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Beneficial Effects of *Murraya Koenigii* Leaves Chloroform Extract (MKCE) on Erythrocyte, Thrombocyte and Leukocyte Indices in Lead-intoxicated Mice

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ABSTRACT

Lead is a well-known heavy metal, ubiquitous environmental toxin. Exposure to lead induces a broad range of physiological, biochemical and behavioural dysfunctions in the body. Although a very few scientific evidences reported the protective effect of plant extracts on the haemotological alternations induced by lead. The study was aimed at evaluating *in vivo* protective role of *Murraya koenigii* leaves against the noxious effects of lead on the hematological parameters. *Murraya koenigii* leaves were shade dried and its chloroform extract was prepared by maceration method. Male albino Swiss mice were divided into three groups. Group I was served as normal control group, Group II was injected intraperitoneally lead acetate at daily once and Group III concurrently received *Murraya koenigii* Chloroform Extract (MKCE) orally and intraperitoneal injection of lead acetate for 7 days. On the 8th day, blood samples were collected for assessing erythrocyte, thrombocyte and leukocyte indices. Blood lead level was determined by atomic absorption spectrometer. Values obtained from group I were considered as normal and were compared in both groups. Significant difference in haemotological indices were observed in lead-intoxicated group compared to MKCE group. Significant decrease of erythrocyte indices like Hb, RBCs count, PCV and RDW values and WBCs count ($p<0.001$) were found in lead-intoxicated group as compared with MKCE group. Significant alterations were observed in thrombocyte indices such as PLT ($p<0.05$) and P-LCR ($p<0.001$) in MKCE group as compared to lead-intoxicated group. Significant improved values of Hb and WBCs count ($p<0.001$) were observed in MKCE group. MKCE has shown possible cardioprotective role against lead induced anaemia, cardiovascular diseases and ischemic heart disease by preventing haemotoxicity and by restoring thrombocytic indices and act as a good candidate for chelating agent of Pb.

Key words: Lead Acetate, Male Albino Swiss mice, *Murraya koenigii*

INTRODUCTION

Acute or chronic exposure to lead induces dysfunction in physiological, biochemical and behavioural parameters in the body. It is one of current interesting studies of different sequestering agents to chelate heavy metals for minimizing toxicities and safe for use. Synthetic chelating agents like EDTA[1] and synthetic antioxidants like Vitamin C and E[1] have been initially used to excrete in case of lead poisoning however it becomes concerned to serious health problems owing to many adverse effects. Naturally occurring plant extracts have been proved since many years for their scavenging properties and restoring capabilities against oxidative stress. These have also capacity for suppressing absorption of heavy metals into the body. Recent studies have been reported for the use of plant extracts like *Withania somnifera*[2] and *Tinospora cordiflora*[3] act as natural chelating agents to scavenge heavy metals *in lieu of* synthetic chelants and antioxidants.

Murraya koenigii is belonging to family Rutaceae[4] which has commonly synonyms such as Curry Leaves, Kadilimb and Kadhipatta. Several

bioactive compounds like euchrestine B, bismurrayafoline E, mahanine, mahanimbicine, mahanimbine[4] and essential oil[5] contributing antioxidative, hypoglycaemic, anti-trichomonal and hepatoprotective effects[6, 7]. On reviewing literature and to our knowledge, scientific evidences are less reported on the beneficial effects of *Murraya koenigii* leaves on lead induced damage in haematological parameters. So this study was aimed for evaluating *in vivo* protective role of *Murraya koenigii* leaves chloroform extract against the toxic effects of lead on the hematological parameters.

MATERIALS AND METHODS

Authentication and Preparation of *Murraya koenigii* Leaves Chloroform Extract (MKCE)

Fresh leaves of *Murraya koenigii* was purchased from the local market areas of Malkapur-Karad and authenticated by botanist. Fresh leaves was thoroughly washed under tap water and shade dried, powdered by using a mechanical grinder. Chloroform extract of *Murraya koenigii* leaves was prepared by maceration method. About 70 g of shade dried leaves of *Murraya koenigii* was extracted in the conical flask by using chloroform. The extraction process will be continued for 6-7 times till the appearance of colourless solvent in the flask. The collected extract was dried out by evaporating chloroform at room temperature to obtain 3% of yield from the crude extract; stored in suitable container for future use and labeled as *Murraya koenigii* Chloroform Extract (MKCE).

