British Journal of Pharmaceutical Research
6(1): 35-43, 2015, Article no.BJPR.2015.047
ISSN: 2231-2919

SCIENCEDOMAIN *international*
www.sciencedomain.org

Ameliorative Potential of Methanolic Extract of *Citrullus lanatus* (Watermelon) Seeds on the Sperm Parameters, Testosterone Level and Testicular Cytoarchitecture of Male Albino Rats Induced with Lead-Acetate

Onyeso Godspower[1], Nkpaa Kpobari Williams[2*] and Nwaka Elochukwu[1]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Madonna University, Elele, Rivers State, Nigeria.
[2]Department of Biochemistry (Toxicology Unit), Faculty of Chemical Science, College of Natural and Applied Science, University of Port Harcourt, P.M.B. 5323, Choba, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OG designed the study, wrote the protocol, carried out the statistical analysis and wrote the first draft of the manuscript. Author NKW managed the literature searches and experimental analysis and author NE also managed the experimental process. All authors wrote, read and approved the final manuscript.

Article Information

DOI:10.9734/BJPR/2015/15358
Editor(s):
(1) Wenbin Zeng, School of Pharmaceutical Sciences, Central South University, Hunan, China.
Reviewers:
(1) Armando Zarrelli, Department of Chemical Sciences, University Federico II, Complesso Universitario Monte S. Angelo, Italy.
(2) Ahmed E. Abdel Moneim, Department of Zoology, Helwan University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=982&id=14&aid=8104

Original Research Article

Received 22nd November 2014
Accepted 31st December 2014
Published 10th February 2015

ABSTRACT

The study aimed to evaluate the ameliorative potential of methanolic extract of *Citrullus lanatus* (*C. lanatus*; watermelon) seeds on lead-acetate induced testicular toxicity considering the sperm parameters, testosterone level and testicular cytoarchitecture on adult male albino rats. The results showed statistically significant ($p<0.05$) decrease in serum level of testosterone and a deleterious effect on the sperm motility, count, morphology, viabilityand seminiferous tubular derangement on lead-acetate treated rats when compared with the control group. The methanolic extract of *C. lanatus* seed from the results showed a corrective effect as against the lead-acetate treated group in relative to the control group. In conclusion it was discovered that methanolic extract of *C. lanatus*

**Corresponding author: Email: nkwilly@gmail.com;*

seed has ameliorative potentials to correct the deleterious effect of lead-acetate on male reproductive system.

Keywords: Citrullus lanatus seeds; lead-acetate; testicular toxicity; sperm parameters; testosterone level; testicular cytoarchitecture.

1. INTRODUCTION

In recent years, more and more attention is being paid to the regulation of spermatogenesis and to the possibilities of influencing therapeutically this somatic function in a positive or negative way. However, human fecundity appears to be on the decline [1], which cannot solely be attributable to an increase in contraception. Rather, a body of data suggests that poor semen quality and low sperm count is markedly increasing and is likely to be a contributing factor [1]. Although many people still think of infertility as a problem that affect only women, but in about 40% to 50% of infertile couples, the man is the sole cause or a contributing cause of the inability to conceive [2]. Exposure of heavy metals during pregnancy has been associated with adverse effects on development of gonads. These substances may act as testicular toxicants and correspond to different compounds, which are related to social habits, life conditions, working hazards or use of drugs and medicines [3,4]. Although, many studies have reported the toxic and carcinogenic effects of metals in human and animals, it is also well known that these metals form a crucial part in normal biological functioning of cells [3].

Lead represents a significant ecological and public health concern due to its toxicity and its ability to accumulate in living organisms. Earlier studies have demonstrated that lead can pass through the blood testis barrier, accumulate in the testis and/or epididymis and seriously affect the spermatogonia, primary spermatocytes, spermatids or spermatozoa (germinal cells different levels of differentiation) [5,6]. Several studies assessed the genotoxic effect of lead acetate (LA) by means of chromosomal aberrations and micronucleus test. Regarding the induction of chromosomal aberrations, LA induced significant increase of aberrant cells and numerical distortion in bone marrow cells of Wistar rats [7,8]. Additionally, Aboul-Ela [9] detected a significant increase of structural chromosomal aberrations in bone marrow cells and primary spermatocytes of albino mice treated with LA. In addition, LA proved to be a potent micronuclei inducer *in vivo* and *in vitro* test systems: LA induced micronuclei in kidney cells of Sprague–Dawley albino rats [10]; in human melanoma cell [11]; in Chinese hamster V79 cells [12,13]; in Wistar rats' leukocytes, reticulocytes and erythrocytes [14,15]; in rats' erythrocytes [16-18]; in peripheral blood erythrocytes tissues, gill and fin epithelial cells of *Carassius auratus* [19] and in bone marrow cells of Algerian mice [20]. More so, the results of the studies indicated that occupational exposure to Pb has adverse effects on sperm parameters (decreased sperm counts, lower and a lesser motility and altered sperm morphology), studies showed that exposure to inorganic lead greatly impaired male reproductive functional activities by decreasing sperm count or distorting sperm motility and morphology [21].

