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Research Article

AMELIORATIVE EFFECT OF BRAHMI ON HAEMATOLOGICAL PARAMETERS IN SUB-ACUTE CADMIUM INDUCED TOXICITY IN WISTAR RATS

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ABSTRACT

The present experimental study was conducted to assess the ameliorative effect of Brahmi (*Bacopa monnieri*) ethanolic extract on cadmium (Cd) induced haematological changes in male Wistar rats for a period of 28 days. The study included forty eight male rats which were divided into eight groups viz. G-I(Control, distilled water), G-II ($1/5^{th}$ LD_{50} of $CdCl_2$), G-III ($1/10^{th}$ LD_{50} of $CdCl_2$), G-IV ($1/20^{th}$ LD_{50} of $CdCl_2$), G-V ($1/5^{th}$ LD_{50} of $CdCl_2$ + Brahmi extract @ 50 mg/kg body weight), G-VI ($1/10^{th}$ LD_{50} of $CdCl_2$ + Brahmi extract @ 50 mg/kg body weight), G-VII ($1/20^{th}$ LD_{50} of $CdCl_2$ + Brahmi extract @ 50 mg/kg body weight) and G-VII (Brahmi extract control @ 50 mg/kg body weight) with 6 rats in each group, respectively. Haematological parameters in all the Cd intoxicated groups (G-II, G-III and G-IV) showed decrease in mean Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) indicated anaemic changes (Microcytic hypochromic anemia) and Platelet count (Thrombocytopenia). However, non-significant decrease in mean MCH values in groups (G-II, G-III and G-IV) in dose dependent manner at the end of experimental study. Mean total leukocyte (TLC) count was significantly ($P<0.05$) decreased and Differential Leucocyte Count (DLC) revealed lymphocytopenia and neutrophilia in groups (G-II, G-III and G-IV) in dose dependent manner, as compared to their respective control groups (G-I and G-VIII). Cd intoxicated groups (G-V, G-VI and G-VII) supplemented with Brahmi ethanolic extract showed improvement in haematological parameters and proved ameliorative effect in sub-acute Cd induced toxicity at different doses in male Wistar rats.

Keywords: Brhami, Cadmium, Haematological changes, Wistar rats

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In the present global scenario, India has emerged as the fastest growing economy in the world backed by rapid growth of industrialization. The increased industrial activities are the major source of land, air and water pollution. Excessive dumping of industrial wastes in the environment mainly water bodies and soil lead to accumulation of various heavy metals. The heavy metals are major concern for health of all living organisms around the world (Dixit *et al.*, 2015). Cadmium is recognized as a significant heavy metal that poses a serious health hazard (Kaplan *et al.*, 2011). According to Agency for Toxic Substances and Disease Registry, Cd is the seventh important toxic heavy metal present in environment (Zhang *et al.*, 2014). Various human activities are common source of Cd in the environment such as use of fertilizers, plastic, battery, pigment industries, electroplating and electronic waste such as computer batteries (Vig *et al.*, 2003; Song and Li, 2014). Experimental (nude mouse model and BALB/c-3T3 cells) and epidemiological studies (in humans) have shown that occupational exposure of Cadmium may causecancer in various organs or system such as lungs, liver, kidneys, urinary bladder, prostate gland, testis, pancreas, stomach and haematopoietic system (Waalkes, 2000; Joseph *et al.*, 2001). Herbal plants and their extracts can be used to prevent the lethal effect of Cd (Graham *et al.*, 2005, Jayakeerthi, 2022). Brahmi (*Bacopa monnieri*) of *Scrophulariaceae* plant family known by various names such as Indian penny wort/Herb of grace/Water hyssop/Thyme leaved gratiol (Shalini *et al.*, 2021). It possesses various pharmacological properties like antioxidant, anti-bacterial, anti-fungal, anti-ulcer, anti-diarrhoeal, anti-hypertensive, analgesic, anti-depressant, anti-anxiety, anti-convulsant, anti-cancer, anti-inflammatory and anti-toxicity activity (Lal and Baraik, 2019). Hence, the present study was carried out to investigate the ameliorative effect of Brhami plant extract in sub-acute Cd-induced toxicity in Wistar rats.

