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Research Article

AMELIORATIVE EFFECTS OF *TINOSPORA CORDIFOLIA* AGAINST CYTOGENOTOXICITY INDUCED BY LEAD ACETATE IN *MUS MUSCULUS*

[1*]Rahul Nayan and [2]Ashok Kumar Thakur

[1,2]Eco-Genetics Lab-University Department of Zoology, T.M. Bhagalpur University Bhagalpur, Bihar, India

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ABSTRACT

Studies concerning exposure of humans to lead (Pb) during daily activities are sometimes leading to many complications. Pb is a poisonous heavy metal; its toxicity can change the antioxidant balance in biological systems. *T. cordifolia* is rich in phenolic acids and flavonoids which exhibit a wide range of biological effects and act as a natural antioxidant. The objective of the research work- first is to investigate the genotoxic potential of Pb-acetate on Bone marrow cells (BMCs), Second is to investigate the antigenotoxic potential of *T. cordifolia* against genotoxic induced by Pb-acetate in BMCs of *Mus musculus* (Swiss albino mice). For the research experiment, Adult *Mus musculus* of the same age group were selected andwere distributed into four groups (Group I, II, III &IV). After the completion of dose treatment, the experimental animals were euthanized by cervical dislocation and exposed both femora for the analysis of Micronucleus (Mn) in Polychromatic and Normochromatic erythrocytes. For the assessment of frequency of Mn in polychromatic or normochromatic erythrocytes 800 - 1000 cells were screened randomly under the compound microscope at 40X. and screening was done by image J microscopy software. Results of the experiment showed that the total frequency of Mn in polychromatic and normochromatic erythrocytes was 0.27 ± 0.07 in Group I, 0.18 ± 0.06 in Group II, 4.32 ± 0.32in Group III, and 0.94 ± 0.15 in Group IV and it is a significant difference to control and treated group at $p < 0.05$ or < 0.01. Hence, the results of the research experiment indicate that *T. cordifolia* stem aqueous extract has the potency to ameliorate and minimize the genotoxic induced by Pb-acetate in *M. musculus* (Swiss albino mice).

Keywords: Erythrocytes, Lead (Pb), Micronucleus, *Mus musculus*, *Tinospora cordifolia.*

INTRODUCTION

Heavy metals (HMs) including soft grey-blue HM-lead (Pb) are naturally occurring elements that have a high atomic weight and a density at least five times greater than that of H_2O. Most HM salts are soluble in H_2O and form aqueous solutions and consequently cannot be separated by ordinary physical means of separation (El-Zahrani and El-Saied, 2012). Their several industrial, domestic, agricultural, medical, and technological applications have led to their wide distribution in the environment, raising concerns over their potential effects on human health and the environment (Tchounwou *et al*., 2012). Although the existence of Pb is indicated in nature but human activities have been found as the main reason for increasing Pb content in the environment (Shahid *et al*., 2015). Pb poisoning is among the oldest and the most widely studied occupational and environmental hazards. Pb is known to induce a broad range of physiological, biochemical, and behavioural dysfunctions in laboratory animals and humans (Kumar *et al*., 2018; Nayan & Thakur, 2023), including central and peripheral nervous systems (Araki *et al*., 2000), haemopoietic system (Moore, 1988) cardiovascular system (Feng, 2018) reproductive systems (Shabani, 2015). Furthermore, this HM- Pb will act as the cause of mutagens, teratogens, clastogens, or carcinogens in both animals and humans. Their toxicity depends on several factors including the dose, route of exposure, and nutritional status of exposed individuals (Sheydaei *et al*., 2020). Teratogenic/mutagenic effects of HM- Pb have been demonstrated and reviewed in man as well as other

*Corresponding Author: Rahul Nayan, Research Scholar, Eco-Genetics Lab- University Department of Zoology, T.M. Bhagalpur University Bhagalpur, Bihar, Email: rahulnayan1993@gmail.com.

mammals and lower vertebrates, but many of the results are inconsistent (Gerber *et al*., 1980, Winder and Bonin 1993). Several mechanisms have been proposed to explain Pb-induced toxicity. One possible mechanism is the disturbance of antioxidant balance by the generation of (ROS) reactive oxygen species (Yiin, 1995; Patra, 2011). The toxic effect of several HMs is controlled by the different natural plant products of different species. The use of herbal extract or medicinal plant extract both ethanolic and aqueous used for the treatment of adverse effects is as old as humanity. The main benefits of herbal-based medications include wide availability, their low price, and no incidences of serious harmful effects. According, to the number of experimental research work on natural remedies and species of medicinal plant that could protect both humans and animals against H.M. toxicity has increased.