The fruits of *C. lanatus* (watermelon) help in Boosting Antioxidant Levels because it is exceptionally rich in carotenoids such as lycopene, lutein and β carotene [22]. A regular watermelon juice consumption result in significant increases in blood plasma concentrations of lycopene and β carotene [23]. Also, lycopene is known to have over 40 potential health benefits and β carotene with equally plentiful health benefits, which make this finding very plausible. Moreover, watermelon-induced is also known to increase plasma antioxidant levels and may explain why epidemiological studies of the Chinese found greater watermelon intake to be associated with a lower risk of cancer [24]. A study found that 6 weeks of treatment with a watermelon extract containing 6 grams of L-citrulline and L-arginine daily on middle-aged obese subjects with prehypertension or stage 1 hypertension experienced reduced ankle blood pressure and altered carotid wave reflection, an indication of improved arterial function of the individuals [25]. If watermelon can cure or ameliorate this process, it would certainly provide a breakthrough to many of the drugs used in the market for primary prevention, such as the cholesterol-lowering statin drug class, whose side effects are numerous [26].

It is very important to note that all parts of the watermelon have something to offer. For

example, the seeds are excellent source of protein. The good nutritional and functional properties of watermelon seed meal proteins suggest their potential use in food formulations and diets [27]. *C. lanatus* possesses numerous bioactivities from natural source which is of better advantage than conventional therapies. This study aimed to evaluate the ameliorative potential of methanolic extract of *C. lanatus* (watermelon) seeds on Lead-acetate induced testicular toxicity considering the sperm parameters, testosterone level and testicular cytoarchitecture on adult male albino rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total number of 20 male Albino rats weighing between 155 to 328 grams were used for the study. The animals were bought from the animal house of the Department of Pharmacology and Toxicology, located in Niger Delta University Wilberforce Island. It was kept in the animal house of Department of Human Physiology Madonna University, Elele Campus for 3 weeks to acclimatize. The animals were kept under normal room condition of temperature 25±2°C, humidity of 50±5% and 12 hour light and dark cycles. The animals were randomized into experimental and control groups and housed in sanitized wooden cages containing saw-dusts as bedding. They were also fed with standard rat chow pellet as diet and clean water ad-libitum was supplied.

2.2 Seeds Collection and Preparation

Ripe watermelon pods were obtained from the local market in Elele (Eke-Onuma Market), Rivers State, Nigeria (May, 2013). The seeds extracted from the pods, only healthy looking seeds were collected. The collected seeds were oven-dried at 35°C, to a constant weight. The dried seeds were reduced into fine powder using a Laboratory grinding hand mill. The powder was weighed and kept away from light before extraction.

2.3 Seeds Extraction and Concentration

Extraction was by maceration over a 72 hours period. 500 g of the powdered seeds material was extracted with 1.5 liters of Methanol in 3 successive extractions (500 ml every 24 hours). The jar was tightly closed and thoroughly shaken intermittently. After 72 hours, the different portions were combined and filtered using filter paper. The filtrate was collected in a glass jar. The extract was concentrated using a Rotary Evaporator. The concentrated methanolic extract of *Citrullus lanatus* seeds were then transferred into bottles covered with aluminium foil and stored in a refrigerator at 4°C before use. This is to prevent it from losing its potency.

2.4 Experimental Procedure

On commencement of the experiment, the animals were divided into 4 groups of 5 animals each. The first group served as the control group while the last 3 groups served as the experimental groups.

2.4.1 Group 1

This group was the control group fed normal rat chow and water.

