ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
Research Article
www.ijptonline.com

EFFICACY OF *SIDA RHOMBIFOLIA* LINN. ROOT ON CADMIUM CHLORIDE INDUCED HAEMATOLOGICAL ALTERATIONS IN RATS

Logeswari.P*[a], Usha. P.T.A[a], Dineshkumar.V [a] and Prathap Kumar. S.M [b]
[a] Department of Veterinary Pharmacology and Toxicology,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651,
[b] Department of Safety Assessment, Advinus Therapeutics Ltd,
Peenya Industrial Area, Bangalore- 560058.
Email: loges1986@gmail.com

Received on 12-09-2012 *Accepted on 25-09-2012*

Abstract

Cadmium, a potent environmental pollutant, is toxic to various tissues. The current study was undertaken to investigate the protective effect of ethanolic extract of root of *Sida rhombifolia* against cadmium chloride induced haematological alterations in male wistar rats. Cadmium as cadmium chloride ($CdCl_2$) at the dose rate of 5 mg/ kg body weight followed by ethanolic extract of root of *Sida rhombifolia* Linn. at the dose rate of 500, 750 and 1000 mg/ kg body weight were administered orally for 30 days. Haematological parameters such as total erythrocyte count (TEC), haemoglobin (Hb) concentration, volume of packed red cells (VPRC), total leukocyte count (TLC) and differential leukocyte count (DLC) were estimated using standard methods. Administration of $CdCl_2$ significantly ($P<0.05$) reduced the total erythrocyte count, volume of packed red cells and Hb concentration and significantly ($P<0.05$) induced leukocytosis with neutrophilia and lymphocytopenia. Treatment with ethanolic extract of root of *Sida rhombifolia* Linn. at different dose levels effectively attenuated the $CdCl_2$ induced haematological changes in a dose dependent manner when compared with Cd-alone-treated group. Thus it concluded that *Sida rhombifolia* Linn. root extract can be effectively used in various haemotoxic conditions.

Key words: Cadmium chloride, haematological parameters, oxidative damage, rats, *Sida rhombifolia* Linn.

Introduction

Cadmium (Cd) is one of the most environmentally abundant xenobiotic metals found in air, drinking water, soil, plant and animal products. It is toxic to various tissues of humans and animals [1]. Haematological parameters are very sensitive indicators of cadmium chloride induced toxicity [2]. Cadmium binds to the erythrocyte

membranes [3,4] and generates reactive oxygen species thus causing oxidative damage in erythrocytes [5]. Cadmium has previously been shown to induce anemia. Some of the proposed mechanisms include decreased iron absorption, distortion of erythropoiesis [6], haemolysis [7, 8] and accompanying changes in antioxidant network [9, 10]. Free radical scavengers and antioxidants are useful in protecting against cadmium toxicity [11, 12, 13].

Sida rhombifolia Linn. is a well known medicinal plant (family: Malvaceae) found as a shruby weed in marshy places and has considerable reputation for its medicinal value in traditional medicine [14, 15]. Making an allowance for the growing incidence of oxidative damage and its impact on health and production and as a part of continuing hunt for naturally occurring antioxidant molecules, the present study was carried out to evaluate the effect of ethanolic extract of root of *Sida rhombifolia* Linn. against $CdCl_2$ induced alteration in haematological parameters in rats.

Materials and Methods

Plant materials/ Chemicals

The plant *Sida rhombifolia* Linn. was collected locally from Thrissur district of Kerala and was authenticated by College of Horticulture, Kerala Agriculture University, Vellanikkara. The root of plant *Sida rhombifolia* Linn. was dried at room temperature and coarsely powdered using an electrical pulverizer. The powder obtained were extracted using Soxhlet apparatus with 95% ethanol. The ethanolic extracts were then concentrated in a rotary vacuum evaporator under reduced pressure, temperature and preserved for further study.

Cadmium chloride was procured from Sigma - Aldrich Co. Solvents used were of analytical grade.

Experimental Animals

The study was conducted in 40 adult male Wistar rats weighing 150-200 g. The rats were procured from Small Animal Breeding Station, Thrissur. The animals were housed in appropriate cages in a well ventilated room with temperature ranges from 21-24° C, relative humidity 65-68 % with 12 hours light and 12 hours dark cycle. They were maintained under identical feeding and management practices in the laboratory. This study was approved by Institutional Animal Ethics Committee (No. Acad [3] 6554/04).

