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PROXIMATE ANALYSIS OF *SIDA RHOMBIFOLIA.* L ROOT AND ITS EFFECT ON CADMIUM CHLORIDE INDUCED ALTERATIONS IN BODY WEIGHT OF WISTAR RATS

*Logeswari.P *[a], Dineshkumar.V [a], Usha. P.T.A[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
***Corresponding Author Email: loges1986@gmail.com**

ABSTRACT

Medicinal plants are of great significance and have long been scientifically exploited. The current study was carried out with the aim to investigate the nutritional value, mineral contents and the possible protective role of Sida rhombifolia.L root on cadmium chloride ($CdCl_2$) induced changes in body weight in Wistar rats. Proximate analysis of powdered root was performed. Inorganic elements such as calcium, sodium, zinc, magnesium, lead and cadmium were analyzed were analyzed using Atomic Absorption Spectrophotometer. Various doses of ethanolic extract of Sida rhombifolia. L root were evaluated for its protective effect on orally administered cadmium chloride induced changes in body weight. Proximate analysis of Sida rhombifolia. L root sample indicated the presence of moisture (7.5%), total ash (7.8%), crude protein (5.7%), crude fibre (36.7%), crude fat (2.8%) and nitrogen free extract (47%). Inorganic mineral analysis of Sida rhombifolia. L root shown that the root contains antioxidant micronutrients such as calcium, sodium, zinc and magnesium in considerable levels. Cadmium chloride at the dose rate of 5 mg/ kg body weight orally for 30 days resulted in significantly ($P \leq 0.05$) decreased mean body weight (193.75 ± 1.83 g) and per cent change in body weight (-1.39 %), on day 30. In the current investigation, negative effect of cadmium chloride on body weight was significantly ($P \leq 0.05$) ameliorated by Sida rhombifolia. L root extract in a dose dependent manner. The protective effect of Sida rhombifolia. L root might be attributed to the antioxidant elements present in it.

KEYWORDS

AAS, antioxidant, cadmium chloride, ROS, Sida rhombifolia.

INTRODUCTION

Upsurge in environmental and occupational settings ending in a variety of exposures to different forms of metals. Among all the heavy metals, cadmium (Cd) is considered to be one of the potent toxic, xenobiotic metal that affects almost all organs of body. Substantial evidence has emerged in recent years implicating Reactive Oxygen Species (ROS) and its effect on health. The instantaneous consequence of *in vivo* exposure to cadmium is spur of reactive oxygen species (ROS) production[1] and depletion of physiological antioxidants.

Accumulation of Cd in tissues may result in decreased body weight gain and growth rate [2, 3]. Oxidative damage by free radicals can be barred by the use of antioxidants. Herbals with antioxidant and adaptogenic property shown to significantly reverse the Cd induced oxidative damage and body weight alterations [4, 5]. Spices and herbs are recognized as natural antioxidants that can protect body from oxidative and other insults.

The pharmacological activities of *Sida rhombifolia* L. are well cited in literatures. In Ayurveda, it is reputed as “Mahabala” or “Atibala” and is useful against fever,

tuberculosis, burning sensation, urinary disorders, piles, arthritis and inflammation. It has been shown to have significant analgesic[6], antimicrobial[7], anti- inflammatory [6,8], hepatoprotective [8], lipid lowering [9] and *in vitro* antioxidant[10] properties. The interest toward explicating the molecular mechanism of medicinal plants is also expanding as commercial exploitation rises. In sight of these facts an experimental study was designed to perform the nutritive evaluation of *Sida rhombifolia* L. root and its effect on $CdCl_2$ induced body weight alteration in rats.

MATERIALS AND METHODS

Plant Materials / Chemicals

The plant *Sida rhombifolia* L. was collected locally from Thrissur district of Kerala and was authenticated by College of Horticulture, Vellanikkara, Kerala. A herbarium specimen was deposited in Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Thrissur, Kerala, India. The root of plant *Sida rhombifolia* L. was separated, cut into small pieces and air dried at room temperature and coarsely powdered using an electrical pulverizer. One half of the powdered root samples were subjected proximate analysis while the other halves of the samples were used for ethanolic extraction. The powdered root was stored in an air tight container for further use.

Cadmium chloride (CAS-10108-64-2) was procured from Sigma - Aldrich Co.

Proximate Analysis and Mineral determination

Powdered *Sida rhombifolia* L. root was evaluated by proximate analysis. The contents of ash, crude fibre and crude lipid were estimated by Association of Official Analytical Chemist[11] (AOAC, 2005) methods. Nitrogen content of the powdered root was estimated by the micro-kjeldhal method [12] and crude protein was calculated (N x 6.25). Nitrogen free extract (NFE) was obtained by the Difference method by subtracting the sum of the ash, fibre, protein and lipid from the total dry matter basis. The energy value (KJ) of the root was computed by multiplying the percentages of crude protein, crude lipid and NFE by the factors 16.7, 37.7, and 16.7 respectively.

