RESEARCH ARTICLE

Beneficial effect of *Moringa oleifera* on Lead induced Oxidative stress

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ABSTRACT

Moringa oleifera is a tree belonging to family Moringaceae. Its leaves and seeds are reported to have ameliorative effects against metal toxicity. *Moringa oleifera* leaves are used in many physiologically adverse conditions and are to be an excellent remedial agent; it needs to be studied comprehensively. In the present investigation, the phytochemical analysis, anti-cytotoxic and anti-hemolytic effect of *Moringa oleifera* leaf extract and crude flavonoid extract was tested against Lead acetate induced cytotoxicity in onion root tips and hemolysis in Human Blood Erythrocytes. The anti-cytotoxic and anti-hemolytic activity increases with increase in the concentration of leaf extract and flavonoid extract of *Moringa oleifera.*

Keywords: Lead toxicity, anti-cytotoxic, anti-hemolytic, *Moringa oleifera* leaves powder

INTRODUCTION

In the last decades natural plant extracts as a form of medical treatment have become a subject of intensive research. There is growing interest in the search for an investigation of natural substances of plant origin that possess anti-mutagenic, anti-genotoxic and anti-oxidant activities against inducers of reactive oxygen species (ROS). Reactive oxygen or nitrogen species (ROS or RNS), such as O2**-**, NO**.**, RO**.**, ROO**.**, OH**.** which are generated either endogenously or exogenously by various environmental and metabolic factors (Halliwell B.1997). These reactive species induce DNA damage and thus play instrumental role in apoptosis or some immune mechanism which ultimately lead to the etiology of many pathological conditions including lethality, mutagenesis, carcinogenesis and aging.

Apart from the DNA damage free radicals interact with polyunsaturated fatty acids (PUFA) of the biological membranes and leads to a chain reaction called lipid per oxidation (LPO). Aldehydes are formed from lipid peroxidation that produce adducts and perturb Watson Crick base pairing of DNA (Kamat *et al.,* 2000). Antioxidants act as a major defense

against radical mediated toxicity by protecting the damages caused by free radicals. Recently special attention has been paid towards edible plants, especially those that are rich in phytochemicals. The phytochemicals are capable to combat with the free radicals efficiently. The phenolics are major chemical identity among the phytochemicals. This group of compounds is principally correlated with their antioxidant property (Wangensteen *et al.,* 2004, Hong *et al.,* 2008; Saito, *et al.,* 2008). Phenolic compounds are widely distributed in fruits and vegetables. Polyphenols can donate hydrogen to free radicals and generate relatively non reactive reduced forms, thus, acting as chain-breaking antioxidants (Nhukarume L, *et al.,* 2010).

Red blood cells (RBCs) are the primary targets of free radicals, owing to their high membrane concentrations of polyunsaturated fatty acids (linoleic and arachidonic acids in particular) and O2 transport associated with redox active haemoglobin molecules, which are potent promoters of ROS. Oxidation depletes membrane protein content, deforms RBCs, and disturbs microcirculation (Yang *et al.,* 2006; Rice-Evans *et al.,* 1986; Yu. 2001; Flynn *et al.,* 1983). It is also implicated in hemolysis (Ko, Hsiao, Kuo, 2006). Hemolysis has long been used to measure free radical damage and counteraction by antioxidants. It is useful for screening for oxidising or antioxidising agents (Djeridane *et al.,* 2006). Several herbal secondary metabolites such as flavonoids have been found to protect cells from oxidative damage. These compounds have been evidenced to stabilise RBC membrane by scavenging free radicals and reducing lipid peroxidation (Yu, 2001& Ebrahimzadeh *et al.,* 2010).

