In-vitro AND *In-vivo* ASSESSMENT OF TOXIC EFFECTS OF *Parthenium hysterophorus* LEAVES EXTRACT

ADIL HUSSAIN [a], AYAZ ALI KHAN [a], MUHAMMAD ALI [b], GHAZALA YASMIN ZAMANI [c], ZAFAR IQBAL [d], QARIB ULLAH [d], JAVID IQBAL [a], MUHAMMAD SHAHZAD [d] AND TARIQ AZIZ [d*]*

[a]*Department of Biotechnology, University of Malakand, Chakdara Pakistan.*
[b]*Department of Biotechnology, Quaid-e- Azam University Islamabad Pakistan.*
[c]*Department of Biotechnology, Bacha Khan University Charsadda Pakistan.*
[d]*Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan.*

ABSTRACT

Parthenium hysterophorus is an invasive plant variety found in around 50 countries. Phenolic compounds in the P. hysterophorus leaves, HPLC analysis was carried out. In addition, methanolic extract of P. hysterophorus leaves was evaluated for total phenolic content (TPC), total flavonoid content (TFC), DPPH free radical scavenging activity, and hemolytic activity. The leaves crude extract was orally administered to rabbits (n = 5) at four doses (10, 20, 40 and 80 mg kg^{-1}) for 9 days and its effects on hematological and biochemical parameters were investigated. Statistical analysis was performed using GraphPad Prism 8.0 software. The HPLC data revealed the presence of Chlorogenic acid, Bis-HHDP-hex (pedunculagin), Morin, Ellagic acid, Rutin, Syringic acid, etc., which were detected at various retention times. Among these compounds, Ellagic acid was abundantly present with sample peak area of 9594.909 %. Total phenolic and flavonoid contents in the leaves extracts at a concentration of 80 µg were, 57.35 ± 4.12 µg GAE/µg and 39.44 ± 0.41 µg QE/µg. DPPH free radical scavenging activity was 72.82 % with IC_{50} value of 168 µg/µL at 80 µg of the extract. In the hemolysis assay, 200 µg of extract had highest cell inhibition of 76.90 % with IC_{50} >500. Significant ($P<0.05$) variation in the hematological and biochemical parameters was observed in the extract fed groups. It has been concluded that P. hysterophorus leaves extract had toxic effects on the hematological and biochemical parameters in rabbits which cause abnormal blood profile.

Keywords: *Parthenium hysterophorus* leaves, biochemistry, toxicity, HPLC, hematology.

INTRODUCTION

Parthenium hysterophorus L. (Asteraceae) is commonly known as Skha-bootey (Pakistan), Parthenium weed (Australia), Congress weed (India), Carrot weed, Star weed, Feverfew (Caribbean), Ragweed Parthenium (USA) and White top. It is a fast growing, multi-branched annual weed. There are approximately 19 known species of *P. hysterophorus* present all over the world [1]. *P. hysterophorus* left its native range more than two centuries ago and now this weed has invaded 46 countries and territories [2]. Local spreading has been achieved through various pathways including water, wind, road, vehicles, agricultural machinery, and contaminated seeds. It can survive throughout the year, can grow up to 2 meters when germinated in a healthy soil, and produces flowers after 4 to 6 weeks of seedling [3]. Under ideal conditions, *P. hysterophorus* can form dense monospecific stands, with densities up to 370 plants m^{-2} [2]. It is resistant to drought and salt stress, enduring low water contents through drought tolerance response (Cowie et al., 2020). Health mature plants can produce 12,000 – 28,000 seeds which are small (2-3 mm in length, 1-2 mm in width and light in weight – 40-50 µg) and therefore, they are easily dispersed by water and wind. Furthermore, these seeds can germinate over a wide range of temperature – 5-40ºC, and they can remain viable for over a decade, forming persistent seed banks [4]. Autopsy revealed ulceration of alimentary tract, and extensive pathological changes were noticed in liver, kidney and skin [5]. *P. hysterophorus* methanolic extracts have different chemical compounds which are used to test the cytotoxic effect through different assays. *P. hysterophorus* extracts show cytotoxic effect. Studies indicate that many extract fractions of leaf, flower, stem, root, and whole plant extracts exhibited cytotoxic potential as it is tested on erythrocytes taken from rabbits and tested for different hematological and biochemical parameters [6].