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MATERIALS AND METHODS

The present study was conducted in male Wistar rats of 6-8 weeks old procured from Disease free animal house LUVAS, Hisar. The rats were housed in polyacrylic cages under hygienic conditions at Animal House Facility in Department of Veterinary Pharmacology, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar. The animals were allowed to acclimatize to the local vivarium for 7 days. All the rats were provided with fresh, clean drinking water and fed *ad libitum* with standard balanced ration throughout the experimental study.

Chemical: Cadmium chloride of Alfa Aser company ($CdCl_2$, anhydrous, ACS, 99.0% min, FW: 183.32) was used. For rats, oral 50 per cent lethal dose (LD_{50}) for cadmium was already determined as 90 mg/kg body

weight (El-Refaiy and Eissa, 2013).

Preparation of Brahmi (*Bacopa monnieri*) ethanolic extract: The Brahmi plant was collected in the month of April, 2022 and authenticated from Ministry of Environment, Forest and Climate change, Government of India, wide letter no. Tech./Herb (Ident.)/2022-23/482, Herbarium sheet number 1164. The ethanolic extract of Brahmi was prepared as per method described by Phrompittayarat *et al.* (2013).

Experimental design: The experimental design, general procedure and use of experimental rats were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the university (VCC/IAEC/2022/1624-1651, Dated: 10/05/2022). A total of 48 male Wistar rats of approximately 6-8 weeks old were randomly divided into 8 groups i.e., G-I, G-II, G-III, G-IV, G-V, G-VI, G-VII and G-VIII with six rats in each group. G-I was kept as control, administered with distilled water; G-II was orally administered with high ($1/5^{th}$ LD_{50}) dose of $CdCl_2$; G-III was orally administered with medium ($1/10^{th}$ LD_{50}) dose of $CdCl_2$; G-IV was orally administered with lower ($1/20^{th}$ LD_{50}) dose of $CdCl_2$; G-V was orally administered with high ($1/5^{th}$ LD_{50}) dose of $CdCl_2$ along with Brahmi extract supplementation @ 50 mg/kg body wt., orally; G-VI was orally administered with medium ($1/10^{th}$ LD_{50}) dose of $CdCl_2$ along with Brahmi extract supplementation @ 50 mg/kg body wt., orally; G-VII was orally administered with lower ($1/20^{th}$ LD_{50}) dose of $CdCl_2$ along with Brahmi extract supplementation @ 50 mg/kg body wt., orally; and G-VIII was kept as Brahmi control and supplemented with Brahmi extract @ 50 mg/kg body wt., orally.

Estimation of Haematological parameters: At the end of experimental study (28^{th} day), blood samples were collected in sterile Ethylene Diamine Tetra Acetate (EDTA) coated vials from Wistar rats through retro-orbital venous plexus. Blood samples were collected in sterile EDTA coated vials for evaluation of haematological parameters i.e., Hb, PCV, TEC, MCV, MCH, MCHC, TLC and Platelet count by using haematological analyzer (MS4Se-Melet Schloesing Laboratories-France). The thin blood smears were prepared from fresh blood and stained with Giemsa staining method (Himedia). The counting of different leucocytes was done by battlement method.

Statistical analysis: The data obtained from haematological parameters were subjected to statistical analysis by using one way analysis of variance (ANOVA). Statistical analysis of experimental data was determined by SPSS 20.0 version software.

RESULTS AND DISCUSSION

The present study was conducted to evaluate the ameliorative effect of Brahmi in cadmium induced haematological changes in 48 male Wistar rats.