Lead is a dangerous heavy metal which is widely spread in the environment. Lead content in the air, food and tap water has increased several folds during recent years due to extensive use of this metal in petrol, paints, battery and other industries (Tuormaa, 1995). Despite of attempts for reducing the exposure to this metal, there are still some reports of cases with severe lead toxicity (Hershko, 2005; Roche *et al.,* 2005, Coyle *et al.,* 2005). On the other hand, chronic lead poisoning is a problem which threats mankind's life and seems to be an unknown reason for some diseases during aging (Coyle *et al.,* (2005) ; Vig & HU, 2000). The toxic effects of lead on blood indices are well known. Significant decrease in RBC count, hematocrit (Hct) and hemoglobin (Hb) were seen in rats and human with high blood lead levels. (Alexa *et al.,* 2002; Othman *et al.,* 2004; Toplan *et al.,* 2004; Hofmann & Segewitz, 1975; Noori *et al.,* 2003, Klauder & Petering, 1977). Exposure to lead is known to causes intravascular hemolysis and is known to be a factor behind lead induced anemia. Oxidative stress has been reported as a mechanism behind lead induced hemolytic changes both in human and laboratory animals. Lead is known to generate free radicals and hence reduces the antioxidant defence system in erythrocytes.

PLANT DESCRIPTION

Moringa native to Asia and spread in most parts of Africa is the sole genus in the flowering plant family *Moringaceae*. This genus is made of 12 species (Steinitz., *et al.,* 2009). *Moringa oleifera* Lam. is one of the most economically important species indigenous to dry tropical areas in the Northwestern India, at the Southwestern foot of the Himalayas (Lalas *et al.,* 2012).

An extensive variety of nutritional and medicinal uses have been attributed to its roots, bark, leaves, flowers, fruits and seeds (Mbikay, 2012). Almost all parts of this plant have been used for various diseases in the folk medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, haematological and hepatic and kidney disorders (Fahey, 2005; Mbikay, 2012). Leaves of *Moringa oleifera* are traditionally used as purgatives and in the treatment of headaches, haemorrhoids, fever, inflammation of nose and throat, bronchitis, eye and ear infections, and to combat vitamin C deficiency. The leaf juice is believed to control glycaemia and is applied for swollen glands.

Leaves of *Moringa oleifera* are cooked and eaten like spinach or used to prepare soups and salads. Fresh leaves have been reported to contain vitamin C and vitamin A, more than those reported in carrots and oranges (Mukunzi, 2011). They are also used to contrast hypertension and cholesterol; indeed, anticancer, antitumor, anti-inflammatory, diuretic properties as well as antihepatotoxic, antifertility, antiurolithiatic and analgesic activities were reported (Asare *et al.,* 2012). *Moringa oleifera* is also known for its antioxidant activity, essentially due to the presence of high amounts of polyphenols (Fahey, 2005).

MATERIALS AND METHODS

Collection of plant material

The Moringa leaves were dried in a hot air oven. The dry weight was calculated as 30 gms. The dried leaves were pulverized using a mechanical grinder. Further tests were performed using only the dried leaf powder. The chemical extraction was done by following the method of Sreenivasa-Rao and Parekh (1981).

Preliminary phytochemical screening of the moringa leaves

Presence of Alkaloids, Saponins, Tannins, Phenols, Steroids, Cardiac glycosides, Anthraquinones, Flavonoids, Amino acids, Reducing sugars, Monosaccharides in the extract and various fractions was confirmed individually by the method given by Sofowara (1994) Siddiqui and Ali (1997).

Estimation of total flavonoid content

Total flavonoid contents were measured with the aluminum chloride colorimetric assay (Kumar *et al.*, 2008),

Extraction of flavonoids from leaf extract of *moringa oleifera*.

The dried powder sample was weighed and extracted in 80% ethanol for 24 hrs and filtered. The filtrate obtained from each sample was subsequently extracted in petroleum ether, diethyl ether and ethyl acetate following the method of Subramanian and Nagarajan (1969).

Qualitative estimation of flavonoids thin layer chromatography

Thin glass plates were coated with Silica gel G and were dried at room temperature. Thereafter were kept at 100°C for 30 minutes for activation and were then cooled at room temperature prior to loading of sample. The plates were developed in an airtight chromatographic chamber saturated with solvent mixture, Benzene: Acetic Acid: Water (125:72:3). Developed plates were air dried and visualized under UV light in U.V transilluminator and were also exposed to iodine vapors for preliminary detection. Plates were also exposed to NH_4OH bottle so as to make contact with each spot for about 5-10 seconds and fluorescent spot was marked. The fluorescent spot was identified (free and bound) as quercetin (blue). The color of spot changed to bright yellow when exposed to ammonia fumes. (Samariya Krishna and Sarin Renu 2013).

