Vol. 11/1 (2011) 54-59

JOURNAL OF NATURAL REMEDIES

Effect of leaves of *Moringa oleifera* on biochemical and physiological parameters in rats.

R. Nagashree*, R. Latha, V. Karthikeyan.

Department of Physiology, P.S.G. Institute of Medical Sciences & Research,Peelamedu, Coimbatore -641004, Tamil Nadu, India.

Abstract

Medicinal herbs are used in indigenous system of medicine for various diseases. *Moringa oleifera* has a high medicinal value which has been recognized. A study on the protective effect of *Moringa oleifera* leaf extract in acute arsenite induced toxicity in rats was evaluated. *Moringa oleifera* leaf extract (200 mg/kg body weight/day) obtained by cold maceration technique, was administrated orally to the albino rats. Its protective effect was determined on sodium arsenite induced changes in the blood cell counts, hemoglobin, cholesterol and sugar. Blood sample was collected by tail vein puncture. Rats given sodium arsenite only produced significant decrease in blood haemoglobin, red blood cell and white blood cell counts and increased the blood cholesterol and sugar levels when compared to control rats. Pretreatment with 200 mg/kg body weight of *Moringa oleifera* leaf extract markedly increased the blood cell counts and the haemoglobin. It also decreased the cholesterol and sugar values. The extract contains substances that acts as an antioxidant and prevents the damage produced by arsenite on various tissues.

Key words: Lipid peroxidation. Medicinal herbs, *Moringa oleifera.*

1. Introduction

In organic arsenite is considered the most potential human carcinogen and humans are exposed to it from soil, water and food [1]. In seven districts of West Bengal arsenic has been found in ground water above maximum permissible limit (0.05 mg/l) recommended by WHO [2]. Arsenic is a proxidant and thus it can cause lipid peroxidation [3].

In traditional societies, nutrition and health care are interconnected and many plants are consumed as food inorder to benefit health [4]. Focus on plant research has increased all over the world and a large body of evidence exists to show immense potentials of medicinal plants used in various traditional systems [5].

Moringa oleifera commonly known as Zogale in Hausa (Nigeria) is used both as a source of food and medicine. Its medicinal value has been recognized in the indigenous system of medicine. Previous studies have shown that leaves of

* Corresponding author
Email: nagashreephysiology@yahoo.co.in

Moringa oleifera have definite hypo cholesterolic activity [6] and its fruits possesses a hypo lipidemic activity [7]. It was also shown that *Moringa oleifera* has a significant blood glucose lowering activity [8].

Though the leaves have been implicated in the treatment of various diseases there is not much significant evidence for the invivo activity of the leaf extract. So it was decided to study the effect of *Moringa oleifera* leaf extract on the blood cell counts, haemoglobin, cholesterol and sugar levels of the arsenite exposed rats.

2. Materials and Methods

2.1 Animals

The experiments were designed and conducted according to the ethical guidelines of Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) after obtaining clearance from the committees. Procedure for the animal care were confined to the International laws and Guidelines for the Use of Animals in Biomedical Research [9]. The adult wistar strain male albino rats weighing 280 ± 2.9 g were used for the study, food and water were given *ad libitum*. They were fed with commercial pellet rat chow (calcium 1%; phosphate 0.6%) obtained from Gold Mohur Animal feeds (Bangalore, India).

2.2 Chemicals

Cholesterol kit C (Euro Diagnostic systems Pvt Ltd, Chennai), sodium arsenite, ortho toluidine reagent, saline , Turks fluid was purchased from Sigma Chemical Co (St. Louis, Mo. USA).

2.3 Extract of Moringa oleifera

Fresh leaves were procured from a vegetable shop. The leaves were washed with fresh water and dried in shade at room temperature, the dried material was made into powder and was seived with the sieve of 0.3 mm aperture size (Endicott Ltd, London). In cold maceration technique [10] the solid ingredients are placed in a stoppered container with 750 ml of acetone and allowed to stand for a period of 3 days in a warm place with frequent agitation, until soluble matter is dissolved. The mixture is filtered and, after most of the liquid has drained, the residue on the filter is washed with sufficient quantity of acetone and the filtrate are combined to produce 1000 ml.