In this study, the genotoxicity of Pb was tried to mitigate or ameliorate by *T. cordifolia* (Giloy). *T. cordifolia* is often considered to be an important medicinal plant that has been used since ages according to the ancient Ayurvedic literature (Singh *et al*., 2019; Pal *et al*., 2017). Its usage has been found in treating a number of diseases and is recommended for improving the immunity of human beings as it increases body resistance as given by (Panchabhai *et al*., 2008). The extract of different parts of this plant has shown anticancer, antigenotoxic, anti-aging, anti-inflammatory, and immunomodulatory characteristics (Singh *et al*. 2003; Dwivedi and Enespa, 2016).

MATERIALS AND METHODS

Collection of Plant Material

Mature plants of *Tinospora cordifolia* (Giloy) were collected from various locations from Jamui district (Bihar) identified by Tapan Kumar Pan, (Department of Botany, Faculty of Science, Tilka Manjhi Bhagalpur University, Bhagalpur).

Preparation of Plant Extract

Collected plant materials were washed under running tap water. The stem of *T. cordifolia* was dried under shade at room temperature and crushed into a fine powder with an electronic grinder and stored at room temperature. For the administration of *T. cordifolia* stem powder. The concentrated stock solution was prepared according to 200mg/kg of body weight of *M. musculus*. Example for the 30 grams of *M. musculus* - The stock solution of aqueous extract was prepared by soaking 300 mg of dried powder in 50 ml of Glass distilled water (GDW). The solution was left at room temperature for 12 hours and then filtered with the help of Whatman filter paper (Rani *et al*., 2015). Then, the resulting extract was stored, protected from light in a refrigerator at -20 °C in a glass container until use.

Figure1. Dried stem of *T. cordifolia*

Figure 2. Powder of *T.cordifolia* stem

Figure 3. Stem extract of *T. cordifolia*

Maintenance of *Mus Musculus* (Swiss albino Mice)

For the research experiment work, 20 adult M. *musculus* of the same age and an average weight of 25 -30 gm body weight were taken from the animal house of the University Department of Zoology, T.M. Bhagalpur University, Bhagalpur. All the *M. musculus* were kept in a polypropylene cage under hygienic conditions in a well-ventilated room with 6 – 8 hrs photoperiods at an ambient temperature of 25 ± 2 Celsius under animal husbandry conditions. And were divided into equal numbers of mice into four groups one group was considered the control (I) group and the other three were considered treated (II, III&IV) groups.

Experimental protocol

After the acclimatization period, Experimental animals *M. musculus* were weighed and distributed into four groups comprising 5 animals in each group.

The group distribution is as follows,

- **Group I (C)**: - Control with GDW (1 ml).

- **Group II (TC)**: - Animal treated with *T. cordifolia* stem extract (200 mg/kg B. wt./day)
- **Group III (LA)**: - Animal treated with Lead acetate (40 mg/kg B.wt. /day).
- **Group IV (LA+TC)**: - Animal treated with Lead acetate + *T. cordifolia* stem extract (40 + 200 mg/kg/day).

Procedure for the Micronucleus assay

After the completion of 40 days of treatment, the control and treated group mice were euthanized by cervical dislocation, both femora were exposed out and bone marrow cells (BMCs) from both femora were collected in test tubes containing sodium citrate. The slides were prepared directly by smears of bone marrow (Schmid, 1975) with slight modifications in the technique (Das and Kar, 1980; Salamone and Heddle, 1983).

Observation and Screening of the slides

To assess the presence of micronuclei (Mn) within Polychromatic Erythrocytes (PCE) and Normochromatic Erythrocytes (NCE). Slides were coded and scored blind, and 800-1000 PCEs and NCE per animal were observed for the presence of micronuclei. Observations were done by compound light microscopy at 40× magnification and screenings were made by image J cell counter software (Nayan & Thakur, 2022).

Statistical Analysis

For statistical evaluation of the experimental data, a Unpaired 't-test' were performed. The difference between the control and experimental groups was analyzed by using Prism software (PRISM, 1997) or SPSS (Statistical Package for Social Sciences) software. The significance of differences was examined at the *p*-value of $p < 0.05$ or < 0.01. as significant or highly significant.

RESULTS AND DISCUSSION

Animal treated (Group III) with Lead acetate (40 mg/kg B.wt. /day) resulted in an increase in the frequency of micronuclei (Mn) in the PCE and NCE of *M. Musculus* and there was a statistically highly significant difference between these Group III (LA) and Group I (C). Animal treated with *T. cordifolia stem* extract (200 mg/kg B. wt. /day) resulted in a decrease in the percentage of micronuclei in the PCE and NCE in experimental animals *M. musculus* as compared to the control group and there was not a statistically significant difference between Group II (TC) and Group I (C). Animal treated with Lead acetate + *T. cordifolia* stem extract (40 + 200 mg/kg/day). Resulted in a decrease in the frequency of Mn in the PCE and NCE. and there was a statistically significant difference between Group II(TC) and Group IV (LA +TC). These results are summarized in Tables: 1, 2, and Figure 5.