Allium cepa ASSAY

The root tips several of plant species have been used for the study of induced chromosomal aberrations (CAs). Descaled healthy onions (*Allium cepa* L.) bulbs (25) were grown in distilled water for 4 days.

Cytotoxic effect of lead acetate on onion root tips

The onion root tips of 15 – 17 mm in length were treated with20µl, 40µl, 60µl, 80µl and 100µl/ml of lead acetate (1%) for 4 hours. The untreated onion root tips were considered as (control).

Cytotoxic effect of *Moringa oleifera* leaf extract and flavonoid extract on onion root tips

The onion root tips roots of 15 – 17 mm in length were treated with leaf extract and crude flavonoid extract of *Moringa oleifera* with 20µl, 40µl, 60µl, 80µl and 100µl/ml for 4 hours.

Anti-cytotoxic effect of *Moringa oleifera* leaf and flavonoid extract on lead acetate toxicity

To study the anti-cytotoxic potential of *Moringa oleifera* the onion root tips were given conditioning treatment with 100µl concentration of leaf and flavonoid extract and standard drug D-penicillamine and then treated with20µl, 40µl, 60µl, 80µl and 100µl of Lead Acetate solution for 4 hours.

Preparation of root tip squashes

For preparation root tip chromosome slides acetocarmine squash technique has been used to analyse MI, cytotoxic effects and CAs. The root tips of bulbs hydrolysed in 1N hydrochloric acid (HCl) at 60 ºC for 4-5 min. The cell wall has to dissolve by hydrolysis with acid. From HCl the roots are transferred to distilled water and left for a few minutes. The roots then were transferred on clean slide. Three roots tips were used for each slide. On the slide, tips were crushed in drop of 2% acetocarmine with the flat end of metal rod (taper) and squashed under a cover slip. The pressure was applied under several thickness of blotting filter paper during sideways movements of cover slip must be avoided.

Mitotic index

The Mitotic Index (MI) represents the total number of dividing cells in relation to the number of analysed cells in cell cycle. The frequency of CAs was expressed as the number of aberrant cells examined. The *Allium cepa* test enables to estimate CAs in all phases of the cell cycle. The Mitotic index (MI) and the frequency of

chromosomal aberrations (CA) was calculated. (Fiskesjo 1997; Bakare *et al.,* 2000).

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells(n)}}{\text{Total no. of cells counted(N)}} \times 100$$

$$\text{Frequency of (CA)} = \frac{\text{Number of aberrant cells}}{\text{Total no. of cells counted}} \times 100$$

Hemolytic activity of lead acetate on erythrocytes

Healthy Human blood sample was taken and centrifuged at 1000x for ten minutes and erythrocytes were separated from the plasma and were washed three times. The erythrocytes separated were then diluted with phosphate buffered saline (0.2M, pH 7.4) to give 4% suspension (Naim*, et al.*1976). 1% Stock solution of lead acetate was prepared. The RBC suspension 2ml with lead acetate at different concentrations (0.02, 0.06, 0.1ml/ml PBS) was made and the final volume was made to 5ml with PBS and then incubated in a 37° C water bath.

Anti-hemolytic activity of *Moringa oleifera* leaf extract and flavonoid extract against lead acetate induced hemolysis in comparision to the standard d-penicillamineFor Anti-hemolytic assay,

The RBC suspension 2ml with lead acetate at different concentrations(0.02, 0.06, 0.1ml)with presence of leaf extract and flavonoid extract and standard D-penicillamine (1% stock) 0.1ml/ml PBS each was made and the final volume was made to 5ml with PBS and then incubated in a 37° C water bath. RBC suspension in PBS was used as negative control.

Statistical analysis

The statistical analysis was done by One way Variance Of Analysis (ANOVA).

Value was considered significant for p value<0.05.