2.4.2 Group 2

This group was fed normal rat chow and water and received 2.25 mg/kg of Lead-acetate + 100 mg/kg (Low dose) of methanolic extract of *C. lanatus* seeds.

2.4.3 Group 3

This group was fed normal rat chow and water and received 2.25 mg/kg of Lead-acetate + 200 mg/kg (High dose) of methanolic extract of *C. lanatus* seeds.

2.4.4 Group 4

This group was fed normal rat chow and water ad-libitum and they received 2.25 mg/kg Lead-acetate only.

2.5 Analytical Procedure

After 30 days of treatment, the animals were fasted for 24 hours prior to sacrifice. The animals were anaesthetized using chloroform and then sacrificed. Thus, blood was obtained via cardiac puncture and put in labeled EDTA bottles for testosterone assay. The animals were then dissected; the testes were removed along with the caudal epididymis. The caudal epididymis was separated from the testes and lacerated to collect the semen with a microscope glass slide for analysis of sperm characteristic and the

testes for testicular cytoarchitecture (histopathology).

2.5.1 Estimation of serum testosterone

Quantitative measurement of serum total testosterone was carried out adopting ELISA technique using kits specific for rats purchased from Department of Pharmacology and Toxicology, Niger Delta University Wilberforce Island, Nigeria according to the protocol provided with kit.

2.5.2 Histological examination

The testes tissues were collected and immediately fixed in 1 ml PBS (pH 7.4) and embedded in paraffin. Sections (5-µm) were prepared and then stained with hematoxylin and eosin (H & E) stain for photomicroscopic observations.

2.6 Statistics

The result were expressed as mean ± standard error of mean (S.E.M). The statistical evaluation of data was performed by using a one-way ANOVA (analysis of variance). The data were considered significant at $p<0.05$.

3. RESULTS

As shown in Table 3.1, the level of active sperm cells in Lead-acetate + HDE of *C. lanatus* seed (65.00±2.88%) was significantly higher ($p<0.05$) than the Lead-acetate only (11.66±7.26%). The level of dead cells increased significantly ($p<0.05$) in Lead-acetate only rats (81.67±10.14%) but showed no significant difference ($p<0.05$) in the sluggish sperm cell levelsthough it increased (18.33±1.66%) in the Lead-acetate + LDE administered with *C. lanatus* seed.

The total sperm count was significantly ($p<0.05$) higher in the Lead-acetate + HDE administered rats (33.67±2.96X10^6/ml) compared to the Lead-acetate administered rats only (7.00±2.08 X10^6/ml). The level of Sperm morphology (%) in both Head, Mid-piece and Tail defect increased significantly ($p<0.05$) in the Lead-acetate only administered rats (compared 5.33 ± 2.40%, 2.33 ±0.33% and 3.00±0.00% respectively) compared to the Lead-acetate + HDE administered rats (which had 0% Mid-piece defect, 0.66±0.33% and 1.00±0.58% for Head and Tail defect respectively).

Sperm viability (% viable) showed no significant difference in either the Lead-acetate + HDE rats Lead-acetate only rats or control rats. Though the Sperm viability (% non-viable) of the Lead-acetate only rats (10.67±2.33%) was significant higher ($p<0.05$) as compared to the Lead-acetate + HDE albino male rats (1.67±.0.67%). The Testosterone level of the Lead-acetate + HDE rats (5.23±0.23ng/ml) was significantly higher ($p<0.05$) compared to the Lead-acetate only rats (0.73±0.12ng/ml).

Histological examination of rat testis in group 1 (control) as shown in Fig. 1 showed the seminiferous tubules lined with stratified epithelium composed of two major cells, which are the sertoli cells and spermatogenic cells. Seminiferous tubules depict normal architecture with adequate cellularity; on the other hand, histological examination in group 4 as shown in Fig. 2 showed degeneration of the spermatogenic cells, occlusion of the lumen and hypertrophied seminiferous tubules. By contrast, the lead acetate alone group depict shrunken seminiferous tubules and reduced cellularity, while group 2 shows increase in the spermatogenic cells of the rats treated compared with the control. This group showed a mild thickening of the basement membrane as noted in certain seminiferous tubules, but it appeared generally healthy (Fig. 3). Finally, histological examination of group 3 showed matured spermatozoa in the seminiferous tubules of rats treated. This group has a healthy testicular tissue simila to the control, but with a slight increase in the interstitial tissues. This shows that methanolic extract of *C. lanatus* seed administered as a treatment option may be medicinally beneficial (Fig. 4).