2.4 Experimental procedures

The animals were divided randomly into three groups consisting of 10 rats in each group. The age of the rats were one year.

Group I: Control group.

Group II: Sodium arsenite 2.5 mg/kg body weight per day was administered intraperitoneally for 20 days.

Group III: Animals were fed with *Moringa oleifera* leaf extract 200 mg/kg body weight intragastrically with Ryle's tube followed by intraperitoneal administration of sodium arsenite 2.5 mg/kg body weight per day for 20 days. At the end of the experimental period, the animals were sacrificed after ether anaesthesia and 5 ml of blood was collected by tail vein puncture without the use of anticoagulant for serum preparation and with ethylene diamine tetra acetic acid for blood cell counts and haemoglobin. The blood sample collected without the anticoagulant was allowed to stand for 10 minutes and then centrifuged at 2000 rpm for 10 minutes using R8C DX model of centrifuge with 447 G. Serum was collected and used for the analysis.

2.5 Determination of haematological parameters

Red blood cell (RBC) and white blood cell counts

(WBC) were determined by using Haemocytometer consisting of Neubauer's counting chamber with appropriate diluting fluids. Haemoglobin was determined by using Sahli's haemoglobinometer.

2.6 Estimation of biochemical parameters

Cholesterol was estimated by using the cholesterol kit purchased from Euro Diagnostic systems Pvt. Ltd.,Chennai. Blood glucose levels was estimated by using o-toluidine [11] method.

2.7 Statistical analysis

Results were expressed as mean ± S.E.M. One way ANOVA was performed to find whether or not scores of different groups differsignificantly. To test the intergroup significant difference Student's unpaired 't' test was performed. Statistical probability of $p<0.05$ was considered to be significant.

3. Results

3.1 Haematological parameters

Arsenite treatment caused a significant decrease ($p<0.001$) in RBC and WBC counts. Group II animals showed a decrease in haemoglobin ($p<0.001$) when compared with the group I animals. In the animals pretreated with *Moringa oleifera* extract, there was a significant increase ($p<0.001$) in red blood cell,white blood cell counts and haemoglobin (Table I).

Table 1. Effect of *Moringa oleifera* leaf extract on haematological parameters.

Treatment	Hb (gms %)	RBC (millions per cumm)	WBC (cells per cumm)
Control	15.2±0.26	5.36±0.04	2270±32.65
Sodium arsenite (2.5 mg/kg body wt per day)	12.88±0.21***	4.17±0.06***	1450±56.27***
Sodium arsenite + Moringa oleifera (200 mg/kg body wt per day)	18.63±0.27***	6.18±0.04***	2815±158.28***
F ratio	96.01a	367.71a	64.14a

Values are mean ± S.E.M; n=10, *** $p<0.001$, a denotes significance at 1% level (One way ANOVA followed by Student's unpaired 't'- test), Hb, RBC and WBC (Haemoglobin, Red blood cell count, White blood cell count)

3.2 Biochemical parameters

Cholesterol levels in the blood increased in group II rats significantly ($p<0.001$). These levels decreased in group III animals ($p<0.001$) when compared with arsenite only treated rats (Fig 1). Blood sugar levels increased in group II animals ($p<0.001$). These levels decreased ($p<0.001$) in the rats pretreated with the *Moringa oleifera* leaf extract (Fig 1).

