

Preventive effects of *Tephrosia lupinifolia* DC. on haematological alterations induced by cadmium in Sprague-Dawley rats

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Abstract

Introduction: Cadmium is known to be one of the most noxious environmental and industrial pollutant. Cigarette smoke and food products are the major sources of cadmium exposure in non-occupationally exposed people. *Tephrosia lupinifolia* DC. (Fabaceae) is commonly known as Fish poison. It has usage in treatment of diarrhea, stomachache, urinary disorders, asthma and rheumatism.

Objectives: This investigation was aimed to examine the protective potentials of *Tephrosia lupinifolia* DC. against cadmium-induced changes in haematological parameters of Sprague-Dawley rats.

Methodology: In present study, male rats (n=30) were taken and equally divided into five groups. Rats were orally administrated with relevant doses of methanolic extract of *Tephrosia lupinifolia* (25, 50 mg/kg bw) for 7 days and then CdCl₂ (4 mg/kg) was intravenously injected to produce acute toxicity. At the end of experiment, rats were dissected to collect blood for haematological analysis.

Results: Cadmium treatment caused significant decline of RBCs (P=0.002), PCV and Hb concentration (P=0.0001) as compared to control. It also elevates the WBC count (P<0.001), concentration of lymphocytes, neutrophil percentages, MCV, MCHC and MCH values (P=0.05). The changes induced by cadmium in different haematological parameters were improved in the rats treated with *Tephrosia lupinifolia*.

Conclusion: The outcomes of this study recommended that methanolic extract of *Tephrosia lupinifolia* (TLME) ameliorates the harmful effects of CdCl₂ on blood parameters which can be credited to the presence of different phytochemical constituents in it.

Keywords: *Tephrosia lupinifolia*; Cadmium; Haematology; Fabaceae

1. Introduction

It has been acknowledged by all that substantial increase in environmental problems in the last couple of decades is chiefly because of human population explosive growth and ever-increasing demand of various household commodities. Although development in technology has uplifted quality of life but at the same time it has posed many health concerns [1]. Major public health issues visible in industrial communities especially in the developing countries are caused by

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environmental pollutants and occupational-related toxic substances. Global metal pollution has been seriously increased after industrial revolution [2].

Among the most toxic heavy metals, cadmium (Cd) is on seventh number in the world as per ATSDR ranking. It is produced as a byproduct in the process of zinc manufacturing from which animals and humans may get exposure at work or in the surroundings. Cadmium will accumulate within the human body for whole life once it gets absorbed. During World War I, this metal was used for the very first time as a substitute for tin and as a pigment in paint industries. Presently, it is also being used for special alloys formation and in rechargeable batteries. In alkaline batteries, about three-fourth of Cd component is used as an electrode, the rest of the part is used in pigments, coatings, plating and as a plastic stabilizer [3].

The atomic number of cadmium is 48 and it belongs to d-block, period 5 and group 12 of periodic table. Cadmium is a silvery-white, soft and ductile metal. It was discovered in 1817 by Friedrich Strohmeyer, a German chemist, as a constituent of smithsonite ($ZnCO_3$) from zinc ore. Its concentration is 0.15 ppm in the earth crust and greenockite (CdS) is the most common cadmium mineral [4].

Cadmium is present everywhere in environment and its concentration within the food chain is mostly because of its excessive solubility comparatively to other toxic heavy metals. Since cadmium is not degradable, therefore it is moved from soil to plants easily on which human beings and animals mainly rely for existence [5].

Now-a-days, an important public health concern worldwide is the increase in Cd contamination. Weathering of rocks, volcanic emissions, mining processes, industrial applications, human and agricultural practices are the natural sources from which Cd moves into biological systems. Air, soil and water are the main environmental components which normally receive continuous emissions of Cd. The large amount of Cd exposure takes place through ingestion of food materials because of absorption of cadmium through plant from manure, sewage, fertilizers and sludge. Wheat, mushrooms, fruits, potatoes, grains, bran, carrot, sugarbeet fiber, dried seaweeds etc, are the sources of cadmium absorption in vegetarian diet. Likewise, shellfish, mussel, meat and fish are rich in Cd among non-vegetarian diets. The amount of Cd in the human body can be greatly increased by intake of Cd rich foods. Hence, Cd being widely spread environmental contaminant is responsible for the induction of toxicity in different organs, that includes blood, heart, kidney, liver, lungs, testis, brain and bone [6].