Experimental animals

Male Swiss albino mice (n=6 in each group) weighing between 25-30 g were used in the study. Animals were obtained from the Animal House, KIMS, Karad, India. The animals were maintained under standard husbandry conditions temperature 22 ± 2°C, humidity 45-55%, light: dark cycle (12:12h) for an acclimatization period of 15 days before performing the experiments.

The experiments were compiled with the guidelines for animal experimentation of laboratory and Institutional Animal Ethics Committee, KIMS, Karad approved for the study.

Acute Oral Toxicity and Dosage Fixation

According to earlier report, the LD_{50} value for the aqueous extract of leaves of *Murraya koenigii* was found to be 150mg/kg i. p. in rats[8]. The dosage of lead acetate (15 mg/kg i. p) and MKCE (50 mg/kg p. o) were chosen in accordance to previous studies of Ghosh *et al* (2013) [8] and Ghosh *et al* (2012)[9].

Animal Groups

Male Swiss albino mice were randomly divided into three groups with each consisting of six animals and was continued at once daily for the consecutive seven days. Normal diet and water were given.

Group-I (Normal)

Normal diet and water only *ad libitum*

Group-II (Pb treated)

Lead acetate (15 mg/kg i. p)

Group-III (Pb + MKCE treated)

MKCE (50 mg/kg p. o) + lead acetate (15 mg/kg i. p)

On 8th day, mice were anaesthetized under anesthetic ether, blood was collected by retro-orbital and animals were sacrificed by cervical decapitation. Blood samples were collected with bottles containing anticoagulant and Ethylene Diamine Tetra Acid (EDTA) for analyzing hematological parameters.

Erythrocytes Indices Analysis

Erythrocytes parameters viz., Red Blood Cells (RBCs), White Blood Cells (WBCs), Packed Cell Volume (PCV), Hemoglobin (HGB), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width-Standard Deviation (RDW-SD) and Red Cell Distribution Width-Correlation Variance (RDW-CV) were analyzed by using automated analyzer.

Blood Lead Analysis

Blood lead levels in mice were analyzed by employing flame atomic absorption spectrometry

in accordance to Ahmed *et al.* (2013)[10]. All laboratory glassware, polypropylene tubes, and disposable micropipette tips were immersed for several hours in 1:1 v/v concentrated HNO_3/H_2O, thoroughly rinsed in deionized water to avoid any possible contamination. Blood samples (2 ml) were added to 8 ml of HNO_3; centrifuged at 15000 rpm for 15 min and 2ml aliquot was taken from the clear solution and diluted (1:5 v/v) with deionized water. Diluted blood samples were injected into the atomic absorption spectrophotometer (PerkinElmer Model 400, Shelton, CT, USA). Hollow cathode lamps of Pb were used at wavelength of 283.3 nm. Levels of blood lead (Pb) were expressed as part per million (ppm).

Statistical Analysis

Data were expressed as the mean ± S.E (n=6). Statistical analysis was done using *post hoc* analysis of one way Analysis of Variance (ANOVA) followed by Dunnett's test and p value < 0.05 was considered significant.

RESULTS

The present study describes the *in-vivo* protective effects of MKCE against lead induced haemotoxicity in male albino Swiss mice.

Erythrocyte Indices

Erythrocytes status of mice were assessed the levels of Red Blood Cells (RBC), Hemoglobin (HGB), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width-Standard Deviation (RDW-SD) and Red Cell Distribution Width-Correlation Variance (RDW-CV) given in Table 1. In table 1, acute lead exposure led to significant decrease in RBC (6.22 ± 0.15), HGB (9.35 ± 0.21), PCV (28.48 ± 0.56), RDW-SD (27.85 ± 0.40) and RDW-CV (15.43 ± 0.45) in lead-intoxicated group as compared with respect to control group ($P < 0.05$). No significant change in MCV (49.53 ± 1.03), MCH (15.19 ± 0.17) and MCHC (30.60 ± 0.59) values were observed in lead-treated mice. MKCE co-administrated with the lead, it could normalize the levels of RBC (7.25 ± 0.11), HGB (11.10 ± 0.3), PCV (34.85 ± 1.66) values ($P< 0.05$) in all the blood indices significantly level. Insignificant result on MCV, MCH and MCHC levels were found in all groups. It is interesting to note that haemoglobin level could normalize to nearby the normal values in MKCE with lead-treated mice.