A very significant decrease in the red blood cells, hemoglobin level, lymphocytes, and platelets count occur by taking *P. hysterophorus* methanolic extracts. A decrease in the amount of WBC count also occurs *in vivo* after feeding of *P. hysterophorus* methanolic extract [7].

The present study reports the toxicity of *P. hysterophorus* leaves both *In-vitro* and *In-vivo*. In addition, characterization of *P. hysterophorus* methanolic extract was made through HPLC to identify different phenolic compounds.

Abaxial surface Adaxial surface

Figure 1. P. Hysterophorus Leaves.

*Corresponding author email: ayazkhan@uom.edu.pk, iwockd@gmail.com

2.5.1 Selection of animals:

Rabbits were bought from different areas of District Malakand and were kept in animal house of the University of Malakand, Department of Biotechnology. Before starting the experiment, the rabbits were acclimatized for 10 days at 25°C and humidity (50% ± 5%). Before and during the experiment, the rabbits were fed with fresh grass and fresh drinking water.

2.5.2 Animal groups and crude extract dosage:

The rabbits were divided into 5 different groups and each group contained 3 rabbits.

Control group: control group received 1 mL distilled water.

Group A: Rabbits treated with LCe at dose of 10 mg kg^{-1} body weight dissolved in 1 mL of distilled water.

Group B: Rabbits treated with LCe at dose of 20 mg kg^{-1} body weight dissolved in 1 mL of distilled water.

Group C: Rabbits treated with LCe at dose of 40 mg kg^{-1} body weight dissolved in 1 mL of distilled water.

Group D: Rabbits treated with LCe at dose of 80 mg kg^{-1} body weight dissolved in 1 mL of distilled water.

2.5.3 Blood collection:

Blood samples of 5 ml from jugular vein were collected at 9th day of treatment for analysis of hematological and biochemical parameters. Aseptic condition and instruments were used during sampling.

2.5.4 Analysis of hematological parameters:

For all groups (n=5), the hematological parameters white blood cells (WBCs), platelets, Mean Corpuscular Hemoglobin (MCH), Hematocrit (HCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), red blood cells (RBCs), hemoglobin, and Packed cell volume (PCV) were measured.

2.5.5 Determination of Biochemical Parameters

The biochemical parameters were determined by using standard kits: Creatinine, Triglycerides, Cholesterol, Blood Urea, Total Protein, High Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDP). To determine the mentioned parameters, ultraviolet (UV) visible spectrophotometer (UV-Vis) (T60, PG instrument UK) was used.

2.6 Statistical Analysis:

Statistical analysis was performed using GraphPad Prism 8.0 software. The data was expressed as mean and standard deviation (SD). The significance of difference was calculated using one-way ANOVA and Tukey test of multiple comparisons. Values of $P<0.05$ were considered significant.

RESULT & DISCUSSION

3.1 In-vitro Toxic Effects of Leaves Crude Extract:

3.1.1 Characterization of leaves crude extract:

To identify different bioactive compounds (phenolic compounds), LCe was subjected to HPLC analysis. Figure 3.5 shows the retention time and peak area of different phenolic compounds which correlates with the available references and standard.

The Chlorogenic acid was identified at the retention time of 6.458 minutes with a peak area of 19.240 %. Bis-HHDP-hex (pedunculagin), Morin, 3-0-caffcoylquinic acid, Ellagic acid, Kaempferol-3-(p-coumaroyl-diglucoside)-7-glucoside, Rutin, Syringic acid, Quercetin-7-O-sophoroside, Kaempferol-3-(caffeoyl-diglucoside)-7-rhamnosyl, Pyrogallol, Mandelic acid, Quercetin-3-(caffeoyl-diglucoside)-7-glucoside, Quercitin-3-O-rutinoside, Glucose, Galactose were detected at retention time of 6.458, 11.309, 12.471, 14.155, 16,204, 18.430, 22.528, 23.335, 24.977, 25.803, 28.536, and 30.382 min, respectively, as shown in Table 1 and Figure 2.