From the triple acid digested sample calcium, sodium, magnesium, zinc, cadmium and lead were analysed using an atomic absorption spectrophotometer (Perkin Elmer 3110, USA).

Preparation of alcoholic extract of *S. rhombifolia* L. root

The powder obtained were extracted using Soxhlet apparatus with 95% ethanol (analytical grade). The ethanolic extracts were then concentrated in a rotary vacuum evaporator under reduced pressure and temperature (55^0 C) to obtain dry extract. Collected extract was preserved for further use.

EXPERIMENTAL ANIMALS

Adult Wistar rats (40 rats weighing 150-200g) procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Thrissur, Kerala. The animals were maintained in appropriate cages in a well ventilated room with temperature (21-24° C), relative humidity (65-68 %) with 12 hours light and 12 hours dark cycle. Identical feeding and management practices were followed.

This investigation was undertaken with an approval [No. Acad (3) 6554/04] by Institutional Animal Ethics Committee.

EXPERIMENTAL DESIGN

The experimental animals were randomly divided into 5 groups consist of eight animals each. The experiment was conducted for a period of 30 days. Group I served as healthy control and was administered with vehicle 3 per cent gum acacia at the dose rate of 5mg/ kg body weight/ day orally for 30 days. Group II was treated with freshly prepared $CdCl_2$ solution at the dose rate of 5 mg/ kg body weight/ day

orally for 30 days. Group III, IV and V were treated with ethanolic extract of *Sida rhombifolia* L. root at the dose rate 500, 750 and 1000 mg/ kg body weight/ day in 3 per cent gum acacia and freshly prepared cadmium chloride solution at the dose rate of 5 mg/ kg body weight/ day orally for 30 days respectively.

Body weight was measured on day zero, thereafter weekly (on day 7, 14, 21) and final body weight on 30th day from all the animals.

Statistical analysis of data

Collected data were analyzed by using SPSS 17.0 software (Statistical Package for Social Sciences) by one-way ANOVA followed by'*t*' test to determine the significant difference between the control and experimental groups. Obtained results were expressed as mean ± standard error. The value of $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Proximate analysis

Table 1: Proximate analysis of *Sida rhombifolia* L. root

Chemical Composition	Moisture %	TA %	AIA %	CP %	CF %	EE (CL) %	NFE %	GE (KJ)
Level in *Sida rhombifolia* L. root	7.5	7.8	1.7	5.7	36.7	2.8	47.0	985.65

TA: Total Ash; AIA: Acid Insoluble Ash; CP: Crude protein; CF: Crude Fibre; EE: Ether Extract (CL: Crude Lipid); NFE: Nitrogen Free Extract; GE: Gross energy, KJ.100^{-1} DM, values are mean of triplicate determinations expressed on dry weight basis.

Table 1. Shows the results of proximate analysis of root of *Sida rhombifolia* L. Plants constitutes number of nitrogen containing substances. The estimated proximate analysis are moisture (7.5 %), total ash (7.8 %), acid insoluble ash (1.7 %), crude protein (5.7 %), crude fibre (36.7 %), crude lipid (2.8 %), nitrogen free extract(47 %) and gross energy (985.65 KJ).

The dry matter of the root of *Sida rhombifolia* L. is as high as 7.8 %. Similar findings have been reported earlier with different plant materials [13, 14]. The energy value and crude protein composition of a plant are of significance as of nutritive values concerned. In the present study GE was 985.65 KJ and CP was 5.7 %.

Plants avail nitrogen in the form of ammonia, formed either from nitrogen fixation in the root or from enzymatic reduction of absorbed nitrate in shoot and leaf [15]. Appearance of nitrogen as amino acids, are involved in the biosynthesis of various phytochemical constituents such as alkaloids, aminess and cytokinins, etc [16]. Earlier studies were reported on the importance of dietary fibre in human health[17].

In the current investigation, *Sida rhombifolia* L. root revealed the presence of crude fibre content of 36.7% on dry matter basis is suggestive of low source of dietary fibre. This is in accordance with earlier study [15] reported low fibre content in extract of corn silk which is poor source of dietary fibre.

Minerals determination

Table 2. Inorganic minerals determination of *Sida rhombifolia* L. root by Atomic Absorption Spectrophotometer

Inorganic minerals	Calcium %	Sodium %	Magnesium %	Zinc (ppm)	Cadmium %	Lead %
Level in *Sida rhombifolia* L. root	**0.85**	**0.28**	**0.32**	**371.1**	**nil**	**nil**

The inorganic mineral compositions of root of *Sida rhombifolia* L. are listed in **Table 2**. Further analysis of triple acid digested ash sample revealed the presence of inorganic minerals in appreciable quantities which are having antioxidant property. Inorganic minerals analysed were calcium (0.85 %), sodium (0.28 %), magnesium (0.32 %) and zinc (371.1 ppm). Cadmium and lead were nil.