Toxicity evaluation of the root extract of *Sida rhombifolia* Linn.

The acute toxicity testing of *Sida rhombifolia* Linn. root extract was carried out according to The Organization

of Economic Co-operation and Development (OECD) Test Guidelines (OECD 423- Limit test procedure) and the critical observations were made on mortality and signs of toxicity.

Experimental Design

The experiment was conducted for a period of 30 days. Animals were randomly divided into five groups comprised of eight animals in each group. Group I animals served as a normal control, which received 3 per cent gum acacia at the dose rate of 5mg/kg body weight/day orally for 30 days. Groups II, III, IV and V animals received freshly prepared cadmium chloride solution at the dose rate of 5mg/ kg body weight/ day orally for 30 days. Group II constituted the untreated cadmium chloride control rats. Group III, IV and V rats were administered with ethanolic extract of root of *Sida rhombifolia* Linn. at the dose rate 500 mg/ kg body weight/ day, 750 mg/ kg body weight/ day and 1000 mg/ kg body weight/ day respectively for 30 days following cadmium chloride administration.

Blood samples were collected from all the animals on day zero, 15 and 30. The blood samples were subjected to various haematological analyses as per the standard technique [16]. Haematological parameters analysed were total erythrocyte count (TEC), haemoglobin (Hb) concentration, volume of packed red cells (VPRC), total leukocyte count (TLC) and differential leukocyte count (DLC).

Statistical analysis of data

Data obtained were analyzed by using analysis of covariance followed by Duncan's multiple range tests for comparison between groups [17]. Student's t test was used for comparison within the treatment group. Results were expressed as mean ± standard error. The value of $P < 0.05$ was considered statistically significant.

Results

Toxicity evaluation of the root extract of *Sida rhombifolia* Linn.

In the present study, the ethanolic extract of root of *Sida rhombifolia* Linn .treated rats did not produce any toxicity signs or mortality even up to seven days indicating the safety of the extract.

Total Erythrocyte Count (TEC), Haemoglobin (Hb) Concentration and Volume of Packed Red Cells (VPRC)

The results obtained were outlined in Table 1. In the present investigation $CdCl_2$ at the dose rate of 5 mg/ kg body weight significantly lowered TEC, Hb and VPRC. Significant fall in TEC, Hb concentration and VPRC of cadmium chloride toxic rats (G_{II}) observed two weeks of the experiment onwards.

In extract treated animals the level of TEC was stable throughout the experiment and insignificant elevation of VPRC was noticed (Table 1). However, the alteration of this value was minor and remained within the normal range. Significant increment of mean Hb value noticed in G_V animals on day 15 onwards and in G_{IV} animals on 30th day (Table 1).

Total Leukocyte Count (TLC): On day 15, significant ($P< 0.05$) changes were observed in TLC between all the groups of rats except G_I normal control when compared to zero day reading (Table 1). Cadmium chloride intoxicated rats were differed significantly ($P< 0.05$) on the 15th day of the experiment from all other group of rats. There was no significant difference observed between extract treated groups.

On day 30, rats belonging to G_{II} and G_{III} groups showed a significantly ($P< 0.05$) higher TLC value, when compared to other group rats. The TLC values of all extract treated group rats were statistically high on day 30 when compared to day zero.

As shown in Table 1, there was a mild raise in TLC of extract treated group animals on day 30 when compared to day zero, but these values were remained within the normal range.

Differential Leukocyte Count (DLC)

Cadmium administration at the dose rate of 5 mg/ kg resulted in significant elevation of total neutrophil count and significant reduction in total lymphocyte count on day 15 onwards and also differed significantly ($P< 0.05$) from all other groups (Table 2). There were no changes in monocyte and eosinophil counts and basophil count was nil. Differential Leukocyte Count of groups treated with the *Sida rhombifolia* Linn. root extract, along with cadmium chloride was unaltered throughout the experiment indicated the protective property of the plant extract (Table 2).

Table 1: Effect of $CdCl_2$ and ethanolic extract of root of *Sida rhombifolia* Linn .on various haematological parameters (Mean ± SE, n = 8).