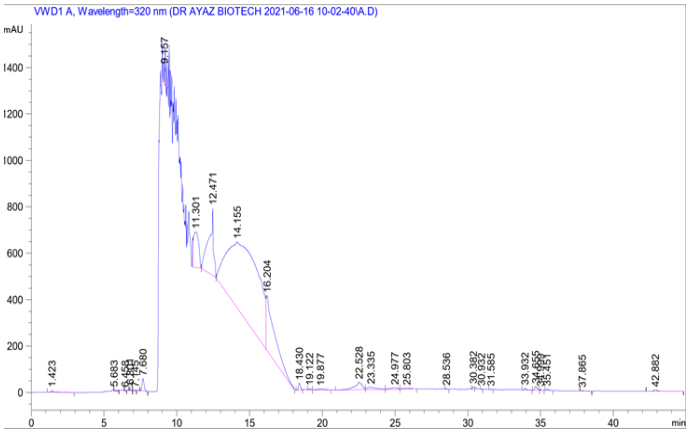

Figure 2. The HPLC Chromatogram of LCe.

Table 1. Identification of Phenolic Compounds in LCe through HPLC.

peak	Retention time, min	Proposed identity of compounds	Peak area %	Identification reference
1	6.458	Chlorogenic acid	19.240	Ref. Stand
2	11.309	Bis-HHDP-hex(pedunculagin)	3796.983	(Suryanti, Marliyana, & Putri, 2016)
3	12.471	Morin	6621.730	Ref. Stand
4	14.155	3-0-caffcovlquinic acid	4.797	(Suryanti et al., 2016)
5	16.204	Ellagic acid	9594.909	Ref. Stand
6	18.430	Kaempferol-3-(p-coumaroyl-diglucoside)-7-glucoside	286.356	(Hamid et al., 2010)
7	22.528	Rutin	1160.873	Ref. Stand
8	23.335	Syringic acid	469.629	(Hamid et al., 2010)
9	24.977	Quercetin-7-0-sophoroside	252.960	(Hamid et al., 2010)
10	25.803	Kaempferol-3-7-rhamnosyl	179.320	(Hamid et al., 2010)
11	28.536	Pyrogallol	27.3526	Ref. Stand
12	30.382	Mandelic acid	90.025	Ref. Stand
13	31.585	Quercetin-3-(caffeoyldiglucoside)-7-glucoside	13.677	(Ngueyem, Brusotti, Caccialanza, & Finzi, 2009)
14	34.999	Quercitin-3-O-rutinoside	21.468	(Hamid et al., 2010)
15	37.865	Glucose	35.255	(Ngueyem et al., 2009)
16	42.882	Galactose	142.129	(Ngueyem et al., 2009)

3.1.2 Total phenolic content (TPC) determination:

The LCe showed 57.00 ± 4.00 µg GAE/µg of phenolics contents followed by flowers and whole plant with 89.36 ± 4.72 and 62.69 ± 3.81 µg QE/µg of extract; stem and roots show 41.52 ± 0.85 and 42.07 ± 1.00 µg QE/µg of extract, as shown in Figure 3. The LCe showed highest flavonoids contents of 39.44 ± 0.41 µg QE/µg of extract, followed by flowers and whole plant with 26.01 ± 1.08 and 25.94 1.93 µg QE/µg of extract, while stem and roots show 15.57 ± 0.25 and 8.92 ± 0.65 µg QE/µg of extract, as shown in Figure 4.

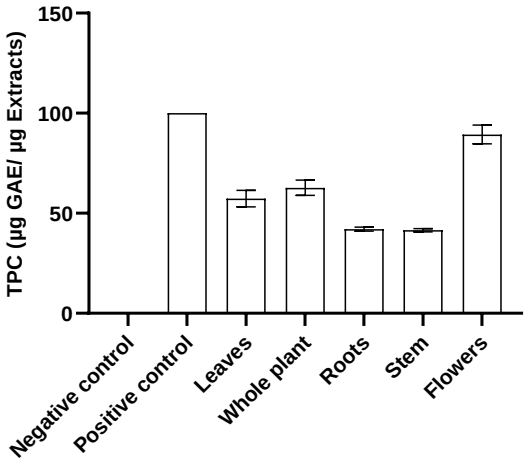

Figure 3. Total Phenolic Content of LCe.

LCe = P. hysterophorus leaves crude extract, GAE= gallic acid equivalent. Different alphabets on bar graph show significant difference (P<0.05) while same alphabets show no significant difference (P<0.05).

3.1.3 Total flavonoids content (TFC) determination:

The LCe showed highest flavonoids contents of 39.44 ± 0.41 µg QE/µg of extract, followed by flowers and whole plant with 26.01 ± 1.08 and 25.94 ± 1.93 µg QE/µg of extract, while stem and roots show 15.57 ± 0.25 and 8.92 ± 0.65 µg QE/µg of extract, as shown in Figure 4.