Table 1: Physical property of leaf extract of *moringa oleifera*

The color, texture and gram yield of the ethanol extract of *Moringa oliefera* leaves

Plant Part	Type of extract	Gram yield	Texture	Color
Leaves	Ethanol extract	1.984	Gummy	Green

Table 2: Phytochemical screening of *Moringa oleifera* leaf extract

	Distilled water	Ethanol	Methanol
Alkaloids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Phenols	+	+	+
Steroids	+	+	+
Glycosides	+	+	+
Anthraquinones	-	-	-
Flavonoids	+	+	+
Amino acids	-	+	-
Reducing sugars	-	-	-
Monosaccharides	-	+	+

Table 3: Total flavonoid content in leaf extract of *Moringa oleifera*

CONCENTRATION IN µl/ml	PLANT EXTRACT O.D at 415 nm	STD QUERCETIN O.D at 415 nm
20	0.032	0.438
40	0.063	0.553
60	0.15	0.653
80	0.155	0.769
100	0.21	0.867

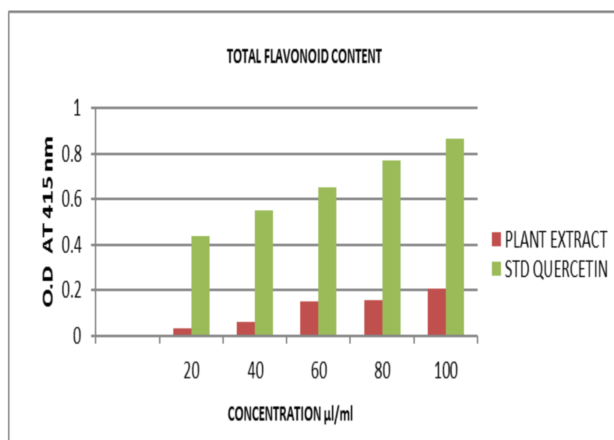
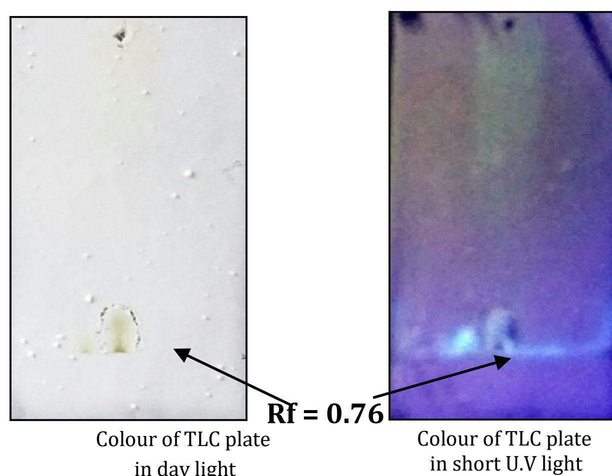


Fig 1: Total flavonoid content in leaf extract of *Moringa oleifera*



Colour of TLC plate in day light

Colour of TLC plate in short U.V light

Fig 2: TLC plate showing presence of quercetin in crude flavonoid extract of *Moringa oleifera*

Table 4: Rf value of plant flavonoid in comparison to standard quercetin

Rf of Plant Flavonoid	Rf of Standard Quercetin	Color of spot in U.V alone without treatment	Color of spot in U.V after exposure to ammonia
0.76	0.78	Blue	Bright yellow

Table 5: % Mitotic index and % Chromosomal aberration at 20µl concentration

Treatment	Conc	No. of Dividing Cells				Total Div Cells	Aberration No.	Total Cells	Ca %	Mi%
		P	M	A	T					
CONTROL		44	18	25	14	101	_	342	_	29.5
L.A	20µl	11	3	2	2	18	14	228	6.14	7.8
LA+STD	20µl	5	_	_	_	5	6	220	2.72	2.27
L.A+ PE	20µl	35	17	2	1	55	2	200	1	27.5
L.A+ FL	20µl	32	15	15	9	71	3	250	1.5	28.4

Table 6: % Mitotic index and % Chromosomal aberration at 40µl concentration

Treatment	Conc	No. of Dividing Cells				Total Div Cells	Aberration No.	Total Cells	Ca %	Mi%
		P	M	A	T					
CONTROL		44	18	25	14	101	_	342	_	29.5
L.A	40µl	7	_	_	1	8	9	145	6.2	5.5
LA+STD	40µl	4	4	_	_	8	17	225	7.55	3.55
L.A+ PE	40µl	28	15	3	2	48	6	180	3.3	26.66
L.A+ FL	40µl	28	13	8	5	54	4	200	2	27