Groups	Day	TEC × 10^6/ µl	Hb (g %)	VPRC (%)	TLC × 10^3/µl
G_1 Healthy control	0	7.29± 0.16	13.92 ± 0.29	41.00 ± 0.33	6.8 ± 0.22
	15	7.36 ± 0.14	13.95 ± 0.25 [b]	40.38 ± 0.46[a]	6.9 ± 0.23[a]
	30	7.25 ±0.12 [a]	14.01 ± 0.26 [a]	40.50 ± 0.19 [a]	6.8 ± 0.20 [a]

G_{II} $CdCl_2$ alone	0	7.09 ± 0.27	13.58 ± 0.25	40.75 ± 0.79	6.9 ± 0.17
	15	6.87 ± 0.28 ^{x}	13.31 ± 0.28 ^{cx}	39.88 ± 0.67 ^{bx}	8.5 ± 0.24 ^{bx}
	30	6.55 ± 0.31 ^{by}	13.19 ± 0. 26 ^{cy}	38.25 ± 0.68 ^{by}	9.5 ± 0.34 ^{cy}
G_{III} $CdCl_2$ + SR extract 500mg/kg	0	7.04 ± 0.12	13.56 ± 0.15	40.25 ± 0.49	7.1 ± 0.22
	15	6.99 ± 0.14	13.48 ± 0.17 ^{b}	40.63 ± 0.63 ^{a}	7.6 ± 0.30 ^{ax}
	30	7.07 ± 0.11 ^{a}	13.67 ± 0.19 ^{b}	40.88 ± 0.55 ^{a}	7.8 ± 0.26 ^{by}
G_{IV} $CdCl_2$ + SR extract 750mg/kg	0	7.08 ± 0.18	13.93 ± 0.22	40.63 ± 0.96	6.9 ± 0.18
	15	7.03 ± 0.17	13.95 ± 0.31^{b}	40.88 ± 1.09 ^{a}	7.2 ± 0.17 ^{ax}
	30	7.08 ± 0.18 ^{a}	14.28 ± 0.31 ^{ax}	41.00 ± 0.98 ^{a}	7.3 ± 0.14 ^{ay}
G_{V} $CdCl_2$ + SR extract 1000mg/kg	0	7.23 ± 0.15	14.02 ± 0.20	40.73 ± 0.64	6.9 ± 0.15
	15	7.28 ± 0.15	14.34 ± 0.24 ^{ax}	41.13 ± 0.35 ^{a}	7.1 ± 0.57 ^{ax}
	30	7.25 ± 0.95 ^{a}	14.64 ± 0.21 ^{ay}	41.88 ± 0.44 ^{a}	7.2 ± 0.13 ^{ay}

Values bearing different superscripts (a,b and c) in columns differ significantly (P≤ 0.05). Means bearing superscripts (x and y) in rows indicate significant (P≤ 0.05) difference between day zero, 15 and 30

SR- *Sida rhombifolia* Linn. root extract, TEC- Total Erythrocyte Count, Hb- Haemoglobin, VPRC- Volume of Packed Red cells, TLC- Total Leukocyte Count

Table-2: Effect of $CdCl_2$ and ethanolic extract of root of *Sida rhombifolia* Linn. on DLC (Mean ± SE, n = 8).

Groups	Day	DLC (%)				
		L	N	E	M	B
G_1 Healthy control	0	67.50 ± 2.04	27.63 ± 1.76	1.00 ± 0.19	3.63 ± 0.33	0
	15	67.50 ± 1.99	28.25 ± 1.79	1.25 ± 0.16	3.63 ±0.42	0
	30	66.50 ± 2.22 ^{a}	28.38 ± 1.95 ^{a}	1.25 ± 0.25	3.88 ± 0.44	0
G_{II} $CdCl_2$ alone	0	68.38±01.46 ^{x}	27.13 ± 1.26 ^{x}	1.25 ± 0.37	3.25 ± 0.37	0
	15	64.63 ± 1.34	30.50 ± 1.32	1.38 ± 0.32	3.38 ± 0.38	0
	30	59.50 ± 1.83^{by}	35.13 ± 1.88 ^{by}	1.50 ± 0.33	3.50 ± 0.33	0
G_{III} $CdCl_2$ + SR extract 500mg/kg	0	67.25 ± 1.03	28.50 ± 0.91	1.00 ± 0.19	3.25 ± 0. 53	0
	15	66.00 ± 1.05	29.25 ± 0.90	1.13 ± 0.13	3.63 ± 0.46	0
	30	66.00 ± 1.41 ^{a}	29.63 ± 1.38 ^{a}	1.25 ± 0.28	3.68 ± 0.42	0
G_{IV}	0	67.25 ± 1.58	28.25 ± 1.46	1.00 ± 0.19	3.50 ± 0.33	0