Furthermore, cigarette smoke is among the other great source of cadmium exposure. It was revealed after taking blood samples of smokers that they had 4 to 5 times more cadmium in their blood comparatively to non-smokers [7]. Markiewicz-Górka *et al.*, [8] has investigated that people take in additional 1-3 μg of Cd who smoke one pack of cigarettes a day. Among the different kinds of plants used in herbalism, some have medicinal activities which are termed as medicinal plants. Compounds employed in drug development and synthesis can be richly acquired from these medicinal plants. Medicinal plants are commonly used as raw materials for extraction of active compounds which are utilized in the formation of various medicines [9].

These medicinal plant species considerably contain many flavonoids, flavonols and phenolic compounds having antioxidant potentials, comparatively to the synthetic antioxidants that have side effects and are reported to be carcinogenic. It has been observed that the herbal drugs strengthen the body system specifically without side effects. In developed countries, the significance of old-style herbal medicinal system has now gained vital importance. Therefore, depending upon this, medicinal plants, which possess good antioxidant potential, are the top supplements for the treatment of diseases linked with oxidative stress [10].

Tephrosia lupinifolia DC. commonly known as Fish poison, is extensively spread in Pakistan, Angola, Namibia, Congo, northern Nigeria, Malawi, Zambia, Zimbabwe and South Africa. Its family is "Fabaceae". Its roots, leaf, stem and bark are essentially used as ingredients in medicines. It also has usage in treatment of stomachache, diarrhea, rheumatism, asthma and urinary disorders [11].

2. Material and methods

2.1. Collection of plant material

Tephrosia lupinifolia DC. (whole plant) was collected on September 17, 2017 from Head Marala, Sialkot, Pakistan. Plant specimen was identified and submitted (#042573) at Natural History Museum, Islamabad, Pakistan. It was shade dried for one month. The dried plant material was powdered and stored in polythene bags at room temperature.

2.2. Preparation of whole plant extract

One kilogram dried powdered sample of *Tephrosia lupinifolia* DC. was extracted and filtered two times with 2 liters of 95% methanol for seven days. Filtrates of *Tephrosia lupinifolia* DC. were evaporated to obtain a crude extract which was stored at 4°C.

2.3. In vivo cadmium induced toxicity in rats

Six weeks old Sprague-Dawley rats having weights 140-160 g were placed in conventional cages under 12 h light/dark cycle at 20±3 °C. The rats had *ad libitum* access to water and standard basal diet.

2.4. Experimental protocol

The study on rats carried out according to institutional instructions for the use of laboratory animals. Sprague-Dawley rats were taken from National Institute of Health, Islamabad (NIH) and acclimatized for one week. To study the hematological effects of *Tephrosia lupinifolia* DC., rats were given plant extracts (25, 50 mg/kg) for seven days, and eventually exposed to cadmium (single injection) twenty-four hours after the last dose of *Tephrosia lupinifolia* DC. Cadmium chloride (4 mg/kg body weight) dissolved in normal saline, was injected (intravenously) into the rats to induce acute toxicity. Rats were dissected and blood samples were obtained at twenty-four hours after cadmium exposure [12].

2.5. Haematological studies

Different haematological parameters including haemoglobin (g/dl), mean corpuscular haemoglobin concentration, percent of packed cell volume, RBC ($\times 10^6/\text{mm}^3$), mean corpuscular volume (MCV; fL), mean corpuscular haemoglobin (MCH; pg), white blood cells (WBC, $10^3/\text{mm}^3$), lymphocytes (%), basophils (%) and neutrophils (%) were estimated.

2.6. Statistical analysis

The values were expressed as mean±standard error. The results were evaluated by one way analysis of variance (ANOVA) using computer software, Statistics 8.1. Multiple comparisons among different treatments were made by Tukey HSD method at $p\text{-value} \leq 0.05$.

3. Results

3.1. Effects of *Tephrosia lupinifolia* DC. extract on complete blood count

3.1.1. Haematological parameters (R.B.C., P.C.V and Hb)

The effect of *Tephrosia lupinifolia* DC. on haematological parameters of CdCl₂ administered male rats are summarized in Table 1. Treatment of CdCl₂ resulted in significant decline in Hb ($P < 0.001$), R.B.C count ($P < 0.05$) and P.C.V concentration ($P < 0.001$) comparatively to control group. Treatment of TLME at both doses (25 and 50 mg/kg) reduces the cadmium effects and the concentration of red blood cell count, P.C.V and Hb were increased comparatively to CdCl₂ administered group but these values were less as compared to normal group values. However, treatment of TLME alone had non-significant effect ($P > 0.05$) on haematological values like red blood cell count, Hb and P.C.V as compared to control group (Fig 1).