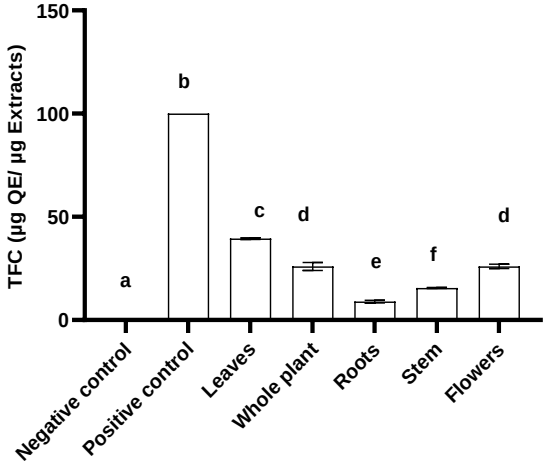

Figure 4. Total Flavonoids Content in LCe.

LCe = *P. hysterophorus* leaves crude extract, QA = quercetin equivalent. Different alphabets on bar graph show significant difference (P<0.05) while same alphabets show no significant difference (P<0.05).

3.1.4 DPPH assay:

LCe was tested for their antioxidant potential using DPPH standard assay at concentration of 20, 40, 60, and 80 µg. The crude extract increases their free radical scavenging activity as their concentration increase with an IC_{50} of 168.68 µg AAE /µg of the extract. It was found that at concentration of 80 µg of the crude extract, it possesses highest free radical scavenging activity with 72.8 % inhibition, followed by 60, 40, and 20 µg of the crude extract with scavenging activity 57. 7, 50.2, and 46.9 % respectively. Ascorbic acid was taken as a standard, and percent DPPH free radical scavenging activity of the LCe are shown in Table 2 and Figure 5.

Table 2. The Percent DPPH Free Radical Scavenging Activity of the LCe Using Ascorbic Acid as the Standard.

Sample	Concentration, µg	DPPH percent inhibition	DPPH IC_{50}, µg AAE /µg
LCe	80	72.827225	
	60	57.748691	168.68
	40	50.26178	
	20	46.910995	

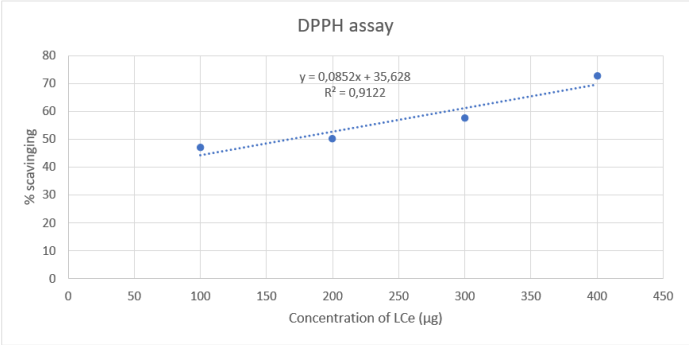

Figure 5. DPPH Free Radical Scavenging Activity of the LCe.

3.1.6 Hemolytic assay:

The hemolytic activity of LCe was assessed against human erythrocytes in order to elucidate the potential hemolytic activity. The activity of the plant LCe was expressed in percent hemolysis. Four different concentrations (80, 120, 160, 200 µg) of the LCe were used. All the concentrations caused hemolysis except 80 µg of LCe which showed only 6.09 % cells lysis. The LCe at 120, 160, and 200 µg concentrations lyzed 38.45, 59.72, and 76.90 % of cells with IC_{50}>500. The erythrocytes showed high cell viability as LCe was low, as shown in Figure 6.

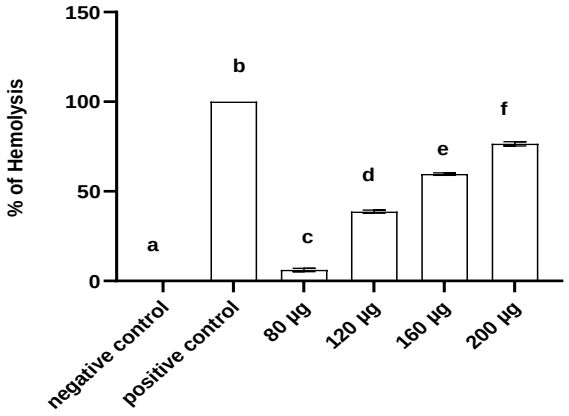

Figure 6. Hemolytic Activity of LCe.