Haematological studies conducted in the present experimental trial have been shown in Table 1 and 2. No significant alterations were observed in haematological parameters in both the control groups *viz*. G-I and G-VIII, throughout the experimental study. It shows that Brahmi extract @ 50 mg/kg body weight has no adverse effects when given as supplement to rats.

Haematological studies revealed that mean Hbcount, PCV, TEC, MCV and MCHC in all the cadmiumintoxicated groups (G-II, G-III and G-IV) were lower as compared to their respective control groups (G-I and G-VIII) in dose dependent manner at the end of experiment, respectively. Cadmium can interact with red blood cells and significantly reduced their activity which is responsible for lowering values of red blood cells in our study. Group received high dose of Cd (G-II) showed significant ($P<0.05$) decrease in mean values of all the parameters studied. However, groups received medium and low dose of cadmium (G-III and G-IV) showed non-significant decrease in all the parameters studied as compared to control groups (G-I and G-VIII), respectively. Earlier authors also reported that, Cd induced microcytic hypochromic anaemia might be due to intravascular haemolysis and direct damage of Cd to erythrocytes (Angmo *et al.*, 2015, Kour *et al.*, 2017; Orororo and Asagba, 2022; Udi *et al.*, 2022). It inhibits erythrocyte production due to reduction of haem synthesis (James and Sampath, 1999). Represented Cd groups supplemented with Brahmi (G-V, G-VI and G-VII) showed non significant increase in haematological values as compared to Cd intoxicated groups (G-II, G-III and G-IV). Almost similar changes were observed in rats supplemented with Centella asiatica (variety of Brahmi) in Cd induced toxicity in rats (Ghosh and Indra, 2015), methotrexate intoxicated rats (Tummala Srinivas *et al.*, 2021). This improvement in haematological parameters might be due to chelation of metal ions, scavenging of free radicals and enhanced antioxidative defense enzymes by Brahmi (Bhattacharya *et al.*, 2000; Ayyathan *et al.*, 2015).No significant difference was noticed in mean MCH values in all the groups (G-II, G-III, G-IV) as compared to control groups (G-I and G-VIII), at the end of experiment. Similar changes were observed in Cd intoxicated rats in earlier study (Kisadere *et al.*, 2022). Represented Cd groups supplemented with Brahmi (G-V, G-VI and G-VII) also showed non significant changes in haematological values as compared to Cd intoxicated groups (G-II, G-IIIand G-IV). Mean platelets count was significantly ($P<0.05$)

Table 1. Haematological parameters (Mean ± SE) of rats in different experimental groups at 28 days

Group	Hb (g/dl)	PCV (%)	TEC ($x10^6$/cu mm)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets ($x10^3$/cu mm)
G-I	11.75^{ab} ±0.36	35.47^{a} ±0.99	7.23^{ab} ±0.41	49.94^{ab} ±3.25	17.69±0.80	35.12^{a}±0.90	380.50^{ab} ±26.95
G-II	9.63^{c} ±0.49	26.83^{c} ±0.50	5.49^{c} ±0.27	49.41^{b} ±2.51	16.98±0.71	33.00^{c}±1.02	287.33^{c} ±47.21
G-III	10.53^{bc} ±0.36	27.88^{bc} ±1.64	5.71^{c} ±0.41	49.49^{ab} ±3.02	17.56±0.95	33.95^{b} ±0.87	355.67^{abc} ±23.67
G-IV	10.94^{abc} ±0.25	29.78^{bc} ±0.41	5.92^{c} ±0.18	50.55^{a} ±1.85	17.74±0.59	35.40^{a} ±0.69	382.33^{ab} ±17.33
G-V	11.02^{abc}±0.40	30.52^{b} ±0.72	6.25^{bc} ±0.20	49.51^{b} ±2.41	17.46±0.67	33.86^{ab} ±0.45	317.17^{bc} ±22.95
G-VI	11.30^{ab}±0.49	35.97^{a} ±0.84	7.36^{ab} ±0.39	49.44^{ab} ±2.31	17.97±1.04	34.05^{ab} ±0.97	394.00^{ab}±19.35
G-VII	11.70^{ab}±0.75	37.27^{a} ±1.46	7.88^{a} ±0.53	50.79^{a} ±1.74	17.78±1.05	34.49^{a} ±1.37	396.33^{ab} ±19.69
G-VIII	12.03^{a}±0.36	37.78^{a} ±1.40	8.06^{a} ±0.51	50.82^{a} ±3.29	17.84±1.12	34.98^{a} ±1.15	406.00^{a} ±17.91