Table 1: Effects of MKCE on Erythrocyte Indices in Lead-intoxicated Mice

Groups	I	II	III
Treatment	**Control**	**Pb**	**Pb + MKCE**
Hb	13.68 ± 0.12	9.35 ± 0.21^{a***}	11.10 ± 0.3^{b***}
RBCs	8.14 ± 0.27	6.22 ± 0.15^{a***}	7.25 ± 0.11^{b*}
PCV	40.65 ± 0.72	28.48 ± 0.56 ^{a***}	34.85 ± 1.66 ^{b**}
MCV	48.3 ± 0.82	49.53 ± 1.03	48.40 ± 0.87
MCH	14.86 ± 0.43	15.19 ± 0.17	14.36 ± 0.40
MCHC	31.20 ± 0.32	30.60 ± 0.59	31.01 ± 0.37
RDW-SD	31.58 ± 0.57	27.85 ± 0.40 ^{a***}	30.16 ± 0.50
RDW-CV	22.05 ± 0.45	15.43 ± 0.45 ^{a***}	19.78 ± 0.55 ^{b*}

Values are expressed=Mean ± SEM. Number of animals =6, Hb-Haemoglobin (g/dl),
RBCs-Red Blood Cells ($\times 10^6$ /mm), PCV-Packed Cell Volume (%), MCV-Mean Cell Volume (cu microns),
MCH-Mean Cell Haemoglobin (pg), MCHC-Mean Cell Haemoglobin Concentration (g/dl),
RDW-SD-Red Cell Distribution Width-Standard Deviation (%),
RDW-CV-Red Cell Distribution Width-Correlation Variance (%)
^{a}P<0.05-compared to group I (control), ^{b}P<0.05 compared to group II (lead exposed)
*P<0.05-Significant, **P<0.01-Significant, ***P<0.001-High significant

Thrombocyte Indices

Platelet status of mice were estimated the levels of Platelet (PLT), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (P-LCR), Platelet Crit (PCT) given in Table 2. In table 2, lead intoxication led to significant decrease in P-LCR (6.98 ± 0.79) in lead-intoxicated group compared with respect to control group ($P < 0.05$) whereas MKCE treated group has shown statistically significant improvement ($p < 0.001$). No significant change in PLT, PDW, MPV and PCT values were observed in lead-treated mice. MKCE co-administrated with lead group, it could elevate the level of PLT (710.33 ±107.66) above normal value which is interesting.

Leukocyte Indices

Leukocyte status of mice were evaluated the levels of White Blood Cells (WBC), Neutrophil (N), Lymphocyte (L), Monocyte (M), Eosinophil (E) and Basophil (B) given in Table 3. In table 3, statistically significant increase in W (15.04 ± 0.5)

Table 2 : Effects of MKCE on Thrombocyte Indices in Lead-intoxicated Mice

Groups Treatment	I Control	II Pb	III Pb + MKCE
PLT	992.16 ± 108.82	1446.83 ± 224.32	710.33 ±107.66^{b*}
PDW	8.18 ± 0.17	7.53 ± 0.08	7.71 ±0.31
MPV	7.65 ± 0.09	7.48 ± 0.09	7.45 ±0.08
P-LCR	9.81 ± 0.61	6.98 ± 0.79^{a}	12.66 ±0.38^{b***}
PCT	0.79 ± 0.08	1.01 ± 0.16	0.58 ±0.09

Values are expressed=Mean ± SEM. Number of animals =6,
PLT-Platelet (×10^3 /m mm), PDW-Platelet Distribution Width, MPV-Mean Platelet Volume (fl),
P-LCR-Platelet-Larger Cell Ratio (%), PCT-Platelet Crit (%), ^{a}P<0.05-compared to group I (control),
^{b}P<0.05 compared to group II (lead exposed)*P<0.05-Significant, **P<0.01-Significant, ***P<0.001-High significant

Table 3: Effects of MKCE on Leukocyte Indices in Lead-intoxicated Mice

Groups Treatment	I Control	II Pb	III Pb + MKCE
WBCs	6.16 ± 0.7	15.04 ± 0.5 a***	10.58 ± 0.68 b***
Differential Leukocyte Count			
E	0.15 ± 0.01	0.11 ± 0.04	0.16 ± 0.01
B	8.55 ± 0.94	17.46 ± 2.05a**	7.22 ± 0.78b***
N	4.18 ± 0.31	5.31± 0.3	4.48 ± 0.29
L	40.5 ± 4.5	49.1± 2.82	35.03 ± 2.1b*
M	5.8 ± 0.92	11.91± 2.22a*	5.98 ± 0.34b*