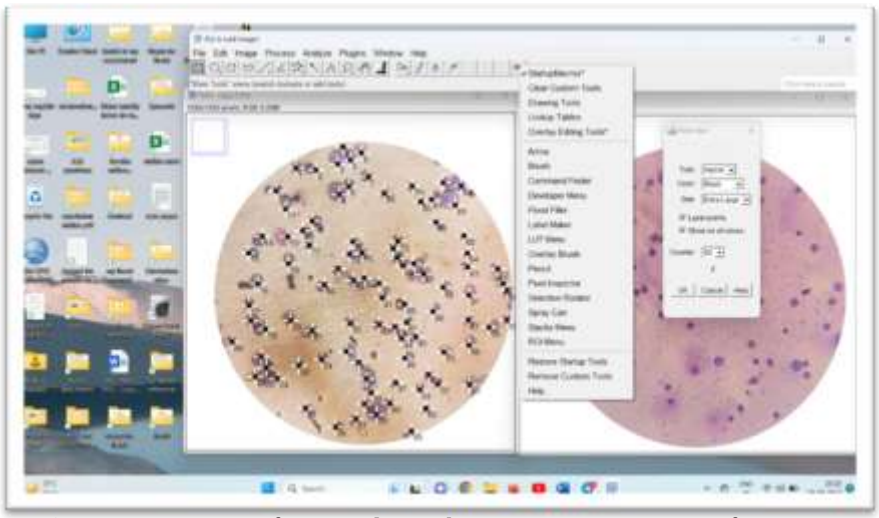

Figure 4. Screening of PCE and NCE by Image J Microscopy software.

Table 1. Result of frequency of micronucleus (Mn) in the bone marrow erythrocytes of *Mus musculus* for 40 days treatment durations.

Exp.	**Result of PCEs and MnPCEs.**			**Result of NCEs and MnNCEs**			**Result of total Mn in PCEs and NCEs.**		
Variant	Score	Mn	Mean% ± SE	Score	Mn	Mean% ± SE	Score	Mn	Mean% ± SE
C	2341	5	0.21±0.09	2075	7	0.33±0.12	4416	12	0.27±0.07
TC ^{NS}	1963	3	0.15± 0.08	1849	4	0.21± 0.10	3812	7	0.18± 0.06
LA**	2035	103	5.06± 0.48	1846	65	3.52± 0.42	3881	168	4.32± 0.32
LA + C*	2114	21	0.99± 0.21	1904	17	0.89± 0.21	4018	38	0.94± 0.15

Abbreviations
C-Control**, TC**- *Tinospora cordifolia***, LA**-Lead acetate**, -MN**- Micronucleus, **PCE**- Polychromatic erythrocyte, **NCE**- Normochromatic erythrocyte, **MNPCE**- Micronucleus polychromatic erythrocyte, **MNNCE** – Micronucleus normochromatic erythrocyte.

***, **,** ^{NS}indicates the significant, highly significant, and non-significant difference to control (C), Lead acetate (LA), *Tinospora cordifolia* (TC), and LA +TC respectively at $p < 0.05$ or $p < 0.01$.

Table 2. Summary of the result of Unpaired't' test of experimental variant (C, TC, LA, LA +TC) of Mn in PCE and NCE.

Effect	t value	df	Result of p-value at (< 0.05)
C **Vs.** LA	12.36	8	Significant
C **Vs.** TC	0.97	8	Insignificant
C **Vs.** TC + LA	4.04	8	Significant
TC **Vs.** LA	12.71	8	Significant
LA**Vs.** TC + LA	9.56	8	Significant
TC **Vs.** TC + LA	4.70	8	Significant