Means ± S.E. - Different superscripts (a, b, and c) in the same column differ significantly (P 0.05)

G-I: Control group, G-II: 1/5th dose of LD_{50} of $CdCl_2$, G-III: 1/10th dose of LD_{50} of $CdCl_2$, G-IV: 1/20th dose of LD_{50} of $CdCl_2$, G-V: 1/5th dose of LD_{50} of $CdCl_2$ + Brahmi extract @ 50 mg/kg body wt., G-VI: 1/10th dose of LD_{50} of $CdCl_2$ + Brahmi extract @ 50 mg/kg body wt., G-VII: 1/20th dose of LD_{50} of $CdCl_2$ + Brahmi extract @ 50 mg/kg body wt., G-VIII: Brahmi extract @ 50 mg/kg body wt.

Table 2. Total Leukocyte and Differential Leukocyte Count (Mean ± SE) of rats in different experimental groups at 28 days

Parameters	WBC ($x10^3$/cu mm)	Neutrophils (%)	Lymphocytes (%)	Monocyte (%)	Eosinophils (%)	Basophils (%)
G-I	11.11^{a} ±1.04	19.67^{d} ±2.67	76.17^{a} ±2.89	2.5^{a} ±0.43	1.17±0.31	0.50 ±0.22
G-II	4.66^{d} ±0.42	58.83^{a} ±4.5	40.17^{c} ±4.5	0.67^{b} ±0.33	0.33±0.21	0.01 ±0.00
G-III	6.66^{cd} ±1.07	49.33^{ab} ±4.27	48.33^{bc} ±4.5	1.5^{ab} ±0.34	0.83±0.31	0.01 ±0.00
G-IV	8.57^{bc} ±0.76	42.83^{b} ±3.43	55.00^{b} ±3.15	1.67^{ab} ±0.33	0.33±0.21	0.17±0.07
G-V	6.85^{cd} ±0.93	54.33^{a} ±3.59	43.5^{c} ±3.56	1.17^{ab} ±0.48	1.00±0.45	0.00 ±0.00
G-VI	9.49^{ab} ±0.58	29.67^{c} ±1.33	67.17^{a} ±1.35	1.83^{ab} ±0.75	1.00±0.45	0.33 ±0.21
G-VII	10.60^{ab} ±0.32	21.67^{cd} ±2.03	73.67^{a} ±2.35	2.17^{a} ±0.4	1.33±0.31	0.50 ±0.21
G-VIII	10.98^{a} ±0.15	19.83^{d} ±2.18	76.17^{a} ±2.09	2.33^{a} ±0.49	1.17±0.48	0.50 ±0.06

Means ± S.E. - Different superscripts (a, b, c and d) in the same column differ significantly (P 0.05)

G-I: Control group, G-II: 1/5th dose of LD_{50} of $CdCl_2$, G-III: 1/10th dose of LD_{50} of $CdCl_2$, G-IV: 1/20th dose of LD_{50} of $CdCl_2$, G-V: 1/5th dose of LD_{50} of $CdCl_2$ + Brahmi extract @ 50 mg/kg body wt., G-VI: 1/10th dose of LD_{50} of $CdCl_2$ + Brahmi extract @ 50 mg/kg body wt., G-VII: 1/20th dose of LD_{50} of $CdCl_2$ + Brahmi extract @ 50 mg/kg body wt., G-VIII: Brahmi extract @ 50 mg/kg body wt.