LCe = *P. hysterophorus* leaves crude extract. Different alphabets on bar graph show significant difference (P<0.05).

3.2 Hematological Analysis:

Table 3 shows variations in hematological parameters in various rabbit groups. There was a significant difference in some hematological parameters among various groups treated with LCe (10, 20, 40, and 80 mg kg^{-1}) when compared to the control group.

Table 3. Hematological Parameters of Animals from Control Group and Treated LCe.

Groups					
		A	**B**	**C**	**D**
Parameters	**Control**	**10 mg/kg**	**20 mg/kg**	**40 mg/kg**	**80 mg/kg**
Erythrocyte (/mm^3)	5.29 ± 0.05 a	5.32 ± 0.09 a	5.10 ± 0.10 a	4.77 ± 0.06 B	4.20 ± 0.08 C
Hemoglobin (g/dl)	10.0 ± 0.5 a	10.1 ± 0.5 a	10.3 ± 0.6 a	10.5 ± 0.3 B	11.0 ± 0.1 C
MCV (%)	64.3 ± 0.15 a	63.9 ± 0.66 a	60.1 ± 0.10 c	58.3 ± 0.49 D	51.6 ± 0.10 E
MCH (%)	19.8 ± 0.10 a	20.5 ± 0.10 b	20.8 ± 0.10 c	20.2 ± 0.10 D	20.6 ± 0.10 E
MCHC (%)	25.5 ± 0.10 a	33.0 ± 0.10 b	32.3 ± 0.10 c	28.3 ± 0.10 D	27.3 ± 0.10 E
HCT (%)	38.8 ± 0.05 a	39.0 ± 3.43 a	40.9 ± 4.01 b	42.1 ± 0.057 C	43.9 ± 1.21 D
WBC (/l)	9.7 ± 0.05 a	9.4 ± 0.10 b	8.9 ± 0.05 c	8.6 ± 0.05 D	8.1 ± 0.11 E
Neutrophils (%)	63.6 ± 0.57 a	66.3 ± 0.57 b	69.0 ± 1.00 c	73.0 ± 1.00 D	77.0 ± 1.00 E
Lymphocyte (%)	31.6 ± 0.57 a	28.6 ± 0.57 b	25.0 ± 1.0 c	21.0 ± 1.0 D	17.0 ± 1.0 e
Platelets (mm^3)	240667±577.4 a	163667±577.4 b	160667±577.4 c	134000±1000 D	125333±574 e

MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration. LCe = *P. hysterophorus* leaves crude extract. Different alphabets on bar graph show significant difference (P<0.05) while same alphabets show no significant difference (P<0.05).

3.3 Biochemical Analysis:

The variations in biochemical parameters of different groups are shown in Table 4. There were significant (P<0.05) increases in ALP, S.G.P.T/ALT, LDL, Urea and Creatinine levels while Cholesterol, Billrubin total, and Triglyceride level significantly (P<0.05) decreased in rabbits treated with LCe when results were compared to the control group. The HDL parameter was significant (P<0.05) and was not found different when it was compared to the control group.

Table 4. Blood Biochemical Parameters of Animals from Control Group and Treated LCe.

Groups					
		A	**B**	**C**	**D**
Parameters	**Control**	**10 mg/kg**	**20 mg/kg**	**40 mg/kg**	**80 mg/kg**
ALP (U/L)	80.33 ± 0.57 a	91.67 ± 1.52 b	103.00 ± 1.0 c	118.33 ± 0.57 D	129.00 ± 1.0 e
ALT (U/L)	220.0 ± 1.0 a	233.0 ± 1.0 b	239.7 ± 1.1 c	257.7 ± 0.57 D	265.0 ± 1.0 e
Cholesterol mg/ dL	156.0 ± 1.0 a	143.7 ± 0.57 b	141.0 ± 1.0 c	133.3 ± 0.57 D	128.0 ± 1.0 e
Triglyceride mg/ dL	130.0 ± 1.0 a	124.0 ± 1.0 b	116.0 ± 1.7 c	100.0 ± 1.0 D	91.0 ± 1.0 e
HDL mg/dL	49.0 ± 1.0 a	48.0 ± 1.0 a	51.0 ± 1.0 a	50.0 ± 1.0 A	50.2.0 ± 1.0 a
LDL mg/ dL	80.6 ± 0.57 a	77.9.6 ± 0.57 a	71.0 ± 1.00 a	63.0 ± 1.00 A	56.3 ± 0.57 a
Billrubin total (mg/dl)	0.7 ± 0.10 a	0.7 ± 0.10 a	0.5 ± 0.05 b	0.4 ± 0.09 C	0.2 ± 0.05 d
Urea (mg/dl)	22.00 ± 1.00 a	24.00 ± 1.00 b	32.00 ± 1.00 c	38.00 ± 1.00 D	48.00 ± 1.00 e
Creatinine (mg/dl)	0.8 ± 0.15 a	0.9 ± 0.05 a	1.0 ± 0.05 a	1.2 ± 0.10 B	1.5 ± 0.10 c