Presence of various inorganic elements in plant parts preclude that the plant could be a fine source of nutrition for body immune system[18]. Magnesium, an essential inorganic element takes part in various metabolic processes[19]. Calcium in combination with magnesium activate many enzyme systems and maintains the electrical potential in the nerves [20, 21].

Body weight

In order to monitor the efficacy of $CdCl_2$ and subsequent *Sida rhombifolia* L. root extract treatment, body weight was measured at regular interval. The weekly mean body weight of rats in all the groups (G_I, G_{II}, G_{III}, G_{IV} and G_V) from day zero to 30 of the experiment is presented in Table 3.

Table 3. Effect of $CdCl_2$ and *Sida rhombifolia* L. root on body weight (g) in different study period (Mean SE, n = 8)

Groups	Body weight (g)					% change in BW
	Day 0	Day 7	Day 15	Day 21	Day 30	Day 30
G_I	195.00 ± 2.67	203.75 ± 3.75 ^{b}	216.25 ± 6.25 ^{ab}	217.50 ± 5.90 ^{b}	232.5 ± 7.50 ^{abx}	19.23
G_{II}	196.50 ± 1.64	201.25 ± 1.25 ^{b}	201.25 ± 1.25 ^{c}	196.25 ± 1.83 ^{c}	193.75 ± 1.83 ^{cx}	-1.39
G_{III}	197.50 ± 1.65	207.50 ± 5.26 ^{b}	208.75 ± 6.66 ^{bc}	216.25 ± 8.00 ^{b}	223.75 ± 9.25 ^{bx}	13.29
G_{IV}	196.75 ± 1.64	221.25 ± 2.27 ^{a}	226.25 ± 5.65 ^{ab}	235.00 ± 4.63 ^{ab}	246.25 ± 6.80 ^{ax}	25.16
G_V	197.00 ± 3.27	222.50 ± 5.90 ^{a}	233.75 ± 8.44 ^{a}	240.00 ± 8.66 ^{a}	250.00 ± 7.07 ^{ax}	26.90

Values bearing different superscripts (a,b and c) in columns differ significantly (P≤ 0.05). Means bearing superscript (x) in rows indicate significant (P≤ 0.05) difference between day zero and day seven, 15, 21 and 30.

% change in Body Weight (BW) from final (F) weight to initial (I) weight on day 30: % change= [(F-I)/I×100]

G I- vehicle control; G II- $CdCl_2$ (5 mg/ kg body weight) control

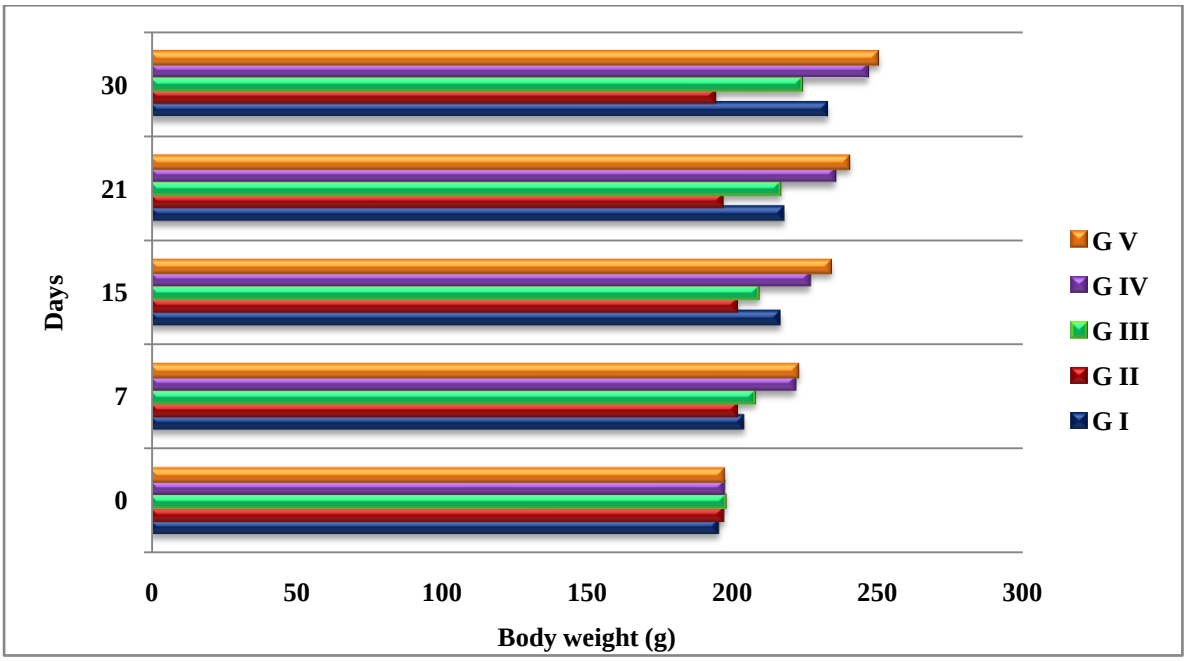
G III- $CdCl_2$ (5 mg/ kg body weight) + ethanolic extract of *Sida rhombifolia* L. root (500 mg/ kg body weight)