decreased in G-II while G-III and G-IV showed non-significant changes as compared to control groups (G-I and G-VIII). Similar changes of decreased platelet count were observed in Cd intoxicated rats in earlier studied at different doses of Cd administration (Olajide *et al.*, 2020; Orororo and Asagba, 2022). Andjelkovic *et al.* (2019) suggested that most common cause of lymphopenia in Cd toxicity might be due to direct toxic effect of Cd on platelets in spleen and cause destruction. Represented Cd groups supplemented with Brahmi (G-V, G-VI and G-VIII) showed non-significant increase in platelet count as compared to Cd intoxicated groups (G-II, G-III and G-IV). This effect may be due to chelation of metal ions (Bhattacharya *et al.*, 2000; Ayyathan *et al.*, 2015).

Mean total leucocyte count (TLC) was significantly (P<0.05) decreased in all the Cd intoxicated groups (G-II, G-III and G-IV) in dose dependent manner as compared to the control groups (G-I and G-VIII) at the end of experiment. Similar results of lower TLC count were observed in Cd intoxicated rats by earlier workers (Lopotych *et al.*, 2020). One possible cause of leucopenia in Cd toxicity might be due to increased destruction of WBC in spleen and thymus (Andjelkovic *et al.*, 2019). Cd intoxicated group (G-VI) supplemented with Brahmi showed significant (P<0.05) increase in TLC values as compared to G-III. However, G-V and G-VII showed non-significant changes as compared to G-II and G-IV. This might be due to anti-oxidative and chelating effect on metal ions (Ayyathan *et al.*, 2015; Velaga *et al.*, 2014). Almost similar results of ameliorative effect of Brahmi at different doses for 15 days was observed by earlier author in methotrexate in rats toxicity (Tummala Srinivas *et al.*, 2021).

Differential leucocyte count revealed that mean relative neutrophil count was significantly ($P < 0.05$) increased and mean relative lymphocyte count was significantly ($P < 0.05$) decreased (lymphocytopenia) as compared to control groups (G-I and G-VIII). Increase in neutrophil count might be due to inflammatory condition which arises due to changes in body tissues by cadmium toxicity (Logeswari *et al.*, 2012). Neutrophils were considered as frontline cells in the immune system as they are capable of recognizing, phagocytosis and destroying foreign agents (Soehnlein *et al.*, 2008). Lymphocytopenia observed in Cd intoxicated rats in present study was in agreement with earlier studies (Rhman *et al.*, 2011). Represented Cd intoxicated groups (G-VI and G-VII) supplemented with Brahmi showed improvement in DLC count as compared to Cd intoxicated groups (G-II,G-III and G-IV). Jain *et al.* (1994) also showed anti-inflammatory effect of Brahmi in experimentally produced inflammatory reaction in rats and Hossain *et al.* (2014) in both carrageenan and histamine induced oedema test models in rats. No significant difference was noticed in mean relative monocyte, eosinophil and basophil counts in Cd intoxicated groups (G-II, G-III and G-IV) as compared to control groups (G-I and G-VIII) at the end of experiment. Similar changes were observed in Cd intoxicated rats in earlier studies (Andjelkovic *et al.*, 2019; Kisadere *et al.*, 2022).

It is evident from the above results that Cd intoxicated groups alone (G-II, G-III and G-IV) induce changes in haematological parameters in dose dependent manner at 28th day of Cd intoxication. Represented Cd intoxicated groups (G-VI and G-VII) supplemented with Brahmi (*Bacopa monnieri*) extract @ 50 mg/kg of body weight showed significant improvement in haematological parameters studied in dose dependent manner at the end of experiment. However, mild improvements in parameters were observed in G-5 (Cd @ 18 mg/kg body weight + Brahmi) as compared to representative Cd administered G-2 (Cd @ 18 mg/kg body weight) indicated mild ameliorative effect of Brahmi at higher doses of cadmium induced toxicity.

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