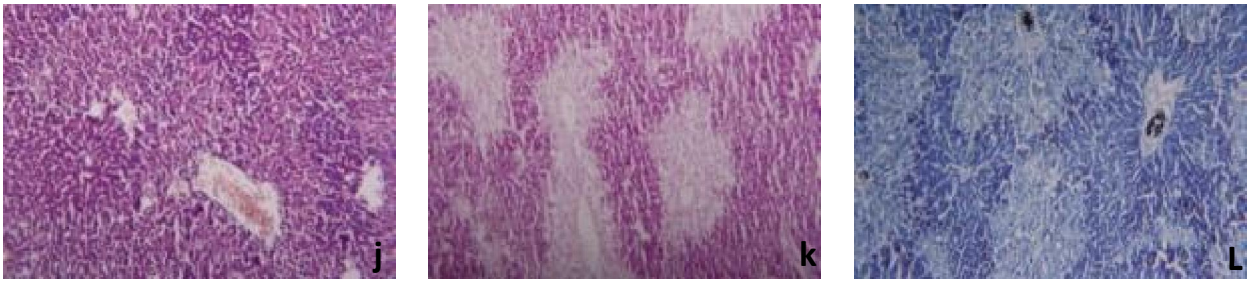

Figure 4. Photomicrograph of liver tissues of rats pretreated with total 70% ethanolic extract of aerial parts of *Lotus Polyphyllos* showing mild degree of cellular degeneration with mild nuclear enlargement, mild cellular infiltration with mildly congested portal vein and mild fatty changes (**j**). PAS staining showing a significant protection against depletion of carbohydrate caused by CCl_4 with normal distribution except in the areas representing fatty changes (**k**). Bromophenol staining showing a significant protection against depletion of protein caused by CCl_4 with normal distribution except in the areas representing fatty changes (**L**).

Figure 5. Photomicrograph of liver tissues of rats pretreated with *n*-butanol fraction showing mild degree of cellular degeneration with mild nuclear enlargement, mild cellular infiltration with mildly congested portal vein and mild fatty changes (**m**). PAS staining showing moderate protection against depletion of carbohydrate caused by CCl_4 with normal distribution (**n**). Bromophenol staining showing moderate protection against depletion of protein caused by CCl_4 with normal distribution except in the areas representing fatty changes (**O**).

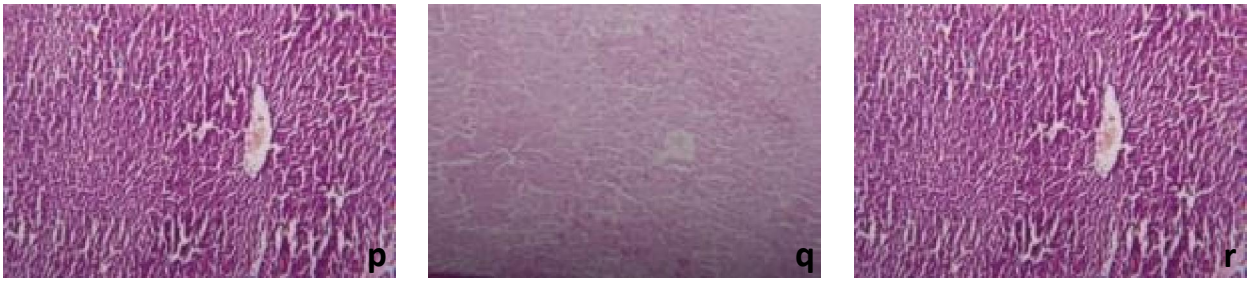

Figure 6. Photomicrograph of liver tissues of rats pretreated with *n*-hexane fraction showing mild degree of cellular degeneration, marked cellular infiltration and moderate fatty changes and loss of cellular architecture (**p**). PAS staining showing moderate protection against depletion of carbohydrate caused by CCl_4 with normal distribution (**q**). Bromophenol staining showing moderate protection against depletion of protein caused by CCl_4 with homogenous distribution (**r**).

Conclusion

Phytochemical screening of the total 70% ethanolic extract of the aerial parts of *Lotus polyphyllos* E.D. Clarke and the fractions thereof was performed. Furthermore, they were also studied for their hepatoprotective activities against CCl_4 intoxicated rates. From the overall results of the biochemical, histological and histochemical examinations, it could be inferred that the total 70% ethanolic extract of the aerial parts of *Lotus polyphyllos* E.D. Clarke showed the highest hepatoprotective activity among the other tested fractions that may be attributed to its high content of phenolic compounds and saponins and thus suggesting its introduction as an efficient and safe phytotherapeutic in complementary medicine. Further studies should be conducted in order to isolate, identify, characterize and elucidate the structure of the hepatoprotective bioactive compounds.