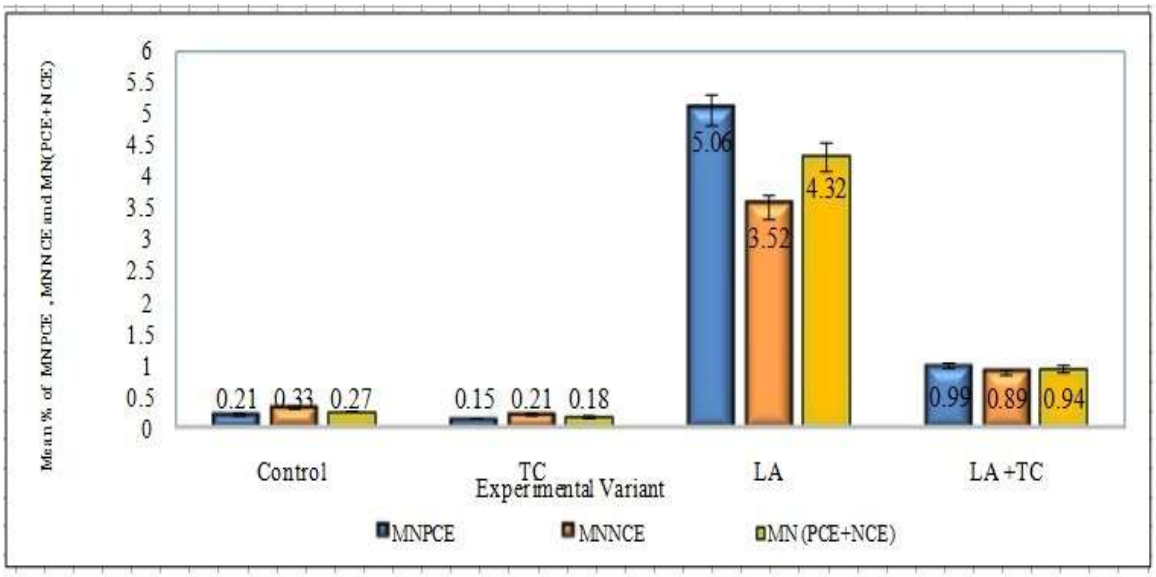

Figure 5. Histogram showing Mean % of Micronuclei (Mn) in PCE, NCE, and PCE +NCE in bone marrow cells (BMCs) of *M. musculus* in 40 days of treatment duration.

Pb is ubiquitously found in environmental and industrial pollutants that have been detected in nearly all phases of the environment and biological system. Its perseverance in human and animal tissues has quite often been connected with considerable health risks. The toxicity of Pb has been studied from many years throughout several end-points but data related to the mutagenic, teratogenic, clastogenic, and carcinogenic properties of Pb and compounds of Pb is still conflicting. The IARC classified Pb as a possible human carcinogen (IARC, 1987), on the basis of sufficient evidence for carcinogenicity in experimental animals but inadequate evidence for carcinogenicity in humans, and the inorganic Pb compounds are classified as probable human carcinogens (IARC, 2006), on the basis of sufficient evidence for carcinogenicity in experimental animals but limited evidence for carcinogenicity in humans. During the past decade, traditional systems of medicine have become increasingly important in view of their safety. Current estimates suggest that, in many developing countries, a substantial proportion of the population relies heavily on traditional practitioners and medicinal and herbal plants to meet primary healthcare needs. Despite all of the advances made by the pharmaceutical industry in the development of novel and highly effective medicines for the treatment of a wide range of diseases, there has been a marked increase in the use of herbal and plant medicines in the more affluent countries of the world. To our acquaintance or knowledge, the present study aimed to evaluate and investigate the ameliorative effect of *T. cordifolia* (Giloy) stem aqueous extract against genotoxicity induced by Pb acetate in *Mus musculus.* It has been suggested that an *in vivo* micronucleus (Mn) test should be carried out to evaluate

the genotoxicity hazard of any substance if it is positive in either a reverse mutation assay or a chromosomal anomalies (CAs) assay or both assays (Lee, 2014). Mn appears in cells due to chromosomal damage during the last mitosis and they are reliable indicators of the genotoxicity of exogenous agents (Fenech, 2016). This research study revealed, that the lead acetate has highly effective inthe formation of Mn in bone marrow cells (BMCs) of intoxicated *M. musculus* (Swiss albino mice) was significantly higher than that of the control group. These results clearly indicated that lead caused a significant increase in micronuclei frequency. These findings support the results from other reports that lead acetate can induce genotoxicity and increase the frequency of Mn in BMCs of *M. musculus* treated with the Pb compound (Abd El-Monem, 2012). However, little is known about plant extract acting as a protective agent against heavy metals-induced genotoxicity. Administration of *T. cordifolia* stem extract with lead acetate treatment clearly restored the genotoxic effect. Accumulating evidence suggests that the protective effect of plant materials could be attributed to their anti-oxidative properties (Elhamalawy, 2018). It is clear from the obtained results that *T. cordifolia* (Giloy) stem aqueous extract hasthe potency to ameliorate and minimize the genotoxic induced by the heavy metals Pb on BMCs of *Mus musculus (*Swiss albino mice*)*.

CONCLUSION

In conclusion, the result of this study demonstrated that lead acetate can be considered an environmentally genotoxic material. Besides, the present result showed that *T. cordifolia* (Giloy) has the potency to ameliorate or minimize lead acetate-induced genotoxicity. Therefore, this experimental study suggests that *T. cordifolia* (Giloy) may be useful as an ameliorating or minimizing agent against genotoxicity induced by lead acetate due to the presence of antioxidant properties.

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