4. DISCUSSION

Several studies show that alkaloids and terpenes are widely spread in the genus *citrullus* [28]. These secondary metabolites are responsible for the pharmacological activities such as antiulcer, antimicrobial, antioxidant, analgesic, aphrodisiac and many other ethno-medicinal uses [29].

Table 3.1. Ameliorative potential of methanolic extracts of *C. lanatus* (watermelon) seeds on sperm parameters and testosterone level of lead-acetate induced male albino rats

Group	Sperm motility (%)			Sperm count (X10^6/ml)	Sperm morphology (%)			Sperm viability (%)		Testosterone (ng/ml)
	AC	SC	DC	Sperm Count	HD	MPD	TD	VC	NVC	Testosterone
Control(1)	82.50±2.50	7.50±2.50	10.00±0.00	43. 50±1.50	0.50±0.50	0.00±0.00	0.50±0.50	99.00±1.00	1.00±1.00	2.75±1.45
Lead-acetate + LDE(2)	43.33±6.00	18.33±1.66*	38.33±4.41	20.00±1.55	2.33±1.20	1.00±0.58	1.00±0.58	95.67±0.33	4.33±0.33	1.77±0.52
Lead-acetate + HDE(3)	65.00±2.88*	15.00±5.00	20.0±2.88	33.67±2.96*	0.66±0.33	0.00±0.00	1.00±0.58	98.33±0.67	1.67±0.67	5.23±0.23*
Lead-acetate only(4)	11.66±7.26	6.66±3.33	81.66±10.14*	7.00±2.08	5.33±2.40*	2.33±0.33*	3.00±0.00*	89.33±2.33	10.67±2.33*	0.73±0.12

Data represented as Mean ± SEM; (*) p<0.05 significant difference; LDE=Low dose extract of C. lanatus seeds, HDE=High dose extract of C. lanatus seed; AC: Active cells; SC: Sluggish cells; Dead cells; HD: Head defect; MPD: Mid-piece defect; TD: Tail defect; VC: Viable cells; NVC: Non-viable cells.