$CdCl_2$ + SR extract 750mg/kg	15	65.88 ± 1.70	29.50 ± 1.31	1.00 ± 0.27	3.88 ± 0.44	0
	30	65.63 ± 1.49 [a]	29.63 ± 1.30 [a]	1.00 ± 0.33	3.87 ± 0.43	0
G_V $CdCl_2$ + SR extract 1000mg/kg	0	67.38 ± 1.68	28.00 ± 1.31	1.00 ± 0.19	3.25 ± 0.25	0
	15	65.88 ± 1.48	28.75 ± 1.20	1.13 ± 0.22	3.38 ± 0.18	0
	30	66.13 ± 1.56 [a]	29.00 ± 1.22 [a]	1.38 ± 0.26	3.50 ± 0.38	0

Values bearing different superscripts (A and B) in columns differ significantly (P≤ 0.05). Means bearing superscripts (X and Y) in rows indicate significant (P≤ 0.05) difference between day zero, 15 and 30.

DLC- Differential Leukocyte Count, N- Neutrophil, L- Lymphocyte, E-Eosinophil, M- Monocyte, B- Basophil.

Discussion

Cadmium has been shown to reduce TEC, Hb concentration and VPRC [18] and significant fall in TEC and Hb level observed two weeks of the experiment onwards [8, 19]. Protective effect of antioxidants has been reported against $CdCl_2$ induced damage to RBC [9, 10, 11]. Reduced TEC, Hb and VPRC noticed in cadmium chloride toxic rats might be due to the oxidative damage of RBC membrane produced by the cadmium chloride.

Minor alterations within the normal range of VPRC in animals treated with *Sida rhombifolia* Linn. plant extract has been reported [20]. Significant increment of mean Hb value in extract treated animals may be ascribed to antioxidative potential [21] and hepatoprotective [22] effect of *Sida rhombifolia* Linn. root extract thus preventing oxidative damage to RBC membrane and have improved the erythropoietic activity of the liver respectively.

Earlier studies have demonstrated the elevated level of TLC in the cadmium chloride intoxicated rats [23, 8, 24]. An increase in the TLC above the normal range (leukocytosis) might be due to intoxications and tissue necrosis. This might be due to the participation of neutrophils and monocytes in the process of phagocytosis as scavengers for a wide variety of particulate material.

Alteration in the differential leukocyte count in cadmium chloride administration might be the result of repairation processes of haemopoiesis as neutrophils are the first line of defence mechanism [23].

Conclusion

Haematological indices involving in oxygen transport such as erythrocytes, haemoglobin and volume of packed red cells (haematocrit) and defense mechanisms such as leucocytes revealed significant differences between cadmium chloride alone treated group and animals treated with *Sida rhombifolia* Linn. root extract. It can be

concluded that treatment with ethanolic extract of *Sida rhombifolia* Linn. root offered protection against cadmium chloride induced alteration in haematopoietic system of rats which might be due to antioxidant potential of the extract studied. However, the specific mechanism of action needs to be further investigated.

Acknowledgement: Authors are thankful to College of Veterinary and Animal Sciences, Thrissur for having provided necessary facilities to carry out the research work.