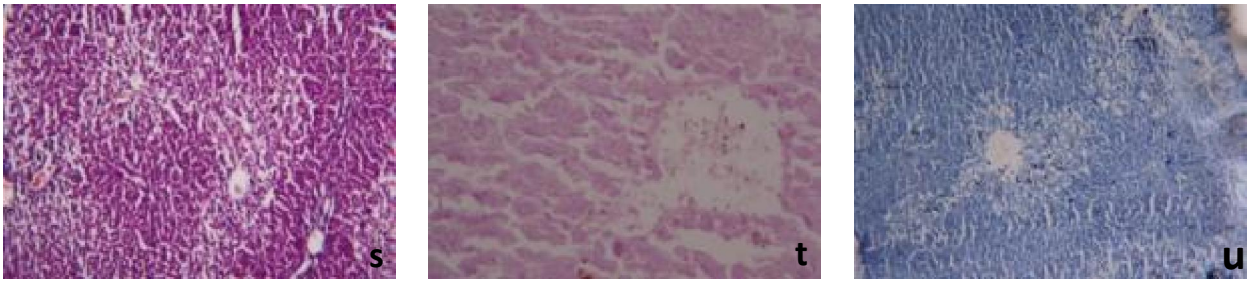

Figure 7. Photomicrograph of liver tissues of rats pretreated with ethyl acetate fraction showing mild degree of cellular degeneration, mild cellular infiltration with moderate congested portal vein and mild fatty changes (**s**). PAS staining showing moderate protection against depletion of carbohydrate caused by CCl_4 with normal distribution (**t**). Bromophenol staining showing moderate protection against depletion of protein caused by CCl_4 with homogenous distribution (**u**).

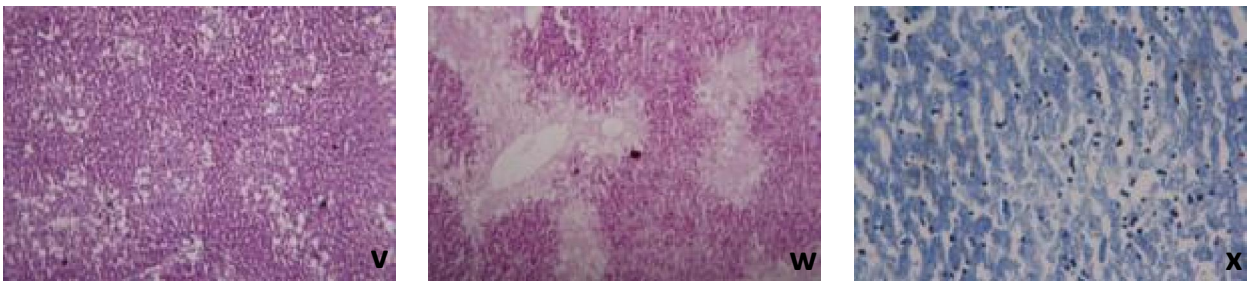

Figure 8. Photomicrograph of liver tissues of rats pretreated with remaining aqueous fraction showing marked degree of cellular degeneration, moderate cellular infiltration with congested portal vein and marked fatty changes (**v**). PAS staining showing marked carbohydrate depletion with abnormal distribution and thus no protection against toxic effects of CCl_4 (**w**). Bromophenol staining showing moderate protein depletion with non-homogenous distribution and thus no protection against toxic effects of CCl_4 (**x**).

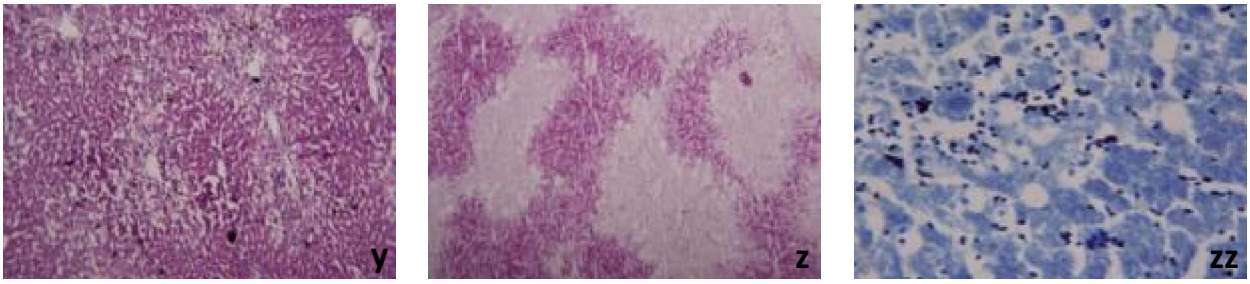

Figure 9. Photomicrograph of liver tissues of rats pretreated with methylene chloride fraction showing total loss of cellular architecture with enlarged nuclease, marked fatty changes with marked degenerative changes and moderate cellular infiltration (**y**). PAS staining showing marked carbohydrate depletion with abnormal distribution and thus no protection against toxic effects of CCl_4 (**z**). Bromophenol staining showing mild depletion of protein with non-homogenous distribution and thus no protection against toxic effects of CCl_4 (**z**).

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