3.1.2. Haematological parameters (WBC, lymphocytes, neutrophils and basophils)

The effects of TLME against cadmium induced alterations in WBC, lymphocytes, neutrophils and basophils are tabulated in Table 2. It was observed that CdCl₂ administration significantly elevates the WBC count ($P < 0.001$) comparatively to the control group's WBC count. It was also observed that the concentration of lymphocytes and neutrophils percentages were increased due to the CdCl₂ administration, while in cadmium treated rats no basophils were found in the blood rat serum. Administration of TLME at both doses (25 and 50 mg/kg) along with cadmium to the experimental rats erased the toxic effects of cadmium and caused a decrease in total neutrophils, lymphocytes and WBC count percentages and normalized the contents of WBC count, neutrophils and lymphocytes near to control group in dose dependent manner (50 and 25 mg/kg). While no abnormal variation in WBC count, neutrophils and lymphocytes percentages was found by administration of TLME alone (Fig 2).

3.1.3. Red blood cell indices (MCH, MCHC and MCV)

The effect of cadmium chloride intoxication on red blood cell indices was observed and effect of TLME on CdCl₂ treated rats are summarized in Table 3. It was observed that there was non-significant elevation in the values of MCH, MCHC and MCV in CdCl₂ administered rats as that of control group values. It was tested that co-treatment of TLME at both doses (25 and 50 mg/kg) along with CdCl₂ to the experimental rats erased the CdCl₂ induced toxicity and reversed the levels of red blood indices in dose dependent manner (25 and 50 mg/kg). However, non-significant variations were found by the administration of TLME alone on the level of these red blood cell indices (Fig 3).

Table 1 Effect of methanolic extract of *Tephrosia lupinifolia* DC. on blood parameters in cadmium induced toxicity in rats

Treatment mg/kg bw	RBC (n×10 ⁶ /mm ³)	PCV (%)	Hb (g/dl)
Control (Saline)	7.83 ± 1.013	34.66 ± 1.35	14.9 ± 0.13
Cadmium 4	3.16 ± 0.65*	23.25 ± 0.87**	12.65 ± 0.31**
TLME 25+CD	5.33 ± 0.76*	30.43 ± 1.71	13.93 ± 0.56*
TLME 50+CD	5.83 ± 0.47*	31.41 ± 1.89	14.71 ± 0.19
TLME 50	7.16 ± 0.79	34.65 ± 1.43	14.96 ± 0.16

Values are expressed as mean ± SEM (06); *P < 0.05; **P < 0.001; CD; Cadmium, TLM; *Tephrosia lupinifolia* DC. methanol extract.

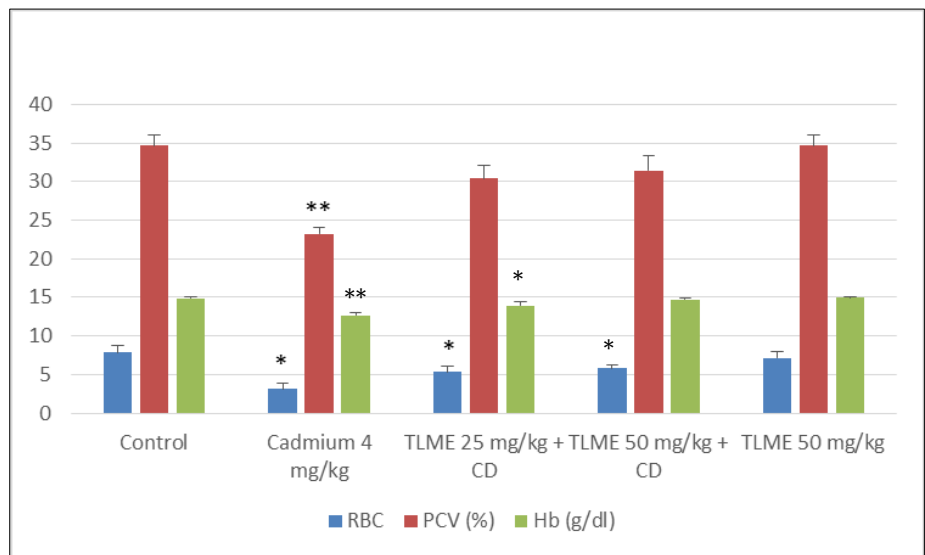


Figure 1 Effect of methanolic extract of *Tephrosia lupinifolia* DC. on blood parameters in cadmium induced toxicity in rats