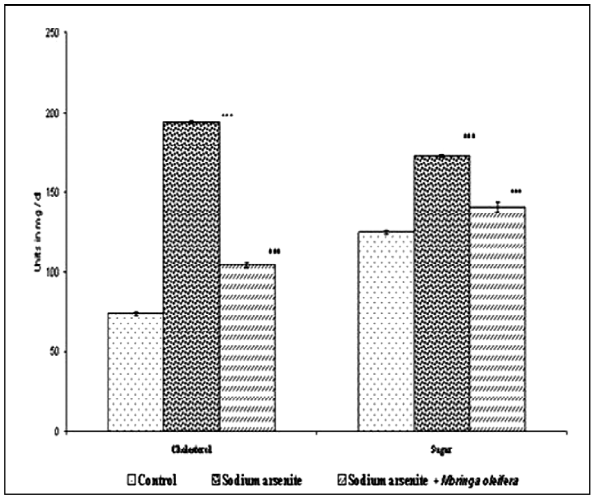

Fig 1: Effect of *Moringa oleifera* leaves on sodium arsenite induced changes in blood sugar and cholesterol levels of rats. Values are mean ± S.E.M (n=10), ***p<0.001.

4. Discussion

Sodium arsenite is the main toxic form of arsenic in environment [12]. So human beings are unavoidably exposed to this toxic metalloid. When arsenite was administered there was significant decrease in the RBC counts. This is in accordance with the previous studies where there was a decrease in RBC production after administration of arsenic and arsenious acid [13]. It was shown that arsenic accumulates in the red blood cell [14]. So red blood cells are very susceptible to its toxicity. This can produce intracellular potassium loss, hemolysis and lactate dehydrogenase leakage [15].

Arsenite also produced decrease in the hemoglobin levels. It could be due to the fact that arsenic interferes with the activities of enzymes of haem biosynthetic pathway [16]. Arsine is also shown to be fixed by hemoglobin in a non-volatile form within red cell, after which lysis occurs [17]. Haemolysis produced by arsenite could be the cause for decrease in RBC count and haemoglobin. This is due to the consequence of the action of compounds formed in oxidation of arsine [18]. Administration of *Moringa oleifera* decreases the lipid peroxidation and increases the antioxidants [19]. It was shown that *Moringa oleifera* seed powder had a significant role in protecting animals from arsenic induced oxidative stress and in depletion of arsenic concentration [20].

The major bioactive compounds in the leaf extract which has the antioxidant effect was found to be the flavinoid groups such as quercetin and kaempferol and the ethanolic extracts of the leaves were found to be capable of scavenging peroxyl and superoxyl radicals [21]. Moreover the leaves are the good source of protein, calcium, iron, copper and zinc [22]. So increase in the red blood cells and hemoglobin when *Moringa oleifera* was administered could be due to its antioxidant effect which prevents the cell lysis caused by arsenite and presence of minerals that is required for erythropoiesis. Arsenite is known to produce leucopenia [23]. In our study also sodium arsenite produced leucopenia. When *Moringa oleifera* was given there was a significant increase in WBC count.

This is in accordance with the previous study [24] which showed that crude extract of *Moringa oleifera* root increased the WBC count.

Treatment with arsenite has produced increase in the blood sugar levels in normal rats. This could be due to the decrease in the GLUT4 concentration in the adipocytes [25]. When *Moringa oleifera* was given prior to the arsenite there was a significant decrease in the blood sugar levels. The leaf extract contains the alkaloid moringine, which is identical to benzylamine and this prevents the hyperglycemia [26] induced by the arsenic. Certain inorganic mineral elements present in the extract could have maintained the normal glucose tolerance and release of insulin from beta cells of Islets of Langerhans [8].

Arsenite produced increase in the blood cholesterol levels. This is in accordance with the previous studies [27] which showed that supplementation with arsenite produced a increase in plasma cholesterol levels. When *Moringa oleifera* was given there was a significant decrease in the cholesterol levels of the blood. This could be due to the flavonoids present in the extract. This is in accordance with the previous studies which showed that leaves have definite hypocholesterolaemic activity in normal animals[6]. Moringa oleifera treated hypercholesterolaemic rabbits also showed decrease in lipid profile [7] and increase in the excretion of faecal cholesterol.