Table 7: % Mitotic index and % Chromosomal aberration at 60µl concentration

Treatment	Conc	No. Of Dividing Cells				Total Div Cells	Aberration No.	Total Cells	Ca %	Mi%
		P	M	A	T					
CONTROL		44	18	25	14	101	_	342	_	29.5
L.A	60µl	5	1	_	1	7	13	180	7.22	3.8
LA+STD	60µl	_	7	_	5	12	20	250	8	4.8
L.A+ PE	60µl	26	6	1	2	35	6	150	4	23.33
L.A+ FL	60µl	32	15	3	12	62	3	232	1.7	26.72

Table 8: % Mitotic index and % Chromosomal aberration at 80µl concentration

Treatment	Conc	No. of Dividing Cells				Total Div Cells	Aberration No.	Total Cells	Ca %	Mi%
		P	M	A	T					
CONTROL		44	18	25	14	101	_	342	_	29.5
L.A	80µl	3	1	_	1	5	16	180	8.88	2.77
LA+STD	80µl	3	2	_	_	5	20	232	8.6	2.15
L.A+ PE	80µl	30	10	3	5	48	11	252	4.1	19.04
L.A+ FL	80µl	33	7	4	6	50	4	200	2	25

Table 9: % Mitotic index and % Chromosomal aberration at 100µl concentration

Treatment	Conc	No. of Dividing Cells				Total Div Cells	Aberration No.	Total Cells	C.A%	Mi%
		P	M	A	T					
CONTROL		44	18	25	14	101	_	342	_	29.5
L.A	100µl	3	_	1	1	5	26	200	13	2.5
LA+STD	100µl	_	2	1	_	3	20	225	8.88	1.33
L.A+ PE	100µl	29	16	2	2	49	11	250	4.4	19.6
L.A+ FL	100µl	20	15	6	13	54	8	220	3.63	24.54

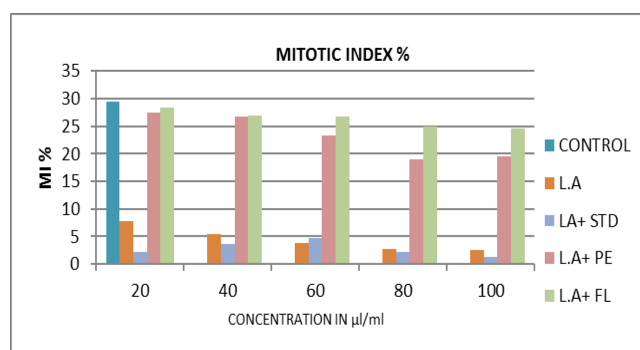


Fig 3: mitotic index in lead acetate, lead acetate+ plant extract and lead acetate+ flavonoids in comparsion to control.

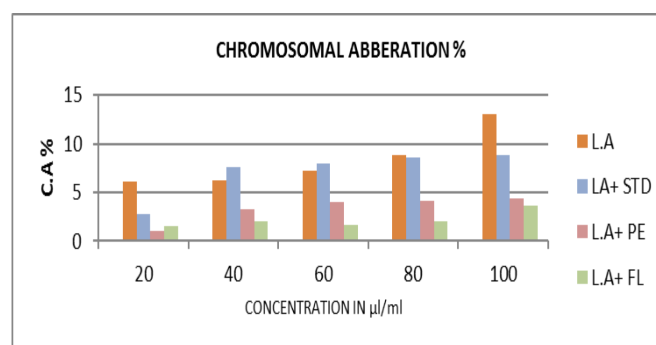


Fig 4: chromosomal abberation in lead acetate, lead acetate+ plant extract and lead acetate+ flavonoids

Table 10: Anti-hemolytic activity of leaf and flavonoid extract of *Moringa oleifera* against lead acetate toxicity

TREATMENT	CONCENTRATION		
	20µl	60µl	100µl
LA	0.472	0.487	0.521
LA+ STD	0.464	0.482	0.495
LA+PE	0.461	0.476	0.484
LA+FL	0.458	0.471	0.475