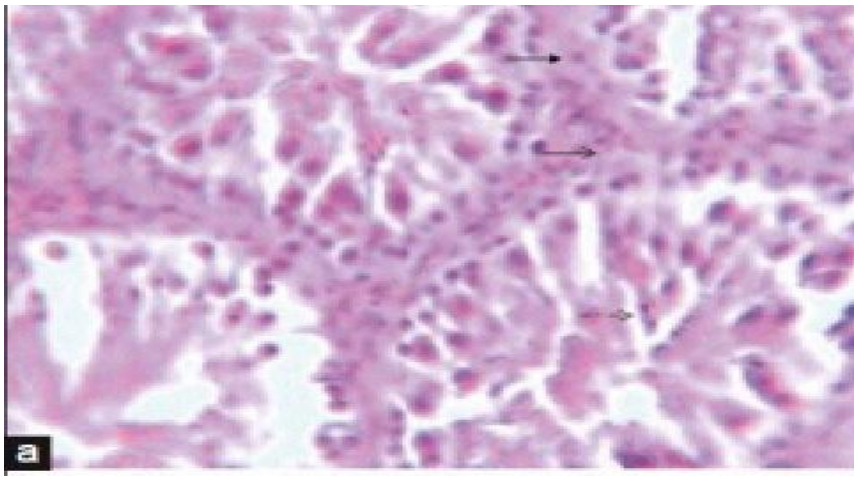

Fig. 1. Photomicrograph of albino rat testis in group 1

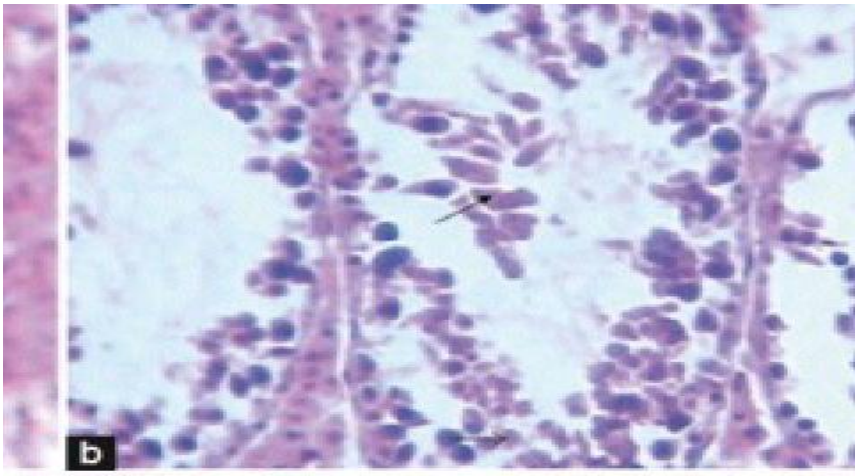

Fig. 2. Photomicrograph of albino rat testis in group 4

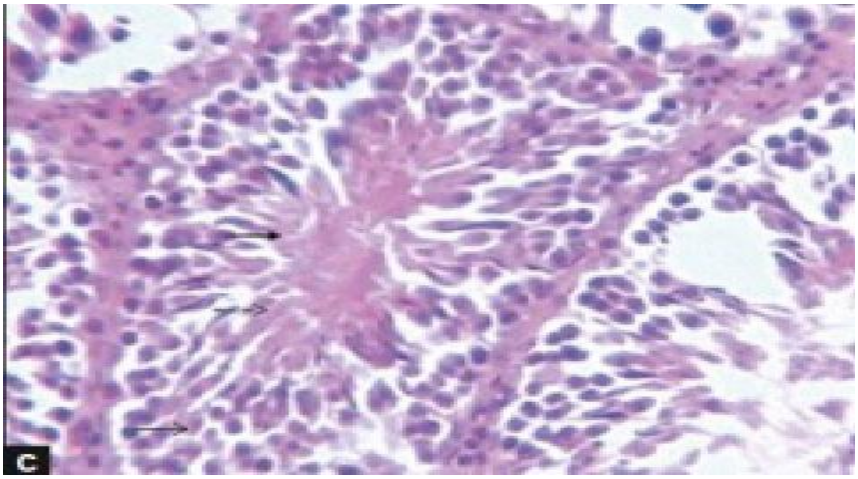

Fig. 3. Photomicrograph of albino rat testis in group 2

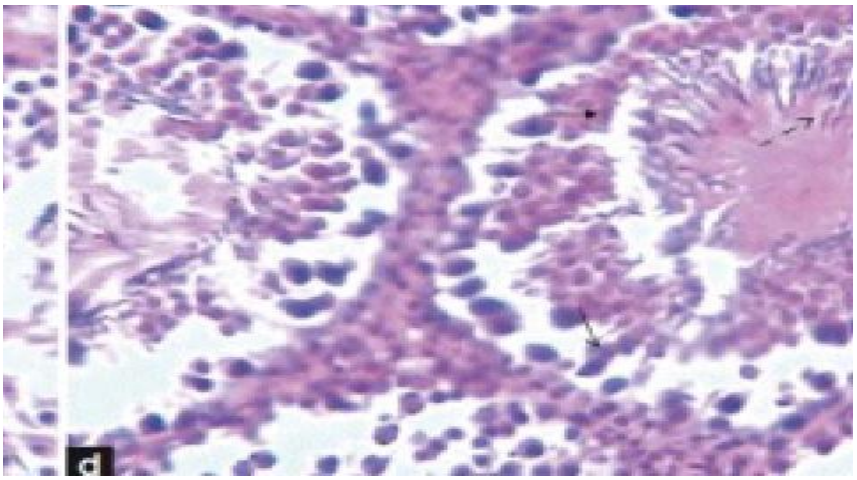

Fig. 4. Photomicrograph of albino rat testis in group 3

This study indicates that even moderate exposure to Lead-acetate can significantly reduce semen parameters, reduce testosterone level and cause toxicity to the testis. The changes observed in the above agree with the previous reports, which demonstrated that lead acetate suppressed testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels along with testicular spermatogenesis, showing that lead acts at all levels of reproduction [30]. The extract administered resulted to improvement of sperm characteristics; this may be as a result of the presence of flavonoids.The presence of steroid in the phytochemical screening of the crude extract could be the resultant improved testosterone level in group 3. The mechanisms by which methanolic extract of *C. lanatus* seeds protect against experimentally induced testicular toxicity may be as a result of the rich source of vitamin C, thiamine and including riboflavin which contains a high level of polyphenolic compounds present in the plant. High concentration of vitamin C in *C. lanatus* seeds provides highly effective anti-oxidants, reversing the negative effect caused by the lead-acetate following the administration of the extracts to the experimental animal as seen in the group III of the different parameters when compared to the group 4. This effect may be influenced by the presence of flavonoids in the extract which contains antioxidants. Saponin that was found to be present in the extract functions majorly at stimulating an increase in the body's natural endogenous testosterone levels which helps to maintain testosterone levels. *C. lanatus* seeds are a rich source of flavonoids and phenol, with ability to scavenge free radical and inhibit hydrolytic and oxidative enzymes and anti-inflammatory action [31]. However, findings from these results are supported by the fact that methanolic extracts of *C. lanatus* seeds contains a high amount of anti-oxidant activity [32], bringing about the promising result obtained in this study.

5. CONCLUSION

Methanolic extracts of *C. lanatus* seeds has shown to be a beneficial treatment option against lead-acetate induced oxidative stress and toxicity in testicular tissue. It was also deduced from the study that among the treatment options, administration of methanolic extracts of *C. lanatus* seeds at a high dose on exposure to lead result to the most beneficial result such as increased sperm motility, well defined cellularity of the testis, increased sperm viability, decreased sperm morphological alterations, increased sperm count, increased testosterone level. There is need for further investigations because methanolic extracts of *C. lanatus* seeds can be a potential complimentary agent in treating lead induced testicular toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that principle of laboratory animal care was dully followed, as well as specific national laws where applicable in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Skakkebaek NE, Jorgensen N, Main K.M, De Metys ER, Leffers H, Andersson AM, Juul A, Carlsen E, Mortensen EK, Jensen TK. Is human fecundity declining? Int J of Androl. 2002;2-11.