5. Conclusion

Our study showed that *Moringa oleifera* leaves produces increase in the blood cell counts and haemoglobin. It also decreased the blood sugar and cholesterol levels which showed a tendency to increase when arsenite was given alone. These protective effects of the extract illustrated that it can be used as a therapeutic agent.

References

1. Pradesh Roy, Anupama saha. (2002) *Current Science*. 82:1.
2. Badal K Mandal, Tarit Roy Chowdhury, Gautam Samanta, Gautam K Basu, Partha P Chowdhury, Chitta R. (1996) *Current Science*. 70:11.
3. Schlenk D, Wolford L, Chelius, Steevens J, Chan KM. (1997) *Comp. Biochem. Physiol.Pharmacol. Toxicol Endocrinol*. 118:117-8.
4. Pieroni A. (1997) *J. Ethanopharmacol*. 70: 235-3.
5. Dahanukar SA, Kulkarni RA, Rege NN. (2000) *Indian Journal of Pharmacology*. 32: S81-S8.
6. Ghasi S, Nwobodo E, Ofili JO, (2000) *J. Ethanopharmacol*. 69: 21-5.
7. Mehta K, Balaraman P, Amin AH, Bafna PA, Gulalti OD. (2003) *J. Ethanopharmacol* . 8: 191-5.
8. Kar A, Choudhary BK, Bandyopadhyay NG. (2003) *J. Ethanopharmacol*. 84: 105-8.
9. Giles AR.(1987) *Thrombosis and Haemostasis*. 58: 1079-4.
10. Lippinott, Williams and Wilkins, Remington.(2000) *The science and practice of pharmacy*, B.I publication pvt. Ltd: Newyork,USA; 750.
11. Varley H, Gowen lock AH, Bell M. (1980) *Practical Clinical Biochemistry*, William

Heinemann Medical Books Ltd: London; 395 – 7.

12. Maiti S, Chatterjee AK. (2001). *Arch Toxicol* .75: 531 -7.

13. Issacs R. (1928) *Folia Haematol.* 37: 389.

14. Lerman S, Clarkson TW. (1983) *Fundam Appl Toxicol*. 3: 309 -14.

15. Ayala-Fierro F, Barver DS, Rael LT, Carter DE. (1999) *Toxicol Sci*. 52: 122-9.

16. Garcia-Vargan GG, Hernandez-Zavala A. (1996) *Biomed Chromatogr.* 10: 278-4.

17. Graham AF. (1946) *Biochem J*. 40: 256.

18. Fowler BA, Weissberg. (1974) *N Engl J. Med* . 291: 1171.

19. Ashok kumar N, Pari L. (2003) *J. Med Food.* 6: 255-9.

20. Richa Gupta, Gurusamy M Kannan, Mamta Sharma, Swaran JS Flora. (2005) *Environmental Toxicology and Pharmacology* . 20: 456-4.

21. Siddhuraju P, Becker K. (2003) *J. Agric Food Chem.* 51: 2144-5.

22. Lockett CT, Calvert CL, Grivetti LE.(2000*) Int J. food Sci Nutr.*51: 195-8.

23. Islam LN, Nabi AH, Rahman MM, Khan MA, Kazi AI. (2004) *Int J. Environ Res Public Health.*1: 74-2.

24. Mazumder UK, Gupta M, Chakrabarti S, Pal D. (1999) *Indian J. Exp Biol*.37: 612-4.

25. Walton FS, Harmon AW, Paul DS, Drobna Z, Patel YM, Styblo M. (2004) *Toxicol Appl Pharmacol*.198: 424-3.

26. Bour S, Visentin V, Prevot D, Daviaud D, Saulnier-Blache JS, Guigne C, Valet P, Carpene C. (2005) *J. Physiol Biochem.* 61: 37-9.

27. Aguilar MV, Martinez –para MC, Gonzalez MJ. (1997) *Ann Nutr Metab* . 41: 189-5.