Values are expressed=Mean ± SEM. Number of animals =6,
PLT-Platelet (×10^3 /m mm), PDW-Platelet Distribution Width, MPV-Mean Platelet Volume (fl),
P-LCR-Platelet-Larger Cell Ratio (%), PCT-Platelet Crit (%), ^{a}P<0.05-compared to group I (control),
^{b}P<0.05 compared to group II (lead exposed)*P<0.05-Significant, **P<0.01-Significant, ***P<0.001-High significant

and B (17.46 ± 2.05) were found in lead-intoxicated group as well as MKCE treated group shown augmentation in WBC (10.58 ± 0.68) and B (7.22 ± 0.78) compared with respect to control group ($p < 0.001$). No significant changes in N and E values were observed in group II and III except LYMPH (35.03 ± 2.1) was found to be significant increase in MKCE treated group. MKCE co-administrated with the lead, the values of L (35.03 ± 2.1), M (5.98 ± 0.34) ($P< 0.05$) shown difference at significant level.

DISCUSSION

Lead is a well-known heavy metal, ubiquitous environmental toxin. Some studies have been reported lead-induced iron deficiency or anemia in animals [2-3] as lead being strong competitive inhibitor of iron/ferric ion [11]. In haem synthesis pathway, lead is responsible to interfere with several functioning enzymes. Decreased Hb level might be linked to inhibiting enzyme ferrochelatase and delta-Amino Levulinic Acid Dehydratase (δ-ALAD), a cytosolic sulfydryl enzyme is averted to convert from delta-Amino Levulinic Acid (ä-ALA) to porphobilinogen [2] and causes avoidance of iron insertion into protoporphyrin [12]. By inactivating enzyme δ-ALAD, it may cause to the accumulation of δ-ALA in the cell which may instigate to the overproduction of Reactive Oxygen Radicals (ROS) and act as a partial agonist for Gamma-Aminobutyric Acid (GABA) [3]. Lead at toxic level disrupts the utilization of oxygen by interfering respiratory cytochromes [12]. Owing to oxidative mechanisms by ROS, it often damages RBCs thus haemolysis is occurred and its significant decreased RBCs count ($p<0.001$) as shown in table 1, is in agreement with other studies reported[2-3].

Attenuated number of PVC level ($p<0.001$) is indicated for the destruction of large number of erythrocytes which is related to the accumulation of pyrimidine nucleotides affecting stability and cellular energetic of RBCs[2]. RDW is reported as inflammatory biomarker[13] and also established for correlated with Cardiovascular Disease (CVD)[14] which may contribute to increased cardiovascular risk factor. Significant decreased levels of RDW-SD and RDW-CV ($p<0.001$) have shown in the present study and upto date, it might be less reported for RDW values (including SD and CV) in lead-intoxicated mice.

Platelets play an important role in maintaining homeostasis and various thrombocytic mechanisms. Thrombocytosis involves in the predisposition of thrombotic stroke and Ischemic Heart Disease (IHD) [15]. P-LCR is a major determinant of IHD [16]. Comparing to lead-intoxicated group, MKCE supplementation in group III has shown progressed level of PLT ($p<0.05$) which is revealed the restoring effects of MKCE to nearby normal level and might be useful in thrombocytosis. An elevated the level of P-LCR ($p<0.001$) in MKCE treated group indicated for increasing oxygen supply to the heart in IHD as shown in table 2.

Decreased WBCs count in blood reflects the anticancer property of drug or extracts [17] and as biomarker of inflammation[18] which imply the antioxidant and anti-inflammatory properties of MKCE where it has shown its significant effect against lead-induced leukocytosis ($p<0.001$) and increased signiûcant count of basophil in table 3, specified for basophilic stippling of red blood cells[19] which is biomarker for the detection of lead poisoning.

CONCLUSION

MKCE may show possible cardioprotective role against lead induced anaemia, CVD and IHD by preventing haemotoxicity and by restoring thrombocytic indices and act as a good candidate for chelating agent of Pb.

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