2. Brugh VM, Lipshultz LI. Male factor infertility. Medicinal clinics of North America. 2004;88(2):367-85. DOI:10.1016/S0025-7125.
3. Bustos-Obregón E. Adverse Effects of Exposure to Agropesticides on Male Reproduction. APMIS Denmark. 2002;109: 233-242.
4. Pomerol JM, Arrondo JL. Practica Andrologica Barcelona Masson-Salvat; 1994.
5. Apostoli P, Porru S, Bisanti L. Critical aspects of male fertility in the assessment of exposure to lead. Scand. J. Environ. Health. 1999;25:40-43.
6. Reshma AM, Sainath SB, Suneetha Y, Sreenivasula. Lead acetate induced reproductive and paternal mediated developmental toxicity in rats. Ecotoxicology and Environmental Safety. 2010;74(4):793-9.
7. Lorencz R, Nehez M, Desi I. Investigations on the mutagenic effects of cadmium and lead on the chromosomes of the bone marrow cells in subchronic experiments. Abstracts of the 27th Meeting of the Hungarian Society of Hygiene, Blatonfoldva Hungary. 1996;116
8. Nehez M, Lorencz R, Desi I. Simultaneous action of cypermethrin and two environmental pollutant metals, cadmium and lead, on bone marrow cell chromosomes of rats in subchronic administration. Ecotoxicol. Environ. Saf. 2000;45:55-60.
9. Aboul-Ela EI. The protective effect of calcium against genotoxicity of lead acetate administration on bone marrow andspermatocyte cells of *mice In vivo* .Mutat. Res. 2002;516:1-9.
10. Robbiano L, Carrozzino R, Puglia CP, Corbu C, Brambilla G. Correlation between induction of DNA fragmentation and micronuclei formation in kidney cells from rats and humans and tissue-specific carcinogenic activity. Toxicol. Appl. Pharmacol. 1999;161:153-159.
11. Poma A, Pittaluga E, Tucci A. Lead acetate genotoxicity on human melanoma *cells In vitro*. Melanoma Res. 2003;13: 563-566.
12. Thier D, Bonacker D, Stoiber T, Bohm KJ, Wang E, Unger E, Bolt HM, Degen G. Interaction of metal salts with cytoskeletal motor protein systems. Toxicol. Lett. 2003;140-141,75-81.
13. Bonacker D, Stoiber T, Bohm KJ, Prots I, Wang M, Unger E, Their R, Bolt HM, Degen GH. Genotoxicity of inorganic lead salts and disturbance of microtubule function. Environ. Mol. Mutagen. 2005; 45(4):346-353.
14. Kasuba V, Rozgaj R, Fucic A, Varnai VM, Piasek M. Lead acetate genotoxicity in suckling rats. Biologia Bratislava. 2004; 59:779-785.
15. Celik A, Ogenler O, Comelekoglu U. The evaluation of micronucleus frequency by acridine orange fluorescent staining in peripheral blood of rats treated with lead acetate. Mutagenesis. 2005;20:411-415.
16. Piao F, Cheng F, Chen H, Li G, Sun X, Liu S, Yamauchi T, Yokoyama K. Effects of zinc coadministration on lead toxicities in rats. Ind. Health. 2007;45:546–551.
17. Garcia-Leston J. Genotoxic effects of lead: An updated review. Environment International. 2010;36:623-626
18. Alghazal M, Sutiakova I, Kovalkovicova N, Legath J, Falis M, Pistl J, Sabo R, Benova K, Sabova L, Vaczi P. Induction of micronuclei in rat bone marrow after chronic exposure to lead acetate trihydrate. Toxicol. Ind. Health. 2008;24: 587-593.
19. Cavas T. *In vivo* genotoxicity of mercury chloride and lead acetate: Micronucleus (MN) assay on acridine-orange stained fish cell. Food Chem Toxicol. 2008;46(1):352-8.
20. Tapisso JT, Marques CC, Mathias ML, Ramalhinho MG. Induction of micronuclei and sister chromatid exchange in bone-marrow cells and abnormalities in sperm of Algerian mice (*Mus spretus*) exposed to cadmium, lead and zinc. Mutat. Res. 2009;678:59-64.
21. Kumar K. Occupational and exposure associated with reproductive dysfunction. J. Occup. Health. 2004;46:1-19.
22. Chandrika UG, Fernando KSSP, Ranaweera KKDS. Carotenoid content and