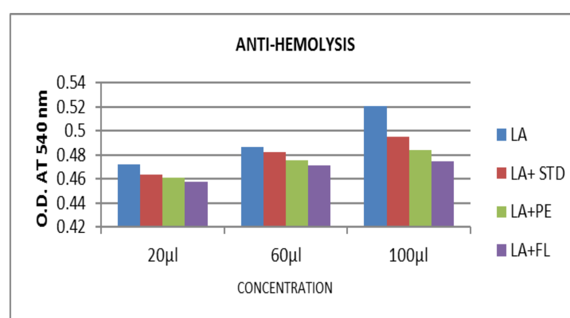


Fig 5: Anti hemolytic activity of plant extract and flavonoid extract on lead acetate induced hemolysis

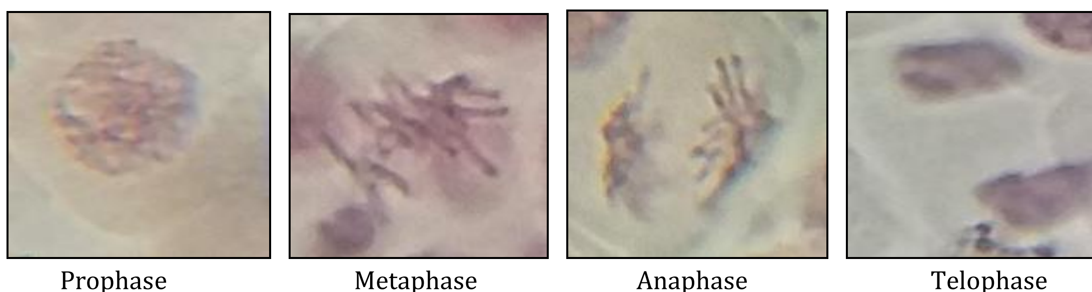


Plate 1: Microscopic photo plates of control group of onion root tips showing normal mitotic phases

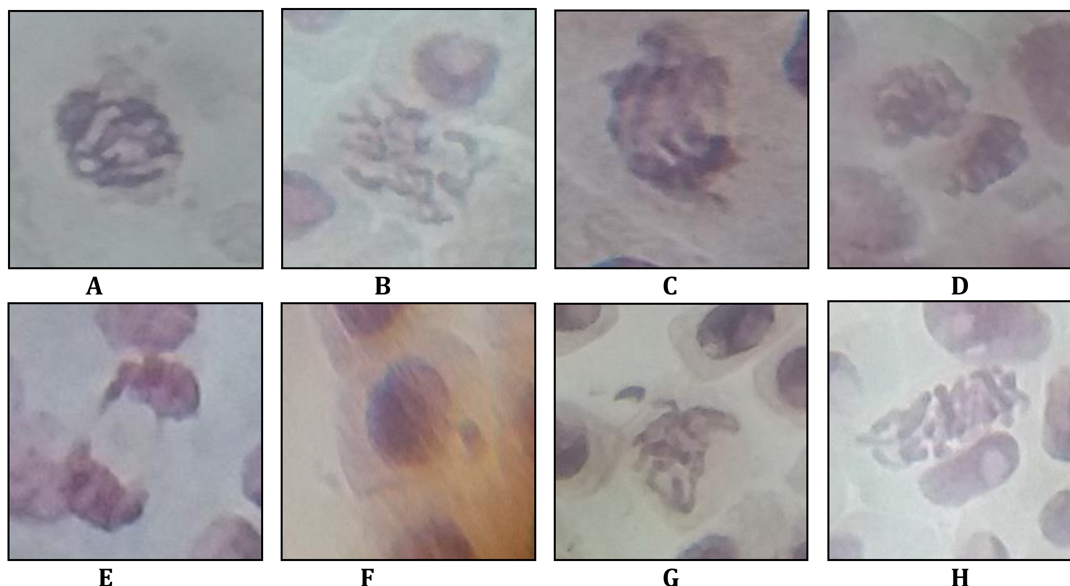


Plate 2: microscopic photo plates of onion root tips showing aberrant mitotic phases treated withn different concentrations of lead acetate (**A.** Spindle disturbance at prophase, **B.** Disoriented metaphase, **C.** Anaphase bridge, **D.** Disturbance at telophase, **E.** Laggard formation, **F.** Micronucleus, **G.** Multipolar anaphase, **H.** Stickiness of chromosomes.

RESULTS

1.Quantification of ethanolic crude leaf extract(Table I) 5gms of powdered leaves gave yield of 1.984 gm with green color and gummy texture. The % yield of crude ethanolic extract was was 39.68%.