G IV- $CdCl_2$ (5 mg/ kg body weight) + ethanolic extract of *Sida rhombifolia* L. root (750 mg/ kg body weight)

G V- $CdCl_2$ (5 mg/ kg body weight) + ethanolic extract of *Sida rhombifolia* L. root (1000 mg/ kg body weight)

There was significant difference ($P \leq 0.05$) in mean body weight of G_{IV} and G_V rats on day seven, 15, 21 and 30 when compared to other groups. Significantly ($P \leq 0.05$) increased mean body weight was recorded in all the group of rats on day seven, 15, 21 and 30 when compared to the body weight on day zero except for cadmium chloride alone treated group rats. Group II rats showed significantly ($P \leq 0.05$) decreased mean body weight (193.75 ± 1.83 g), on day 30 (**Figure 1**).

Figure 1. Effect of $CdCl_2$ and *Sida rhombifolia* L. root on body weight (g) in different study period



G I- vehicle control; G II- $CdCl_2$ (5 mg/ kg body weight) control
G III- $CdCl_2$ (5 mg/ kg body weight) + ethanolic extract of *Sida rhombifolia* L. root (500 mg/ kg body weight)
G IV- $CdCl_2$ (5 mg/ kg body weight) + ethanolic extract of *Sida rhombifolia* L. root (750 mg/ kg body weight)
G V- $CdCl_2$ (5 mg/ kg body weight) + ethanolic extract of *Sida rhombifolia* L. root (1000 mg/ kg body weight)

Considerable alteration in body weight is noted to be one of the most sensitive indicators of an animal's health condition. Increased catabolism of proteins would lead to muscle wasting and reduction in the body weight. Weight gain is influenced by the availability and absorption of nutrients. Recent studies confirmed that cadmium lowers the nutrient digestion and absorption. Consumed Cd is presume to bind to intestinal mucosal metallothionine (MT) and then the cadmium-MT complex is released into the systemic circulation[22].

Normal control rats were found to be stable in their body weight gain whereas significant reduction in the body weight following cadmium chloride administration was noticed in G_{II} rats (**Table 3**). There were reports that the treatment with cadmium chloride significantly reduced the body weight [23, 24]. This loss of body weight might be due increased catabolism of body protein and improper nutrient digestion and absorption.

Animals treated with *Sida rhombifolia* L. root extract at the dose rate of 750 mg/ kg and 1000 mg/ kg significantly increased the body weight by 49.50 g and 53 g respectively. Per cent change in body weight gain at the end of the experiment was higher in G_{IV} (25.15 %) and G_V (26.90 %). Earlier findings showed increase in the bodyweight of *Sida rhombifolia* L. extract treated animals [25]. It has been shown that aqueous extract of *Sida rhombifolia* ssp. *retusa* (400 mg/ kg, 800mg/ kg , orally) produced dose dependent effect on body weight induced by thioacetamide [26].

In the current investigation, negative effect of cadmium chloride on body weight was ameliorated in animals treated with plant extract which might be attributed to the antioxidant property of *Sida rhombifolia* L. root. Presence of inorganic antioxidant elements such

as calcium and zinc may impart appreciable amount of antioxidant property to the plant.

CONCLUSION

This investigation demonstrated that cadmium chloride drastically reduced the body weight in rats which might be due to oxidative damage. *Sida rhombifolia* L. root extract exhibited dose dependent protection against cadmium chloride induced body weight alterations. Presence of inorganic antioxidant elements and other phyto constituents might be involved in the antioxidant property exhibited by *Sida rhombifolia* L. Root. Further insights are needed to delineate the exact mechanism of protection of *Sida rhombifolia* L. root to establish its therapeutic role as a potential protective agent.

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***Corresponding Author:**
Dr. P. Logeswari
Department of Pharmacology and Toxicology
College of Veterinary and Animal Sciences,
Kerala Veterinary and Animal Sciences University,
Mannuthy, Thrissur, Kerala-680651
Phone: 09535812731, 09902052651
Email: loges1986@gmail.com