ALT: Alanine aminotransferase; HDL: High density lipoprotein; LDL: Low density lipoprotein. LCe = *P. hysterophorus* leaves crude extract. Different alphabets on bar graph show significant difference (P<0.05) while same alphabets show no significant difference (P<0.05).

DISCUSSION

In present study, we aimed to find out the phytochemical composition as well as the *In-vitro* and *In-vivo* toxicity potential of LCe. Initially, the LCe was analyzed for qualitative phytochemical analysis. Similar to other plants, the alkaloids, flavonoids, phenols and chlorophyll are the representative phytochemical groups that were detected. For identification of bioactive compounds, the LCe was subjected to HPLC system. Based on the analysis results, Chlorogenic acid, Bis-HHDP-hex(pedunculagin), Morin, 3-0-caffcoylquinic acid, Ellagic acid, Kaempferol-3-(p-coumaroyl-diglucoside)-7-glucoside, Rutin, Syringic acid, Quercetin-7-O-sophoroside, Kaempferol-3-(caffeoyl-diglucoside)-7-rhamnosyl, Pyrogallol, Mandelic acid, Quercetin-3-(caffeoyldiglucoside)-7-glucoside, Quercitin-3-O-rutinoside were identified as bioactive compounds in the LCe. Among these compounds, Ellagic acid was abundantly present with a peak area of 9594.909 %.

Due to phytochemical composition of various plants, biological activities are usually observed (Zahoor et al., 2020). Thus, the phenolic and flavonoid contents are the two main phytochemicals for the biological activities such as antioxidant potential (Swarnalatha, Saha, & Choudary, 2015), (Rahman et al., 2017). Furthermore, high phenolic and flavonoid contents were observed in the LCe at 80 µg (57.35 ± 4.12 µg GAE/µg and 39.44 ± 0.41 µg QE/µg). The antioxidant potential (DPPH assay) was also high at 80 µg of LCe showing relationship between the antioxidant activity and flavonoid and phenolic contents, which has also been reported by (Zahoor et al., 2020). Significant correlation was observed between total phenolic, flavonoid contents and percent DPPH scavenging.

DPPH is synthetic compound which is used for determination of in-vitro antioxidant activity of biological sample. Antioxidant is the first line of defense against the damage that occurs due to formation of free radicals. Antioxidants stabilize free radicals before they attack the cells (Akhtar, Akhtar, Deshmukh, Ahmed, & Khan, 2019). IC_{50} value is a widely used parameter to measure free radical scavenging activity. Low IC_{50} indicates significant activities as compared to high IC_{50} value (Maisuthisakul, Suttajit, & Pongsawatmanit, 2007). The LCe showed the highest percent scavenging activities which are significant as compared to Ascorbic acid. As the concentration of the extract was increased, the percent scavenging activity also increased, thus a dose-dependent response was recorded.