2. The phytochemical analysis of leaves (Table-II) in Aqueous extract showed presence of Alkaloids, Saponins, Tannins, Phenols, Steroids, Glycosides, Flavonoids. Anthraquinones, Amino acids, Reducing sugars, monosaccharides were absent in Aqueous extract. The Ethanolic extract showed presence of Alkaloids, Saponins, Tannins, Phenols, Steroids, Glycosides, Flavonoids, Amino acids and Monosaccharides while Anthraquinones and Reducing sugars were absent. The Methanolic extract showed presence of Alkaloids, Saponins, Tannins, Phenols, Steroids,Glycosides, Flavonoids, and Monosaccharides. Anthraquinones, Amino acids and Reducing sugars were absent in the methanolic extract.

3. The Total flavonoid content in the leaves increases with the increase in concentration of leaf extract. (Table III)(Fig I)

4. The crude flavonoids were extracted from ethanolic leaf extract which were in two fractions, Ether fraction yield free flavonoids and Ethyl acetate fraction yield bound flavonoids. The qualitative estimation of crude flavonoids (free and bound) was done by Thin layer chromatography which reveal presence of Quercetin which was confirmed by comparing with standard Rf value of Quercetin. The Rf value of crude flavonoid was 0.76 (Table IV) which is approximately equal to the Rf of standard Quercetin 0.78 (Samariya Krishna and

Sarin Renu 2013). The colour of the spot observed on Thin layer chromatography plate was fluorescent yellow when seen in U.V illuminator) (Fig II).

5. Cytotoxic and/or genotoxic effect of Lead acetate , *Moringa oleifera* leaf extract and crude flavonoid extract of *Moringa oleifera* leaf was evaluated and it was observed that lead acetate was toxic at 100 µl concentration where as *Moringa oleifera* leaf extract and crude flavonoid extract of *Moringa oleifera* leaf were anti-cytotoxic and anti-genotoxic.

6. To study the anti-cytotoxic and anti-genotoxic potential of, *Moringa oleifera* the most appropriate concentrations were chosen based on our preliminary experiments. Conditioning treatment with *Moringa oleifera* leaf extract and crude flavonoid extract of *Moringa oleifera* leaf (100 µl/ml) followed by 4 h inter-treatment time with different concentration of lead acetate (20,40,60,80,100 µl/ml).

After the treatment, the root tips were fixed in 70% ethanol (70:30), (ethanol:water) and hydrolyzed in 1 N HCl at 60°C and stained with actetocarmine reagent at room temperature.
Lead acetate showed significant cytotoxic effect in *Allium cepa* root tips. The Anti-cytotoxic effect of *Moringa oleifera* leaf extract and crude flavonoid extract caused significant increase in the % Mitotic Index of the lead acetate treated cells.

7. (Table V) shows % Mitotic Index and % Chromosomal aberration for 20µl concentration of Lead acetate - %Mitotic Index for lead acetate was 7.8%, lead acetate+ Standard D- penicillamine was 2.27%, lead acetate+ Plant extract was 27.5% and lead acetate+ flavonoids was 28.4%. %Chromosomal aberration for lead acetate was 6.14%, lead acetate+ Standard D- penicillamine was 2.72, lead acetate+ Plant extract was 1% and lead acetate+ flavonoids was 1.5%.

8. (Table VI) shows % Mitotic Index and % Chromosomal aberration for 40µl concentration of Lead acetate - %Mitotic Index for lead acetate was 5.5%, lead acetate+ Standard D- penicillamine was 3.55%, lead acetate+ Plant extract was 26.66% and lead acetate+ flavonoids was 27%. %Chromosomal aberration for lead acetate was 6.2%, lead acetate+ Standard D- penicillamine was 7.55%, lead acetate+ Plant extract was 3.3% and lead acetate+ flavonoids was 2%.