References

1. Hamada, T., Nakano, S., Iwai, S., Tanimoto, A., Ariyoshi, K. and Koide. O. Pathological study on Beagles after long-term oral administration of cadmium. Toxicol. Pathol. 19(2): 138-147, (1991)
2. Kori-siakpere, O and Ikomi, U. Alterations in some haematological parameters of the african snakehead: *Parachanna africans* exposed to cadmium. Not. Sci. Biol. 3(4):29-34, (2011)
3. Wakchaure, S.U. Evaluation of toxico-pathological effect of some heavy metals in slaughtered swine. M.V.Sc. Thesis. Maharastra Animal and Fishery Sciences University, Nagpur. Maharastra, (2004).
4. Kanter, M., Coskun, O. and Gurel, A. Effect of black cumin (*Nigella sativa*) on cadmium-induced oxidative stress in the blood of rats. *Biol. Trace Elem. Res*. 107(3): 277-87, (2005)
5. Waisberg, M., Josph, P., Hale, B. and Beyersmann, D. Molecular and cellular mechanism of cadmium carcinogenesis: a review. *Toxicol*. 192: 95 –117, (2003).
6. Horiguchi, H., Sato, M., Konno, N. and Fukushima, M. Long term cadmium exposure induces anaemia in rats through hypoinduction of erythropoietin in the kidney. Arch. Toxicol. 71:11–19, (1996).
7. Pawaiya, R.V.S., Charan, K and Manna, J. Cadmium toxicity in rabbits haematological and pathological studies. Indian J. Vety. Pathol. 22 (2): 109-114, (1998).
8. Yamano, T., Shimizu, M. and Noda, T. Comparative effects of repeated administration of cadmium on kidney, spleen, thymus, and bone marrow in 2, 4, and 8 month old male Wistar rats. *Toxicol. Sci*. 46: 393-402, (1998).
9. Ognjanovic, B.I., Pavlovic, S.Z., Maletic, S.D., Zikic, R.V., Stajn, A.S., Radojicic, R.M., Saicic, Z.S. and Petrovic, V.M. Protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium. Physiol. Res. 52: 563–570, (2003).
10. Borde, A.U., Qureshi, M. I., Patil, M. K., Mendhe, M. S. and Athawaley, A. M . Protective effect of *Withania*

somnifera on cadmium chloride induced hematological and biochemical changes in male rats. J. Res. Educ. Indian Med. 15-20, ((2008).

11. Shaikh, Z. A and Tang, W. Protection against chronic cadmium toxicity by glycine. Toxicology. 132: 139–146, (1999).
12. Eybl, V., Kotyzova, D. and Bludovska, M. The effect of curcumin on cadmium-induced oxidative damage and trace elements level in the liver of rats and mice. Toxicol. Lett. 151: 79-85, ((2004).
13. Messaoudi, I., Hammouda, F., Heni, J. E., Baati, T., Said, K. and Kerkeni, A. Reversal of cadmium-induced oxidative stress in rat erythrocytes by selenium, zinc or their combination. Exp. Toxicol. Pathol. 62(3): 281-288, (2010).
16. Schalm, O.W., Jain, N.C. and Carroll, E.J. Veterinary Haematology. 4th edn. Eds. Lea and Febiger, Philadelphia. pp. 45-48, (1986).
17. Snedecor, G.H. and Cochran, W.G. Statistical Methods. Eighth edn. Iowa State University Press, U.S.A. 534 p, ((1994).
18. Asagba, S. O. and Eriyamremu, G. E. Oral cadmium exposure alters haematological and liver function parameters of rats fed a Nigerian-like diet. J. Nutr. Environ. Med. 16(3): 267–274, (2007).
19. Mackova, N., Lenikova, S., Fedorocko, P., Brezani, P. and Fedorockova, A. 1996. Effects of cadmium on haemopoiesis in irradiated and non-irradiated mice: Relationship to the number of circulating blood cells and haemopoiesis. Physiol. Res. 45(2): 101-106
20. Sireeratawong, S., Lertprasertsuke, N., Srisawat, U., Thuppia, A., Ngamjariyawat, A., Suwanlikhid, N. and Jaijoy, K. Acute and subchronic toxicity study of the water extract from root of *Sida rhombifolia* Linn. in rats Songklanakarin J. Sci. Technol. 30: 729-737, (2008).
21. Gangu, A. R., Prapulla, P., Anilkumar, C. H., Chamundeeswari, D. and Reddy, U. M. Free radical scavenging activity of the alcoholic extract of *Sida rhombifolia* roots in arthritic rats. Int J. Res. Pharm. Chem. 1(3): 624-629, (2011).

Corresponding Author:

Dr. P. Logeswari*,

Email:*loges1986@gmail.com*