For identification of the effect of plant extract on blood cells, it is very important to determine the hemolytic activity and is a good way to identify cytotoxicity. Tests for hemolytic activity are commonly used to determine toxic agents and their toxicological activity (Oliveira, Carneiro, Cauper, & Pohlit, 2009), (Franco et al., 2020). Plant extracts are considered toxic to RBCs if the percent hemolysis is above 30% (Kundishora, Sithole, & Mukanganyama, 2020). At concentration of 200 µg of LCe, 76.90 % of cells lysis with IC_{50}>500 was observed which may be due to the significantly high parthenin contents in the leaves. The toxic secondary metabolites are mainly located in *P. hysterophorus* leaf tissues. Parthenin was highly cytotoxic to human erythrocytes by inhibiting the synthesis of key cellular enzyme, DNA, and RNA within 24 hours after exposure (Bajwa et al., 2020), (Narasimhan et al., 1977). Furthermore, other sesquiterpene lactones, such as ambrosin, coronopilin, tetraneurin A and hysterone D, and some acetylated pseudoguaianolides have been correlated with the cytotoxicity of LCe (Das et al., 2007), (Rodriguez, Dillon, Mabry, Mitchell, & Towers, 1976), (Ramesh et al., 2003). The α-methylene-γ-lactone moiety was suggested to be responsible for cytotoxicity in most of these compounds (Bajwa et al., 2020), (Das et al., 2007).

Determining the intensity of the toxic compounds in a plant extract, hematological parameters are very sensitive and can be used as an indicator to intrusion of the toxic compounds (Wu et al., 2018). The toxicity data collected from animal models are considered as relevant risk factors, which could lead to toxicity in humans as well (Nadkarni & Nadkarni, 1976), (Nostro, Germano, D'angelo, Marino, & Cannatelli, 2000). The difference in hematological parameters, such as hemoglobin, WBCs, RBCs, etc. were significantly ($P<0.05$) affected amongst the groups when compared to the control. The decline of RBCs value in the treated group suggested kidney and liver tissue injuries (Wu et al., 2018), or LCe might have inhibited RBCs formation (Shah, Khan, Rizvi, & Siddique, 2007) which resulted in anemia after oral LCe treatment (Saha, Yadav, Kumari, Raipat, & Sinha, 2013). The significant ($P<0.05$) reduced WBC and altered leukocytes count which suggest weak immune system. Parthenin and some other sesquiterpene lactones interact with RBCs membrane and may result in pore formation that increase hemoglobin levels in LCe treated groups which has also been reported by (Das et al., 2007), (Ramesh et al., 2003). Alteration in the MCV level might be due to the presence of ellagic and morin acid in the LCe (Tasaki et al., 2008), (Cho et al., 2006).

Biochemical analysis results showed that alkaline phosphatase, Alanine Aminotransferase, Creatinine, Triglyceride, and Urea levels increased in the animals treated with LCe. These variations in alkaline phosphatase and Alanine aminotransferase levels are indicators of liver damage, as these enzymes are recognized markers (da Silva et al., 2020), (Refaie, Mohafrash, Ibrahim, & Mossa, 2017). The liver damage may be due to altered membrane permeability or liver cell necrosis and cytosol leakage in the serum (Kumari, Raipat, & SKinha, 2015), (Ozer, Ratner, Shaw, Bailey, & Schomaker, 2008). The reason the membrane altered permeability and cell necrosis may be due to the accumulation of toxic compounds in the liver, or might be due to Morin present in the LCe (Cho et al., 2006). A significant ($p < 0.001$) reduction in cholesterol level in the treated group might be due to the presence of Rutin acid present in the LCe (Park et al., 2002), (Fusi, Saponara, Pessina, Gorelli, & Sgaragli, 2003). The increased Creatinine level in the treated animals is a marker of renal function, and its level is usually changed due to clearance of excess substances (Traesel et al., 2014). The high diversity of compounds present in the extract may be linked to this increase. The triglyceride value was remarkably decreased than that of the control, possibly due to the presence of Morin acid in the LCe because Morin has lipolytic activity (Cho et al., 2006).

Moreover, significantly ($P < 0.05$) low effect was observed at the dose of 10 $mg \cdot kg^{-1}$ of LCe whereas a highly significant ($P < 0.001$) effect was observed at 80 $mg \cdot kg^{-1}$ of LCe which clearly indicated the dose-dependent response.

CONCLUSION

In this study, we performed phytochemical analysis using leaves crude extracts to elucidate the toxicity. Using standard protocols, we investigated DPPH free radical scavenging assay to determine its antioxidant potential, cytotoxic effect, total phenolic and flavonoid contents in leaves crude extract. The HPLC analysis was also performed to analyze the phenolic contents. Based on the data obtained, *P. hysterophorus* was concluded to be a toxic plant, having hemolytic activities.

In vivo, the leaves crude extract was proven to be toxic and unsafe, which was evident from the results of the biochemical and hematological parameters.

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