in vitro bioaccessibility of lycopene from guava (*Psidium guajava*) and watermelon (*Citrullus lanatus*) by high-performance liquid chromatography diode array detection. Int J Food Sci Nutr. 2009; 60(7):558-66.
23. Edwards AJ, Vinyard BT, Wiley ER, Brown ED, Collins JK. Penelope Perkins-Veazie, Robert A Baker, Beverly A Clevidence. Consumption of watermelon juice increases plasma concentrations of lycopene and beta-carotene in humans. J Nutr. 2003;133(4):1043-50.
24. Zhang C, Ho SC, Chen Y, Fu J, Cheng S, Lin F. Greater vegetable and fruit intake is associated with a lower risk of breast cancer among Chinese women. Int J Cancer. 2009;125(1):181-8.
25. Figueroa A, Sanchez-Gonzalez MA, Wong A, Bahram H, Arjmandi BH. Watermelon Extract Supplementation Reduces Ankle Blood Pressure and Carotid Augmentation Index in Obese Adults With Prehypertension or Hypertension. Am J Hypertens. 2012;12:342-353.
26. Christensen AS, Viggers L, Hasselström K, Gregersen S. Effect of fruit restriction on glycemic control in patients with type 2 diabetes-a randomized trial. Nutr J. 2013;12:29.
27. Wani AA, Sogi DS, Singh PI, Wani ID, Uma S, Shivhare US. Characterisation and functional properties of watermelon (*Citrullus lanatus*) seed proteins. J Cancer Res Clin Oncol. 2011;137(2):279-86.
28. Ali M, Pandey A. Cucurbitaceae of blhar: Diversity and Conservation. Global Biodiversity: Status and Conservation. 2007;257.
29. Khan MS, Hussain SA, Matjais AM, Zakaria ZA, Khan M. Antiulcer activity of Ficusreligiosa stem back ethanolic extract in rats. J. Med. Plant. Res. 2011;5(3):354-359.
30. Biswas NM, Ghosh PK. Protection of adrenal and male gonadal functions by androgen in lead treated rats. Kathmandu University Medical Journal. 2006;14:1218-21.
31. Frankel E. Nutritional benefits of flavonoids. International Conference on Food Factors Chemistry and Cancer Prevention, Hamatsu Japan, Abstracts C6-2; 1995.
32. Chan EWC, Lim YY, Wong LF, Lianto FS, Wong SK, Lim KK, Joe CE, Lim TY. Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. Food Chem. 2008;109: 477-483.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=982&id=14&aid=8104