Table 2 The effect of *Tephrosia lupinifolia* DC. on blood parameters of cadmium chloride treated male rats

Treatment mg/kg bw	WBC (10 ³ /mm ³)	Lymph (%)	Neut (%)	Basophils (%)
Control (Saline)	6.33 ± 0.42	67.83 ± 2.02	8.16 ± 1.19	0.00 ± 0.00
Cadmium 4	12.33 ± 0.55**	72.16 ± 23.92	19.66 ± 7.32	0.00 ± 0.00
TLME 25+CD	8.33 ± 0.55	70.33 ± 4.83	10.66 ± 10.23	0.00 ± 0.00
TLME 50+CD	8.16 ± 0.60	69.16 ± 3.00	8.83 ± 7.92	0.00 ± 0.00
TLME 50	6.83 ± 0.47	68.33 ± 2.37	9.66 ± 0.66	0.00 ± 0.00

Values are expressed as mean ± SEM (06); *P < 0.05; **P < 0.001; CD; Cadmium, TLM; *Tephrosia lupinifolia* DC. methanol extract.

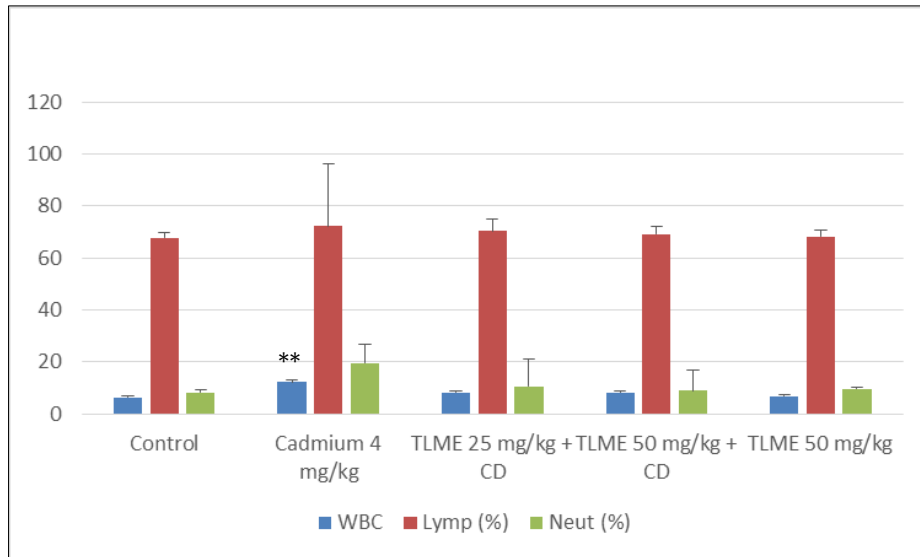


Figure 2 The effect of *Tephrosia lupinifolia* DC. on blood parameters of cadmium chloride treated male rats

Table 3 Effect of methanolic extract of *Tephrosia lupinifolia* DC. on red blood cell indices of cadmium induced toxicity in rats

Treatment mg/kg bw	MCV (fl)	MCHC (g/dL)	MCH (pg)
Control (Saline)	46.68 ± 0.43	34.05 ± 1.41	18.46 ± 0.18
Cadmium 4	49.25 ± 7.51	36.75 ± 1.26	20.91 ± 0.20
TLME 25+CD	45.55 ± 3.28	35.51 ± 1.33	19.95 ± 0.69
TLME 50+CD	45.41 ± 1.66	35.33 ± 1.14	19.63 ± 1.25
TLME 50	46.81 ± 0.86	34.46 ± 1.76	18.36 ± 0.21

Values are expressed as mean ± SEM (06); * $P < 0.05$; ** $P < 0.001$; CD; Cadmium, TLM; *Tephrosia lupinifolia* DC. methanol extract.