9. (Table VII) shows % Mitotic Index and % Chromosomal aberration for 60µl concentration of Lead acetate - %Mitotic Index for lead acetate was 3.8%, lead acetate+ Standard D- penicillamine was 4.8%, lead acetate+ Plant extract was 23.33% and lead acetate+ flavonoids was 26.72%. %Chromosomal aberration for lead acetate was 7.2%, lead acetate+ Standard D- penicillamine was 8%, lead acetate+ Plant extract was 4% and lead acetate+ flavonoids was 1.7%

10. (Table VIII) shows % Mitotic Index and % Chromosomal aberration for 80µl concentration of Lead acetate - %Mitotic Index for lead acetate was 2.77%, lead acetate+ Standard D- penicillamine was 2.15%, lead acetate+ Plant extract was 19.04% and lead acetate+ flavonoids was 25%. %Chromosomal aberration for lead acetate was 8.8%, lead acetate+ Standard D- penicillamine was 8.6%, lead acetate+ Plant extract was 4.1% and lead acetate+ flavonoids was 2%

11. (Table IX) shows % Mitotic Index and % Chromosomal aberration for 100 µl concentration of Lead acetate - %Mitotic Index for lead acetate was 2.5%, lead acetate+ Standard D- penicillamine was 1.33%, lead acetate+ Plant extract was 19.6% and lead acetate+ flavonoids was 24.54%. %Chromosomal aberration for lead acetate was 13%, lead acetate+ Standard D- penicillamine was 8.8%, lead acetate+ Plant extract was 4.4% and lead acetate+ flavonoids was 3.63%. The % mitotic index of control was 29.5% and no chromosomal aberration.

(Plate I) shows normal mitotic phases in control set of root tips.

The standard drug D- penicillamine+ Lead acetate showed cytotoxic effect on root tips.
(Plate II) Chromosomal aberrations shown by lead acetate, Standard drug D- penicillamine + lead acetate were disoriented metaphase, multipolar anaphase, laggards, anaphase bridges, chromosome fragments. The frequency of chromosomal aberrations increased with increase in the concentration of lead acetate.

12. Hemolytic activity of Lead (Table X)
Lead is known to increase oxidative stress in different systems. Since oxidative stress is a pathway of lead

acetate induced hemolysis in erythrocytes, antioxidant treatment must be effective in ameliorative the toxicological effects of lead.

13. Antihemolytic activity of *Moringa oleifera.*
The result of the present study indicated the protective role of *Moringa oleifera* on lead acetate induced hemolysis. The *Moringa oleifera* leaf extract and flavonoid extract inhibited Lead acetate hemolysis significantly. (Table X)(Fig V).

Statistical analysis
All the above results were statistically analyzed by One way ANOVA Single factor and it was observed to be significant with p values <0.05

DISCUSSION

Human beings are exposed to various kinds of radiations and other ways of oxidative stress from natural as well as man-made sources. It includes endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer (Chitra K, Pillai KS., 2002). All these incidents form free radicals which are being scavenged by body's natural antioxidant system. But when formed in excess they can attack the cell membrane, causing lipid peroxidation and consequently damage the DNA (Kohen R, Gati I., 2002). One of the deleterious consequences of DNA damage from exposure to oxidative stress is cancer. Protecting cellular DNA from radical damage might result in the prevention of the cancers. Phytochemicals can neutralize such oxidative stress either by eliciting the synthesis of *in vivo* antioxidants or themselves acting as radical scavengers (Aruoma OI, 1999).

The *Moringa oleifera* leaf extract has shown considerable free radical scavenging potency. The single strand or double strand breaks may prove fatal if not repaired or protected from oxidizing environment Bont RD, van Larebeke N (2004). The *Moringa oleifera* leaf extract reveal the presence of compounds which have strong antioxidant activity. Thus, the preventive potential is lying in the chemical properties present in *Moringa oleifera* leaf extract. The present study shows that *Moringa oleifera* provides substantial protection against environmental toxicological stress with its bioactive components.

CONCLUSION

Our current study revealed that Lead acetate is a potent mutagen which causes oxidative stress in different test systems which showed cytotoxic effect in onion root tips and hemolysis in Human erythrocytes. *Moringa oleifera* shows potent anti-cytotoxic and anti-hemolytic properties against Lead acetate induced cytotoxicity and hemolysis. The crude Flavonoid extract works better than the Crude plant extract.

Further area of research
Further study can be done on purification, quantification, characterization of the crude flavonoid extract of *Moringa oleifera*.

Conflicts of interest: The authors stated that no conflicts of interest.

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