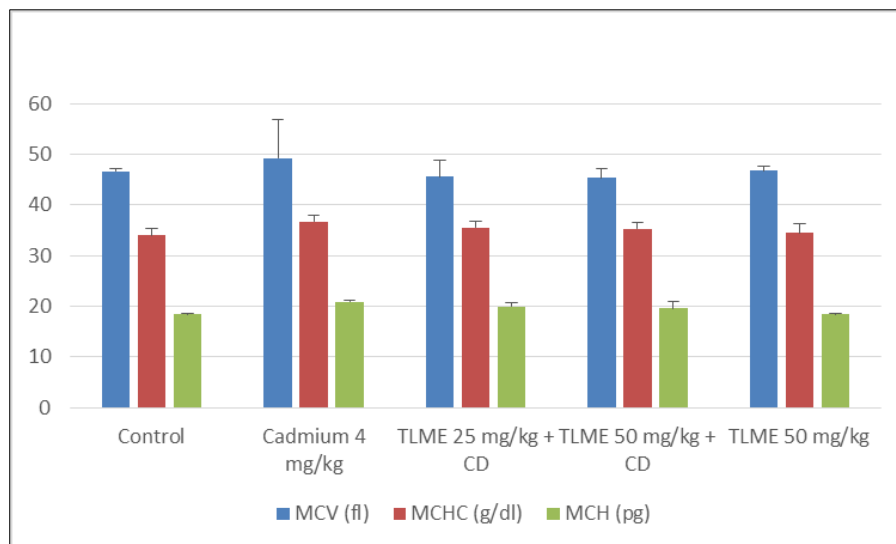


Figure 3 Effect of methanolic extract of *Tephrosia lupinifolia* DC. on red blood indices of cadmium induced toxicity in rats

4. Discussion

Cadmium is one of the predominant occupational and environmental pollutant in industrialized areas. It is acknowledged that Cd is one of the most toxic heavy metal which is able to induce oxidative stress that leads to development of severe pathological conditions, impaired health, such as lungs damage, diarrhea, gastrointestinal problems and intense vomiting, bone fracture, infertility, harm to the central nervous system, mental sickness, possibly DNA damage or cancer development in human beings. All these diseases are being caused to people living in near vicinity of industries or waste sites that discharge Cd into the surroundings and to population working within the metallic refinery industries [13].

Medicinal plants possess different therapeutic properties due to the presence of different types of bioactive compounds in them. Throughout the world, medicinal plants are used to cure different diseases because of the presence of various natural antioxidants in them. Immunomodulatory, anti-cancer, anti-microbial, anti-diabetic and even cardio protective effects are some properties of medicinal plants [14].

In the present study, different toxic effects are caused on the hematological parameters by cadmium exposure. A considerable decline in the packed cell volume, red blood cell count and hemoglobin concentration is observed with reduction in the mean corpuscular haemoglobin (MCH) and increased in the mean corpuscular volume (MCV). Reactive oxygen species can be produced by reduction in haemoglobin (Hb) concentration which causes oxidative stress in the erythrocytes that leads to the destruction of cell membrane as well as change in energy metabolism and results in the appearance of Cd induced anemia [15].

After intake of Cd into the body, it moves into the blood where it binds to the red blood cell (RBC) membranes. Cd induces the production of reactive oxygen species and metallothioneins in the tissues and blood, therefore leads to the oxidative destruction of red blood cells and hence, resulting in a loss of membrane functions in many tissues. Additionally, a number of alterations in antioxidant defense enzymes were reported [16].

In current study, we noticed decline in the haemoglobin concentration, P.C.V and red blood cell count significantly in cadmium chloride treated rats comparatively to the control group value. However, when TLME (25 and 50 mg/kg) was given to rats along with Cd, it increased the R.B.Cs, Hb and P.C.V values. It was also observed that CdCl₂ administration caused a significant increment in the WBCs count comparatively to the WBCs count of the control value, whereas when TLME administered to the Cd treated rats it decreases the WBCs count dose dependently to that of control group. In addition, it also seems from the results that Cd treatment increased the neutrophils, lymphocytes percentages, while no basophils were seen in the serum. It was also observed that administration of cadmium chloride increases the blood indices (MCH, MCHC and MCV) values comparatively to the control group values. Administration of TLME extract caused decrease in blood indices values (MCV, MCH and MCHC) comparatively to CdCl₂ administered group.

5. Conclusion

Our results indicates that methanolic extract of *Tephrosia lupinifolia* DC. has the potential to reduce cadmium induced toxic effects on hematological parameters in rats which might be due to the presence of different phytochemical compounds having therapeutic effects. Further studies can be conducted to isolate these compounds for use in drug formulations.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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Statement of ethical approval

All animal handling and subsequent killings were done according to the guidelines provided by the ethics committee of the Department of Zoology, GC Women University, Sialkot.

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